Attention Deficit / Hyperactivity Disorder (ADHD),

The Human Endocannabinoïd System &

Medicinal Cannabis (phyto-cannabinoïds)

A Patients' Bibliographical Review

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Summary

The present review is a collection of different materials related to ADHD, the human EndoCannabinoïd System **(ECS)** and phyto-cannabinoïds. As the medical use of cannabinoïds for the treatment of ADHD is not fully accepted by the medical community in Europe, a group of patients wanted to have the worldwide view of the current state-of-the-art in that field. A number of patients worked together in order to build up the more exhaustive and complete collection of scientific articles, papers, internet resources, doctors and patients testimonies available on the internet.

2008 is the first year where **clinical studies** have been published in the field of ADHD and cannabinoïds. Two studies report surprising results, in opposition with expectations, putting in light the apparent **ADHD paradoxal reaction to cannabinoïds**. The first study relates the case of an ADHD patient, medicated with Dronabinol (THC) and cannabis, and driving performance evaluation. The second study is partly related to ADHD adolescents and reveals a positive effect of cannabis on mental health and behavior.

The review is divided in two main parts. The first one is a short review of the ECS theory. It outlines in particular an underlying neurological mechanism called **Inhibition Retrograde Signaling.** It reviews the implication of the ECS in emotional responses through **CB1 receptors**. In this part is also pointed out the importance of the ECS regarding chronic symptoms expressed in ADHD: impulsivity, anxiety, fear, and distraction. It finally suggests that the ECS is probably closely involved in the neuro-immunity and that **CB2 receptors, in addition to CB1 receptors,** also appear to be interesting pharmacological targets for ADHD treatment.

The second part of the review is much more practical. It is mainly based on California doctors' experiences and patients testimonies. Indeed, in 1996, California was the first US states to tolerate medicinal cannabis (MC). Since then, a large number of doctors had the opportunity to prescribe cannabis to treat patients. To date, the estimative number of Californian patients treated with MC is 350 000. In that state, many doctors approve, from their patients real experiences, the **viability and efficiency** of MC to treat ADHD symptoms in comparison to standard medications. At the end of the report are presented the 3 MC grades available, under prescription, in the Netherlands' pharmacies since 2005.

Acknowledgements

We would like to address particular warm words of gratitude to Dr Franjo Grotenhermen for having helping patients to get in contact, through the intermediate of the **International Association for Cannabis as Medicine (IACM)**. We appreciate your open mind and comprehensive listening of our turbulent life Odysseys. Think you also for your important contribution to the first publication ever done on ADHD and cannabis: “Cannabis improves symptoms of ADHD” (Dr. P. Strohbeck-Kuener).

We also would like to acknowledge Dr K. Muller-Vahl, Medical Hospital Hanover (Germany), for her comprehensive listening and kind invitation to the Hanover Congress on Tourette Syndrome, TIC and hyperactivity (9-11 oct. 2008). Think you for having permitted us to express our positive experience with medicinal cannabis.

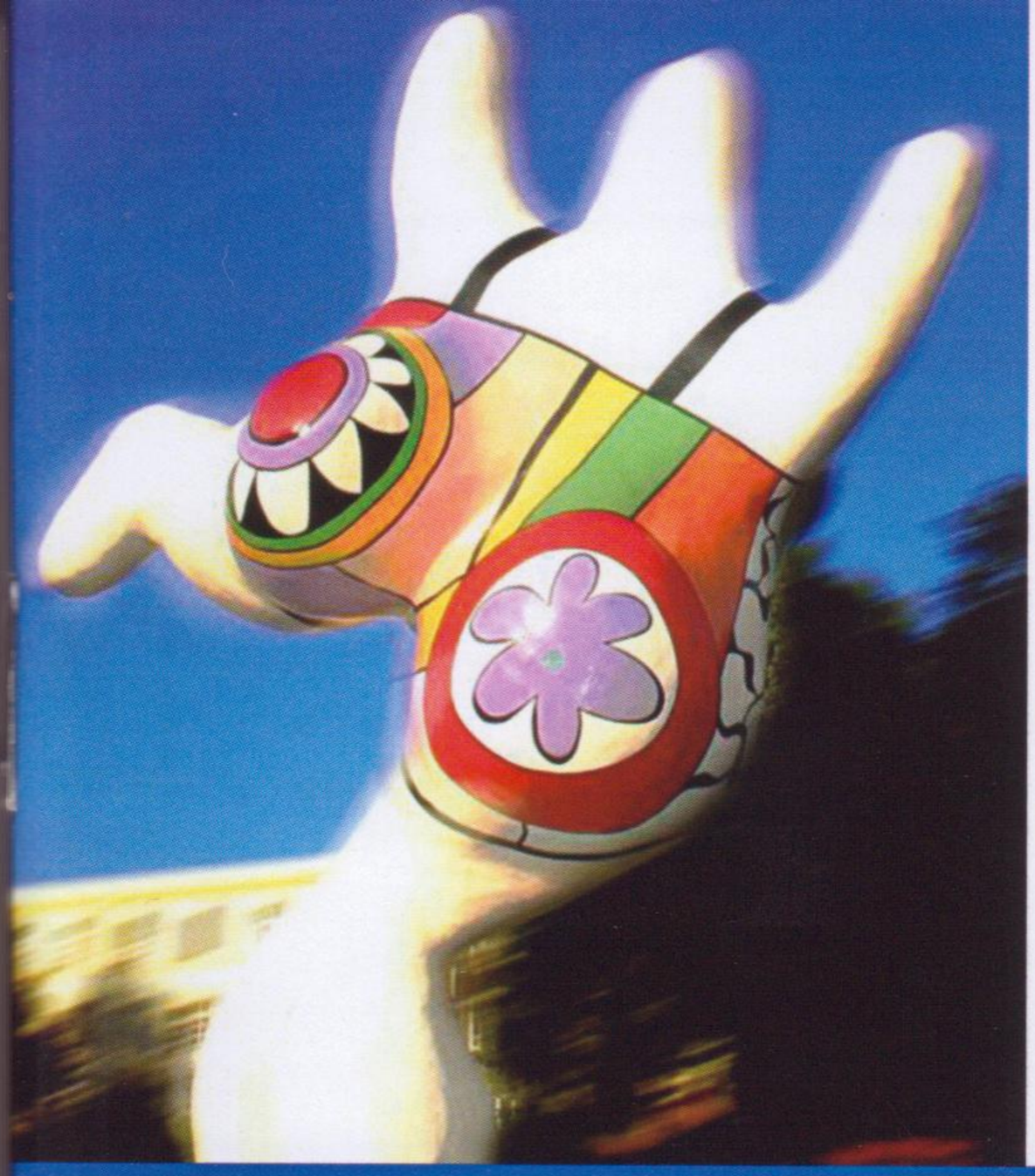
We also would like to address an immense “thank you” to Dr Robert W. Gorter, Medical Center Cologne (Germany), for its support and advises in our projects.

We would like to take this occasion to address special congratulations to Dr Strohbeck-Kuener, Heidelberg University (Germany), for the discovery of the paradoxal driving performances of ADHD under cannabis effects.

We would like to thank Dr D. Bearman, Santa Barbara (California), for having introduced to us the concept of inhibition retrograde signaling, and for having written so much on ADHD and medicinal marijuana. Think you for speaking in the name of all the ADHD patients who use cannabis to reduce their symptoms and live a better life.

Finally, we would like to address acknowledgements to all the persons, doctors, patients, who have been cited in this review. Thank you for your direct and indirect contributions.

A. Jeffrey, M.Sc.A.



Medizinische Hochschule
Hannover

Abstracts

Wider das Stigma – ADHS, Tic und Zwang im Spiegel von Gesellschaft und Forschung

9. – 11. Oktober 2008 | Medizinische Hochschule Hannover
Klinik für Psychiatrie, Sozialpsychiatrie
und Psychotherapie

ADHS DEUTSCHLAND e.V.
Selbsthilfe für Menschen mit ADHS

disorders. Amalgam fillings in teeth, maternal fish consumption and vaccinations prenatal expose and placental transfer are the main source for presence of this heavy metals in our body's who are damaging our and our children organs, enzyme system and brain. These intoxications are shooting our immune system down and causing inflammations like middle ear infection. Antibiotics given against the inflammation are destroying our gastrointestinal flora (Leaky gut syndrome). The damaged enzyme system is not able to digest food like containing gluten / casein the intestines, damaged by candida albicans, allow incompletely digested food, pathogens, and toxins passing into the bloodstream and reaching the brain, trigging an immune response. The inappropriate activation of receptors in the brain are provoking behavioural patterns associated with autism and ADHD.
Treatment and interventions for ADS / ADD / ADHD: Analyses: Diagnose (psychologic), Food intolerance (Cytotest), Peptide test, Acids, vitamins and minerals, Oxidative stress, Bacteriological inventory, Accumulation in our body's of viruses, contained in vaccination shots (MMR).

Still in 2008, we are depending on psychologic diagnoses for metabolic disorder problems. (In past it was different.)
May inflammations in the body's can be reduced an calmed by CBD / THC, the best and successfully effect will be showed by our scientific experiences in future with the easement from the usual biomedical treatment and a mix of CBD / THC (official, usual medical THC = Dronabinol).
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Cannabinoids, a possible treatment for ADD/ADHD: views of patients

Brodusch L, Wagener N

ASD/ADD/ADHD: Complex Neuro-Immune Disorders Autistic Spectrum Disorders, including Attention Deficit Disorders (with or without Hyperactivity), are multi-modal caused disorders. Genetic predispositions are often implicated, as well as a defective dopaminergic system. In addition, emotional and immune hypersensibilities are often associated (micro-traumas, allergies), suggesting that the causes lie at the neuro-immune system. For that reason, in addition to a healthy lifestyle (sport ...), additional medical treatments are required, integrating medication, controlled food intake and psychological support.
Implication of the Endocannabinoids System (ECS) Clinical studies on animal models show that the ECS is involved in neuro-transmission and in the regulation of neuronal activity. Through the retrograde signalling, the ECS controls inhibitory neurons activity that modulates behaviour and

emotional responses and learning. tudies support the idea that ECS receptors CB1/CB2 could be targeted with exo-cannabinoids to address a large number of ADD/ADHD symptoms: attention, hyper-impulsivity, hyper-emotionality, hyper-anxiety, depression, addictions and tics.
Medical Cannabis: Contrasted Legal Situation In 2008, thousands of ADD/ADHD patients receive medical cannabis legally in North America (Canada, 9 USA states) and Europe (The Netherlands, Spain). A growing number of physicians have been able to evaluate the therapeutic interest for ADD/ADHD. Many interviews report positive results, improved conditions and side effects decrease. Phytocannabinoids, used under medical control, are becoming progressively available in European countries (Bedrocan®, Bedrobinol®, Bediol®), but prescriptions rarely address psychiatric disorders comparing to North America. Could Cannabinoids be the cure rather than the threat, as it is experienced efficiently by many ADD/ADHD patients?

Hirnveränderungen bei erwachsenen, männlichen Patienten mit ADHS-Syndrom - eine strukturelle voxelbasierte MRT-Studie

Buddensiek N, Bents S, Glahn A, Ohlmeier MD, Grosskreutz J, Peschel T, Emrich HM, Müller-Vahl KR

Hintergrund: Die Aufmerksamkeitsdefizit-Hyperaktivitätsstörung (ADHS) zeichnet sich durch eine Kombination von Störungen der Aufmerksamkeit und Konzentration sowie motorischer Unruhe aus. Die Störung beginnt in der Kindheit und persistiert in etwa einem Drittel der Fälle bis ins Erwachsenenalter. Häufig sind psychiatrische Erkrankungen wie das Gilles de la Tourette-Syndrom assoziiert. Mittels voxelbasierter MRT-Techniken, wie der voxelbasierten Morphometrie (VBM), dem Magnetisation-Transfer-Imaging (MTI) und dem Diffusion-Tensor-Imaging (DTI) stehen neue strukturelle Kernspintomographiemethoden zur Verfügung, die es ermöglichen, makroskopische sowie histo-pathologische Hirnveränderungen in vivo sichtbar zu machen.
Methodik: Ziel dieser Studie war es, hirnpathologische Veränderungen bei Patienten mit einer ADHS ohne wesentliche Komorbiditäten („ADHS only") nachzuweisen. Hierzu wurden 25 ADHS-Patienten und 25 alters- und geschlechtsentsprechende, gesunde Kontrollpersonen mittels VBM, MTI und DTI untersucht. Um zusätzliche Einflussfaktoren auszuschalten, wurden ausschließlich erwachsene, unbe-

Wider das Stigma – ADHS, Tic und Zwang im Spiegel von Gesellschaft und Forschung

Cannabinoids 2008;3(1):1-3

Case report

Cannabis improves symptoms of ADHD

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Abstract

Attention-deficit/hyperactivity disorder (ADHD) is characterized by attention deficits and an altered activation level. The purpose of this case investigation was to highlight that people with ADHD can benefit in some cases from the consumption of THC. A 28-year old male, who showed improper behaviour and appeared to be very maladjusted and inattentive while sober, appeared to be completely inconspicuous while having a very high blood plasma level of delta-9-tetrahydrocannabinol (THC). Performance tests, which were conducted with the test batteries ART2020 and TAP provided sufficient and partly over-averaged results in driving related performance. Thus, it has to be considered, that in the case of ADHD, THC can have atypical effects and can even lead to an enhanced driving related performance.

Keywords: ADHD, cannabis, performance, driving

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Introduction

Assessing the performance or impairment of cannabis users is generally problematic as there is no stringent proof of a linear dose-effect relationship between the concentration of delta-9-tetrahydrocannabinol (THC) in blood and THC-induced impairment. The cause of the absence of such a relationship has not been identified. In this context it is rarely considered that the missing correlation may be due in part to a conceivable positive effect of cannabis on the behaviour and performance of individuals. Recently, Adriani et al. [1] gave evidence that cannabinoid agonists reduce hyperactivity in a spontaneously hypertensive rat strain, which is regarded as a validated animal model for attention deficiency hyperactivity disorder (ADHD). There was also a significantly better treatment retention of cocaine dependent patients with comorbid ADHD among moderate users of cannabis compared to abstainers or heavy users [2].

ADHD was long considered a disorder limited to children and adolescents. It has now been proven that ADHD symptoms may persist into adulthood [3,4]. Individuals suffering from ADHD characteristically have an increased drive to move around and are unable to calm down. They are lacking in directed planning of their actions and the ability to assess the impact of their decisions. Their ability to organize day-to-day activities is reduced, they usually have a poor short-term memory, are forgetful and tend to work in a chaotic and inefficient way. Emotionally, they are prone to impulsive outburst, excessiveness and instability [5,6].

This present case study describes a male, 28 years of age, who was diagnosed with attention deficit hyperactivity disorder (ADHD), and whose response to THC suggests that such a positive effect may exist. Considering that the subject applied for the reinstallation of his driving licence gives particular significance to psycho-physical performance deficits caused by ADHD. Numerous studies have shown that various performance functions, such as divided attention, selective attention, long-term attention and vigilance are impaired [7].

Case Description

The subject had a record of several violations of the German drug control law. He also had a record of numerous violations of traffic laws, including speeding, running of a red traffic light and driving under the influence of cannabis during which a high THC concentration in blood had been detected.

Seven years ago, the subject had been diagnosed with ADHD (ICD 10 F90.0) for the first time, and that diagnosis had been assessed repeatedly and independently since by several psychiatric units. There was some

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evidence from his carrier that typical symptoms were already present in childhood, they were, however, not properly recorded. Comorbidities such as addiction, including cannabis, or personality disorders were absent. He had been treated over a period of about 12 months through a combination of methylphenidate (Ritalin®, 20-30 milligram/day) and behaviour therapy. Being not sufficiently efficacious, the medication was stopped. A subsequent certificate by a specialist for general medicine suggests that ADHD symptoms were much improved under cannabis and that dronabinol (THC) had been prescribed, even though ADHD is not indicated for this drug.

Prior to the first contact the subject had been advised not to consume any medicinal or recreational drug. During that first visit he showed grossly conspicuous behaviour. His attitude was pushy, demanding and lacking distance. He expressed impatience, for example by drumming his fingers on the table. He also constantly shifted position, folded arms behind his head or leaned over the table in front of him. He was not open to discussing the potential impairment of driving skills caused by cannabis consumption. As the conversation continued and he was informed of the preconditions for a positive assessment of his suitability to operate a vehicle, his behaviour became even more conspicuous and aggressive. Finally, he got up, grabbed the table, leaned forward and shouted that he needed a driving license and that he needed cannabis. Overall he showed behaviour typical of persons who suffer from ADHD. During this visit, an appropriate performance of the tests was impossible.

He was then offered to undergo, at a later time, a test of the impact of dronabinol on performance. During this appointment he appeared fundamentally changed and was not disturbed at all. He stated that he had stopped smoking cannabis, was taking dronabinol on a regular basis and that he had consumed it just two hours ago. He appeared calm, but not sedated, organized and restrained. Unlike during the first meeting he was able to accept and discuss arguments. When trying to make clear that THC was indispensable for his quality of life he became more engaged but without losing restraint. Rather, he was understanding of the position of the expert and indicated that the path to get back his driver license may be long but that he was willing to undertake it. His behaviour, motor function, mood and consciousness did not give any indications of a prior use of a psychoactive substance.

The tests of performance functions that are relevant to driving skills involved the four subtests of ART2020, a computer-controlled test system, which is commonly used to assess driving performance. These subtests evaluate complex reactions (RST3), sustained attention (Q1), directed attention (LL3) and visual surveying and perception (TT15). In addition the functions of "vigilance" and "divided attention" were tested with the attention test module (TAP).

The results of these tests (see Fig. 1) showed that the subject met, in all of the functions tested by ART2020, not only minimum criteria but that he achieved average or, in some areas, even above-average results. In the very demanding tests for "vigilance" and "divided attention" categories he also showed average performance. ADHD or acute effects of THC by themselves would usually impair performance particularly in these tests.

A blood sample was taken after completion of the tests. It showed a very high concentration of THC (71 ng/mL serum), of the psychoactive metabolite 11-hydroxy-

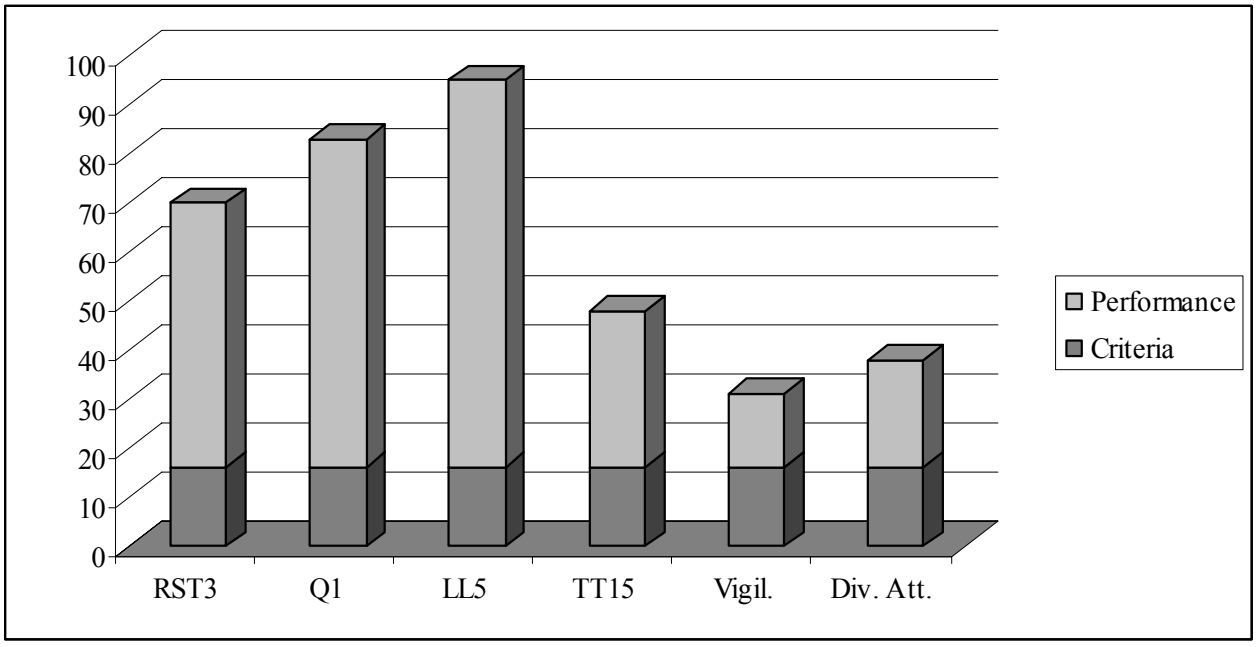

Figure 1: Subjects actual performance and minimum criteria.

THC (30 ng/mL serum) and of the main non-psychoactive metabolite 11-nor-delta-9-carboxy-THC (251 ng/mL serum). Such levels indicate recent as well as frequent consumption of THC-containing matters, and the analyte pattern also suggests smoking. Detection of cannabinol in hair (5.3 ng/mg) along with THC (3 ng/mg) gives evidence that the medication could not have been the only source of the THC.
Only much later did the subject, who had been arrested for a drug offence a few days after the second visit, report that he had not consumed pharmaceutical dronabinol products but instead smoked cannabis just before the tests, since it was much less costly.

Conclusions

The present case report suggests that individuals suffering from ADHD, a dysfunction with a symptomatic change in activity levels, may - in some cases - benefit from cannabis treatment in that it appears to regulate activation to a level which may be considered optimum for performance. There was evidence, that the consumption of cannabis had a positive impact on performance, behaviour and mental state of the subject. The present observation corroborates previous data of Müller-Vahl et al. [8] suggesting that in patients suffering from Tourette syndrome, treatment with THC causes no cognitive defects. Gilles de la Tourette syndrome is a neurobehavioral disorder associated with motor and vocal tics as well as behavioural and cognitive problems. The authors also hypothesized that the effects of cannabinoids in patients may be different from those in healthy users suggesting an involvement of the central cannabinoid receptor systems in the pathology of the disorder. The same conclusion may be drawn from previous studies [1, 2] and the present case report, although more information on these atypical effects should be provided and the underlying mechanisms are still to be elucidated.

References

1. Adriani W, Caprioli A, Granstrem O, Carli M, Laviola G. The spontaneously hypertensive- rat as an animal model of ADHD: evidence for impulsive and non-impulsive subpopulations. Neurosci Biobehav Rev 2003;27:639-651.
2. Aharonovich E, Garawi F, Bisaga A, Brooks D, Raby, WN, Rubin, E, Nunes EV, Levin FR. Concurrent cannabis use during treatment for comorbid ADHD and cocaine dependence: effects on outcome. Am J Drug Alcohol Abuse 2006;32: 629-635.
3. Mannuzza S, Klein RG, Bessler A, Malloy P, Lapadula M. Adult outcome of hyperactive boys. Arch Gen Psychiatry 1992;50:565-576.
4. Murphy K, Barkley RA. Attention deficit hyperactive disorder adults: comorbidities and adaptive impairments. Compr Psychiatry 1993;37:393-401.
5. Wender PH, Wolf LE, Wasserstein J. Adults with ADHD. Ann NY Acad Sci 2001;931:1-16
6. Sobanski E, Alm B. Aufmerksamkeitsdefizit-/Hyperaktivitätsstörung (ADHS) bei Erwachsenen – Ein Überblick. Der Nervenarzt 2004;75: 697-715.
7. Woods SW, Lovejoy DW, Ball JD. Neuropsychological characteristics of adults with ADHD: a comprehensive review of initial studies. Clin Neuropsych 2002;16:12-34.
8. Müller-Vahl KR, Prevedel H, Theloe K, Kolbe H, Emrich HM. Treatment of the Tourette syndrome with delta-9-tetrahydrocannabinol (Δ9-THC): no influence on neuropsychological performance. Neuropsychopharmacology 2003;28.384-388.

The complete case-report was published in 2007 in Archiv fuer Kriminologie 220: 11-19.

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Different psychological effects of cannabis use in adolescents at genetic high risk for schizophrenia and with attention deficit/hyperactivity disorder (ADHD)

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Abstract

Background: Controversy exists regarding whether young people at risk for schizophrenia are at increased risk of adverse mental effects of cannabis use.
Methods: We examined cannabis use and mental health functioning in three groups of young people aged 14–21; 36 non-psychotic siblings of adolescents with schizophrenia (genetic high risk group), 25 adolescents with attention deficit hyperactivity disorder (ADHD) and 72 healthy controls. The groups were sub-divided into 'users' and 'non-users' of cannabis based on how often they had used cannabis previously. Mental health functioning was quantified by creating a composite index derived from scores on the Schizotypal Personality Questionnaire (SPQ), Strengths and Difficulties Questionnaire (SDQ) and Global Assessment of Function (GAF).
Results: A significant positive association between cannabis use and mental health disturbance was confined to young people at genetic high risk for schizophrenia. To determine whether the relationship was specific to particular dimensions of mental health function, a second composite index was created based on scores from the SPQ Disorganisation and SDQ hyperactivity-inattention sub-scales. Again, there was a significant positive association between cannabis use and factor scores which was specific to the genetic high risk group. There was a trend for this association to be negative in the ADHD group ($p=0.07$).
Conclusions: The findings support the view that young people at genetic high risk for schizophrenia are particularly vulnerable to mental health problems associated with cannabis use. Further research is needed to investigate the basis of relationships between cannabis and mental health in genetically vulnerable individuals.
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Keywords: Schizophrenia; High-risk; Cannabis; Mental health; ADHD

1. Introduction

Cannabis use in adolescence is associated with mental health problems in early adulthood (McGee et al., 2000) and is estimated to double the risk of psychosis and schizophrenia in early adult life (Arseneault et al., 2004; Fergusson et al., 2006). While the association with psychosis appears robust, the mechanism and causal direction remains unclear. Lack of direct causality would render ineffective public

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health interventions based on reducing cannabis use (Macleod et al., 2004). An alternative explanation for the association between cannabis and mental health problems is reverse causality where adolescent cannabis use is a consequence of personality factors and psychological disturbance that are themselves risks for psychosis and adult psychiatric disturbance (for a recent review see Degenhardt and Hall, 2006).

So far it is unclear whether being at genetic high risk for schizophrenia increases the risk of exposure to cannabis and/or increases sensitivity to its adverse psychological effects. One example of a gene-environment (cannabis) interaction is the catechol-*o*-methyl transferase (COMT) val^{158} met polymorphism which appears to moderate the link between adolescent cannabis use and psychosis (Caspi et al., 2005). The Edinburgh High Risk Study found that cannabis use increased the risk of psychotic symptoms in young people both at high and low genetic risk of schizophrenia (Miller et al., 2001). Other studies suggest that psychological adverse effects of cannabis are greatest in young people already at high-risk by virtue of pre-existing psychotic symptoms (van Os et al., 2002; Verdoux et al., 2003), although one study of an ultra high-risk group reported no difference in rates of transition to psychosis between users and non-users of cannabis (Phillips et al., 2002).

Here we examined whether adolescents at genetic high risk of schizophrenia (healthy siblings of patients) were more prone to the psychological adverse effects of cannabis than either healthy adolescents at low genetic risk or adolescents with attention deficit hyperactivity disorder (ADHD). Importantly and in contrast to much of the previous work in this field, the high-risk group in the present study were entirely free from any signs or symptoms of psychosis or of the schizophrenia prodrome. This enabled us to measure relationships between cannabis use and mental health without the potentially confounding effects of pre-existing psychotic or prodromal symptoms. Furthermore, previous research has tended to focus on relationships between cannabis use and the development of psychotic symptoms (Phillips et al., 2002) or other aspects of mental health associated with increased risk of psychosis, such as schizotypal personality traits (Barkus et al., 2006). We were interested in measuring relationships between cannabis use and a broad range of mental health domains. We included adolescents with ADHD to determine whether the association of cannabis with mental health problems was specific to adolescents at increased genetic risk of schizophrenia, or whether it was also present in a group of adolescents with a non-psychotic mental health disorder.

2. Materials and methods

2.1. Participants

Participants were recruited as part of a large-scale study assessing neuro-cognitive function and mental health (see Groom et al., 2008 for a detailed description of recruitment and assessment methods). Full ethical approval was granted by the Trent Multi-centre Research Ethics Committee and by the Research and Development department of the Nottinghamshire Healthcare NHS Trust. Three groups of adolescents aged 14 to 21 years were included in the present investigation: i) a group at genetic high-risk for schizophrenia consisting of 36 non-psychotic siblings of patients with adolescent-onset schizophrenia (15 males, mean age 17.50 years ± 2.18); ii) 72 'healthy control' adolescents (30 males, mean age 17.19 years ± 2.03) and iii) 27 adolescents with ADHD (25 males, mean age 15.69 years ± 1.47). Participants gave fully informed consent if aged 16 years or older; parental consent (with participant assent) was obtained for those aged less than 16. There were significant group differences for age ($F(2,132)=7.489$, $p=0.001$) and gender ($\chi^2(2)=27.17$, $p<0.001$). Post hoc Tukey tests showed the ADHD group were significantly younger than the HC and SZ-SIB groups ($p<0.01$). Pairwise chi-square tests showed the ADHD group had a higher male:female ratio than the HC ($\chi^2(1)=26.31$, $p<0.001$) and SZ-SIB ($\chi^2(1)=17.26$, $p<0.001$) groups. There were no significant differences between the HC and SZ-SIB groups for age and gender and no significant differences between any groups for parental Socio-Economic Status (SES) measured using the National Statistics for Socio-Economic Classification (Statistics, 2004). All participants had an IQ score of at least 70 on the Wechsler Abbreviated Scale of Intelligence (WASI) (Wechsler, 1999). Recruitment and assessment procedures for each group are described below.

2.1.1. Schizophrenia-sibling group (SZ-SIB)

Participants in the SZ-SIB group were the full biological siblings of adolescents with a diagnosis of early-onset schizophrenia-spectrum disorder. Selection of patients was based on the following criteria: DSM-IV diagnosis of schizophrenia-spectrum disorder (code 295) (determined by thorough interview using the Schedules for Clinical Assessment in Neuropsychiatry (SCAN) (Wing et al., 1990) and case-consensus conference of 3 psychiatrists); age of onset before 19 years; first psychotic episode within previous 5 years; IQ at least 70; no other neurological illness. Of 85 young

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people referred to the study team with a broad diagnosis of functional psychosis, 50 satisfied the study diagnostic criteria and of these 41 had at least one sibling eligible for inclusion; all patients gave consent for their sibling to be contacted about the study. One sibling was recruited per patient. Sibling pairs did not have to be same-sex. In cases where a patient had more than one sibling in the age range of 14 to 21 years the sibling closest in age to the proband (either younger or older) was approached first for inclusion. If this sibling was not willing to take part, the sibling next closest in age to the proband was approached. Of 41 siblings who initially agreed to take part five later withdrew from the study leaving 36. The mean difference in age between affected and unaffected siblings included in the study was 2.38 (±1.3) years with 70% of participants in the SZ-SIB group being younger than the proband. All siblings were free from psychotic and prodromal symptoms on the Structured Interview for Prodromal Symptoms (SIPS) (Miller et al., 2003) and Psychosis Screening Questionnaire (PSQ) (Bebbington and Nayani, 1995). They were therefore a group at increased genetic risk of schizophrenia-spectrum disorder but showing no signs of clinical risk.

2.1.2. Attention deficit hyperactivity disorder group (ADHD)

Forty-six young people with a clinical diagnosis of ADHD were contacted about the study; 34 volunteered to participate. Psychiatric assessment was conducted using the Parental Account of Childhood Symptoms (Taylor and Hepstinall, 1993). All were diagnosed with DSM-IV ADHD combined type (314.01) following a consensus conference of 3 psychiatrists and all were free from psychotic symptoms. Three were excluded due to IQ less than 70; 3 were excluded due to scoring less than 22 on the Social Communication Questionnaire (Rutter et al., 2003), a screening threshold used to exclude possible pervasive developmental disorder. Contact was lost with 1 participant.

2.1.3. Healthy control group (HC)

Healthy control subjects were recruited from local schools; further education colleges providing non-professional vocational courses, and the University of Nottingham. Of 89 who volunteered to take part, 72 satisfied the inclusion criteria and were willing to take part. Participants were free from personal or family history of psychosis or schizophrenia prodrome (on the PSQ and SIPS) and from symptoms of ADHD measured with the hyperactivity-inattention sub-scale of the Strengths and Difficulties Questionnaire (Goodman, 1997).

2.2. Assessment of mental health function

In addition to the measures completed for group inclusion/exclusion, the following were completed by all participants:

Schizotypal Personality Questionnaire (SPQ) (Raine, 1991): a 72-item self-report measure of schizotypal personality traits. A score of 1 is given for each positive response. A total score ('SPQ Total') is derived and scores can be summed to assess the number of traits related to each of three dimensions: cognitive–perceptual; interpersonal; disorganisation. A higher than average number of traits is associated with increased risk of schizophrenia-spectrum conditions (e.g. Miller et al., 2002).

Strengths and difficulties questionnaire (SDQ): 40-item questionnaire assessing function in 4 domains (hyperactivity-inattention; emotional symptoms; peer relations; conduct problems) using a 3-point Likert scale with '0' denoting absence of problems. Parent- and self-rated versions were completed. A total score ('SDQ-self Total'; 'SDQ-parent Total') reflects problems across all domains. Scores can also be summed to create 4 sub-scales, 1 for each domain.

Global assessment of function (GAF) (WHO, 1992): a rating scale completed by the interviewer on the basis of discussion with the interviewee. A score from 0–100 rates the interviewee's overall functioning in daily life. A score of 0 indicates severe impairment in all aspects of function (occupational; social; emotional; psychological) and a score of 100 indicates excellent functioning.

2.3. Cannabis use

Cannabis use was assessed using the substance misuse interview from Section 12 of the SCAN which begins by asking the interviewee whether they have ever used drugs and if so, whether they have used drugs more than once or twice. In the present sample regular use of drugs other than cannabis was rare; we therefore focussed on cannabis. Those who report using drugs once or twice or more than once or twice are asked a series of further questions to determine whether they have a substance abuse disorder. To assess relationships between cannabis use and mental health function in the present study cannabis use was dichotomised into i) regular cannabis use (i.e. more than once or twice) and ii) never or occasional use (i.e. never used, or only once or twice). For simplicity these groups will henceforth be referred to as 'users' and 'non-users' respectively.

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2.4. Statistical analysis

The primary aim of analysis was to investigate relationships between cannabis use and mental health and to determine whether these relationships differed between a group at high genetic risk of psychosis (SZ-SIB group) and two groups at low genetic risk of psychosis; a healthy control group and a comparison group of adolescents experiencing mental health problems other than psychosis (ADHD group).

Data for the measures of mental health function were not normally distributed. Non-parametric Mann–Whitney *U*-tests were conducted to compare users and non-users on each measure, within the HC, SZ-SIB and ADHD groups. To provide an index of mental health function which captured the overall profile revealed by the non-parametric analysis and which was amenable to parametric analysis, Principal Components Analysis (PCA) was conducted on the square root transform of 4 variables (SPQ Total; SDQ-self Total; SDQ-parent Total; GAF). One component was identified, accounting for 72.6% of variance; all variables loaded highly on the component (>0.8) which was labelled 'Mental Health Disturbance Index'. Univariate ANCOVA (age as covariate) with the factors Group (HC, SZ-SIB, ADHD) and Cannabis (users; non-users) was conducted to investigate differences in factor scores. Significant main effects and interactions were followed with planned independent samples *t*-tests; we predicted significantly higher factor scores (indicating greater mental health disturbance) for users than non-users within each group (HC; SZ-SIB; ADHD). Gender was not included as a covariate in the analyses as there were only 2 females in the ADHD group. Independent-samples *t*-tests were conducted to compare mental health factor scores of males and females within the HC and SZ-SIB groups and within the user and non-user groups. There were no significant differences; for brevity, the results are not reported.

Factor scores deviated slightly from a normal distribution in the HC and SZ-SIB groups (Shapiro–Wilk statistic significant at $p=0.02$ and $p=0.002$, respectively). To ensure the results obtained using parametric statistical methods were reliable, Mann–Whitney *U*-tests were conducted to compare users and non-users within each group. The results were consistent with those obtained using parametric methods; parametric methods are reported.

3. Results

3.1. Cannabis use in the HC, SZ-SIB and ADHD groups

The lifetime prevalence of regular cannabis use was 39% (14/36) in the SZ-SIB group, 33% (9/27) in the ADHD group and 26% (19/72) in the HC group. There was no significant difference in the proportion of users between groups ($\chi^2(2)=1.827$, $p=0.41$). Of those who used cannabis more than once or twice (the 'users' group), the following percentages represent those who used cannabis at least once monthly: HC: 68%; SZ-SIB: 43%; ADHD: 89%.

3.2. Relationships between cannabis use and mental health

Table 1 presents the descriptive statistics for mental health scores of cannabis users and non-users within the HC, SZ-SIB and ADHD groups. There were no significant differences between users and non-users in the HC and ADHD groups but significant differences in the SZ-SIB group for SDQ-self Total ($z=$

Table 1
Descriptive data for measures of mental health function in cannabis users and non-users in all groups

Measure [a]	Cannabis	Group [b, c]		
		HC	SZ-SIB	ADHD
SPQ Total	Non-users	11.00 (4.00–15.00)	8.00 (5.00–15.00)	32.00 (24.00–41.00)
	Users	9.00 (2.50–25.25)	17.50 (5.75–37.75)	29.00 (4.50–40.00)
SDQ-self Total	Non-users	6.00 (4.00–8.50)	6.00 (4.00–8.00)	18.00 (15.00–19.50)
	Users	9.00 (3.25–13.25)	10.00 (7.75–15.00)	17.00 (10.00–23.50)
SDQ-parent Total	Non-users	5.00 (3.00–8.00)	5.00 (3.00–10.00)	24.00 (17.50–28.00)
	Users	2.50 (1.25–10.75)	11.50 (5.75–13.50)	24.00 (15.50–32.00)
GAF	Non-users	87.00 (85.50–90.00)	89.00 (82.00–90.00)	61.00 (57.50–66.50)
	Users	86.50 (73.25–88.75)	83.50 (71.75–86.25)	66.00 (62.50–71.00)

[a] SPQ = Schizotypal Personality Questionnaire; SDQ-self total = Strengths & Difficulties Questionnaire, self-rated version, SDQ-parent total = Strengths & Difficulties Questionnaire, parent-rated version; GAF = Global Assessment of Function.
[b] HC = Healthy control group; SZ-SIB = schizophrenia-sibling group; ADHD = ADHD group.
[c] Data shown are median with 25th–75th percentile in parentheses.

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Table 2
Descriptive data for factor scores on the Mental Health Disturbance Index and Hyperactivity–Disorganisation Index

Group [a]	Cannabis	Factor	
		Mental health disturbance index [b]	Hyperactivity-disorganisation index [b]
HC	Non-users	−0.82 (0.38)	−0.62 (0.63)
	Users	−0.64 (0.62)	−0.58 (0.57)
SZ-SIB	Non-users	−0.77 (0.67)	−0.68 (0.55)
	Users	−0.06 (0.16)	−0.12 (0.81)
ADHD	Non-users	0.91 (0.62)	1.28 (0.64)
	Users	0.78 (0.83)	0.78 (0.72)

[a] HC = Healthy control group; SZ-SIB = schizophrenia-sibling group; ADHD = ADHD group.
[b] Data shown are age-adjusted group means with standard deviations in parentheses.

−2.72, $p<0.01$), GAF ($z=-2.285$, $p<0.05$) and a trend towards significance for SDQ-Parent Total ($z=-1.827$, $p=0.071$).

These results indicate a relationship between cannabis use and mental health function in the SZ-SIB group which is not present in the HC and ADHD groups. Univariate ANCOVA was conducted on factor scores for the Mental Health Disturbance Index derived from PCA (see Table 2 for descriptive statistics of factor scores). There were significant effects of Group ($F(2,101)=56.32$, $p<0.001$), Cannabis ($F(1,101)=4.21$, $p<0.05$) and a significant Group * Cannabis interaction ($F(2,101)=4.11$, $p<0.05$). Independent-samples t-tests were conducted to compare cannabis users and non-users within each group. There was no significant difference in the HC or ADHD groups but a significantly higher factor score (greater mental health disturbance) for users than non-users in the SZ-SIB group ($t(31)=-3.154$, $p=0.004$).

3.3. Relationships between cannabis use and specific dimensions of mental health

To determine whether relationships between mental health and cannabis use were explained by scores on specific sub-scales from the SPQ and SDQ, Mann–Whitney U-tests were conducted to compare users and non-users on the Cognitive-perceptual, Interpersonal and Disorganisation sub-scales of the SPQ and the Hyperactivity-inattention sub-scales of the SDQ, self- and parent-rated versions. The descriptive data are shown in Table 3. There were no significant differences between users and non-users in the HC and ADHD groups. In the SZ-SIB group users had significantly higher scores on the SDQ-self hyperactivity-inattention sub-scale ($z=-2.183$, $p<0.05$) and a trend towards significantly higher scores on the SPQ Disorganisation sub-scale ($z=-1.658$, $p=0.1$). These variables were entered into PCA, from which one component was identified accounting for 83.82% of the variance. The component was labelled ‘Hyperactivity-Disorganisation Index’ (see Table 2 for descriptive statistics of factor scores). Univariate ANCOVA with the factors Group (HC; SZ-SIB; ADHD) and Cannabis (users; non-users) and with age as covariate was conducted on the factor scores and revealed a significant effect of Group ($F(2,109)=46.488$, $p<0.001$) and a significant Group * Cannabis interaction ($F(2,109)=2.684$, $p<0.05$). Independent-samples t-tests revealed significantly higher factor scores for users than non-users in the SZ-SIB group ($t(33)=-2.189$, $p<0.05$) and a trend towards significantly lower factor scores for users than non-users in the ADHD group ($t(24)=1.835$, $p=0.079$). Comparison of users and non-users in the HC group was non-significant.

Table 3
Descriptive data for SPQ and SDQ sub-scales in cannabis users and non-users in all groups

Measure [a]	Cannabis	Group [b, c]		
		HC	SZ-SIB	ADHD
SPQ cognitive-perceptual	Non-users	2.00 (0.00–6.00)	4.00 (1.00–7.00)	13.00 (6.50–19.00)
	Users	2.00 (0.00–7.00)	5.50 (0.75–13.00)	13.00 (3.00–20.50)
SPQ interpersonal	Non-users	5.00 (2.00–9.00)	6.00 (3.00–11.00)	14.00 (10.50–19.00)
	Users	4.00 (1.00–11.50)	9.00 (2.00–18.50)	9.00 (1.50–18.00)
SPQ disorganisation	Non-users	3.00 (0.00–5.25)	1.00 (0.00–5.00)	10.00 (7.00–12.00)
	Users	3.00 (1.00–5.50)	5.00 (0.00–9.00)	7.00 (1.00–11.00)
SDQ-self H-A	Non-users	2.00 (1.00–4.00)	2.00 (1.00–3.50)	8.00 (5.50–9.00)
	Users	3.00 (1.00–5.00)	3.00 (2.75–5.50)	6.00 (4.50–8.00)
SDQ-parent H-A	Non-users	1.00 (0.00–3.00)	2.00 (1.00–3.00)	9.00 (6.50–10.00)
	Users	0.00 (0.00–2.50)	2.50 (0.75–4.25)	9.00 (4.50–10.00)

[a] SPQ = Schizotypal Personality Questionnaire; SDQ = Strengths & Difficulties Questionnaire; H-A = hyperactivity-inattention sub-scale of SDQ.
[b] HC = Healthy control group; SZ-SIB = schizophrenia-sibling group; ADHD = ADHD group.
[c] Data shown are median with 25th–75th percentile in parentheses.

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In response to a reviewer's comment, the first ANCOVA which investigated the relationship between cannabis use and factor scores on the 'Mental Health Disturbance Index' was re-run with cigarette smoking behaviour included as an additional covariate. This procedure was performed to ensure the significant group * cannabis use interaction was not influenced by nicotine use. Cigarette smoking was assessed using Section 12 of the SCAN. Ratings were dichotomised in the same way as cannabis use: 'non-users' reported smoking cigarettes never or rarely (once or twice); 'users' reported smoking cigarettes more than once or twice. The group * cannabis use interaction remained significant at $p < 0.05$ with this covariate included in the model; the results are therefore reported without the covariate and it was not included in the secondary analysis investigating relationships between cannabis use and specific aspects of mental health disturbance.

4. Discussion

Analysis of factor scores from a composite measure of mental health derived from the SPQ, SDQ and GAF revealed a significant positive association between cannabis use and mental health problems in adolescents at high genetic risk for schizophrenia, which was not statistically significant in healthy adolescents in the general population. Further investigation revealed the same pattern when analysing factor scores from a composite index derived from two sub-scales: the Disorganisation sub-scale of the SPQ and the hyperactivity-inattention sub-scale of the SDQ (self-rated version). The effects remained robust with age and nicotine use included as covariates. The young people included in this study were a well-functioning, non-psychotic group who were entirely free from psychotic or prodromal symptoms. This suggests that even among high-risk individuals who are unlikely to develop a psychotic illness, cannabis use is associated with worse mental health problems. The results also highlight the importance of considering a range of mental health domains when assessing the effects of cannabis use in those at increased genetic risk of psychosis.

The cross sectional nature of this study makes it difficult to infer causality. However, our findings do not support the hypothesis that cannabis use is in general related to mental health disturbance because the cannabis users in the ADHD group had similar, or possibly lower, mental health problem scores than non-users. Our results are more consistent with the hypothesis that there is a specific relationship between risk for schizophrenia and cannabis use. The differential effects of cannabis across groups suggest that the psychological effects of cannabis use are moderated by variation in genetic and neuro–chemical vulnerability. Adolescents at high genetic risk for schizophrenia may have a hypersensitive dopaminergic system rendering them more vulnerable to cannabis-stimulating dopamine release in the mesolimbic system (Fergusson et al., 2006). In contrast, this release could attenuate some behavioural symptoms of ADHD by normalising a hypofunctioning dopamine system (Swanson et al., 2007). Hence, the risks of mental health problems associated with cannabis use in young people may differ within the population. Identification of moderating factors and neurobiological mechanisms associated with cannabis use in adolescents is a key target for future research leading to evidence-based risk prevention.

There are a number of methodological limitations of this study. Dichotomising the sample into users and non-users of cannabis meant that some participants included in the users group may have used cannabis not much more frequently than once or twice. This approach might have resulted in a lack of sensitivity to the possibly greater mental health problems experienced by those who use cannabis very regularly. However, within the context of this study, this method was the most appropriate for the following reasons. Firstly, rates of cannabis use were low in all groups meaning that further dividing the samples into those with more regular use yielded too few cases for reliable statistical analysis. Secondly, a large proportion of those who had used cannabis reported doing so at least once per month, indicating fairly regular use for such a young cohort. Thirdly, the method was more likely to hide differences in mental health function between users and non-users than artificially inflate them. The finding of greater mental health problems in users than non-users in the schizophrenia high risk (SZ-SIB) group suggests therefore that even low rates of use might be significant in this population. Further research using larger samples and a measure of cannabis use which provides interval level data is needed.

A second limitation is the cross-sectional design. It is not possible to determine from our analysis whether the mental health problems in high-risk siblings of schizophrenia patients were present before their regular cannabis use, or have emerged since. Longitudinal research assessing all aspects of mental health function from an early age is needed. In relation to this, consideration of other factors such as stressful lifetime events and degree of socio-economic deprivation was beyond the scope of the present study. Previous research has shown these to be important when assessing mental health in those at high risk of psychosis (Miller et al., 2001) and they might also

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have influenced the relationship between cannabis and mental health in our high-risk group.

Finally, there were low rates of cannabis use and of mental health problems in the SZ-SIB group and it is possible that this high functioning sample may not be representative of the wider schizophrenia high-risk population. Further research is needed to determine whether the results can be replicated in larger samples and in those who are deemed to be at high risk of psychosis due to the presence of prodromal or attenuated psychotic symptoms.

4.1. Conclusions

This study revealed a relationship between cannabis use and mental health problems in young people at increased genetic risk of schizophrenia who were free from prodromal or psychotic symptomatology at the time of assessment. This relationship was specific to the high-risk group when comparing them with adolescents at low genetic risk of psychosis and those with ADHD. The findings highlight the importance of assessing broader domains of mental health function in this group rather than focussing solely on prodromal or psychotic symptoms; although further research is needed to investigate causality.

Role of funding source

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Contributors

CH and PL conceived and designed the study. GJ and AB contributed to the study design and analysis. MG, DD, HA, AB and TC recruited participants, collected data and contributed to analysis. CH, MG, DD and PL analysed and interpreted the data and prepared the original draft. CH and MG wrote the final draft and CH is guarantor of the paper.

Conflict of interest

None.

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References

Arseneault, L., Cannon, M., Witton, J., Murray, R.M., 2004. Causal association between cannabis and psychosis: examination of the evidence. Br. J. Psychiatry 184, 110–117.

Barkus, E.J., Stirling, J., Hopkins, R.S., Lewis, S., 2006. Cannabis-induced psychosis-like experiences are associated with high schizotypy. Psychopathology 39 (4), 175–178.

Bebbington, P., Nayani, T., 1995. The psychosis screening questionnaire. Int. J. Methods Psychiatr. Res. 5 (1), 11–19.

Caspi, A., Moffitt, T.E., Cannon, M., McClay, J., Murray, R., Harrington, H., et al., 2005. Moderation of the effect of adolescent-onset cannabis use on adult psychosis by a functional polymorphism in the catechol-*o*-methyltransferase gene: longitudinal evidence of a gene × environment interaction. Biol. Psychiatry 57 (10), 1117–1127.

Degenhardt, L., Hall, W., 2006. Is cannabis use a contributory cause of psychosis? Can. J. Psychiatry-Revue Canadienne De Psychiatrie 51 (9), 556–565.

Fergusson, D.M., Poulton, R., Smith, P.F., Boden, J.M., 2006. Drugs — cannabis and psychosis. BMJ 332 (7534), 172–175.

Goodman, R., 1997. The strengths and difficulties questionnaire: a research note. J. Child Psychol. Psychiatry Allied Discipl. 38 (5), 581–586.

Groom, M.J., Jackson, G.M., Calton, T.G., Andrews, H.K., Bates, A.T., Liddle, P.F., Hollis, C., 2008. Cognitive deficits in early-onset schizophrenia spectrum patients and their non-psychotic siblings: a comparison with ADHD. Schizophr. Res. 99 (1–3), 85–95.

Macleod, J., Oakes, R., Copello, A., Crome, L., Egger, M., Hickman, M., et al., 2004. Psychological and social sequelae of cannabis and other illicit drug use by young people: a systematic review of longitudinal, general population studies. Lancet 363 (9421), 1579–1588.

McGee, R., Williams, S., Poulton, R., Moffitt, T., 2000. A longitudinal study of cannabis use and mental health from adolescence to early adulthood. Addiction 95 (4), 491–503.

Miller, P., Lawrie, S.M., Hodges, A., Clafferty, R., Cosway, R., Johnstone, E.C., 2001. Genetic liability, illicit drug use, life stress and psychotic symptoms: preliminary findings from the Edinburgh study of people at high risk for schizophrenia. Soc. Psychiatry Psychiatr. Epidemiol. 36 (7), 338–342.

Miller, T.J., McGlashan, T.H., Rosen, J.L., Somjee, L., Markovich, P.J., Stein, K., et al., 2002. Prospective diagnosis of the initial prodrome for schizophrenia based on the structured interview for prodromal syndromes: preliminary evidence of inter-rater reliability and predictive validity. Am. J. Psychiatry 159 (5), 863–865.

Miller, T.J., McGlashan, T.H., Rosen, J.L., Cadenhead, K., Cannon, T., Ventura, J., et al., 2003. Prodromal assessment with the structured interview for prodromal syndromes and the scale of prodromal symptoms: predictive validity, inter-rater reliability, and training to reliability. Schizophr. Bull. 29 (4), 703–715.

Office for National Statistics, 2004. The National Statistics Socio-economic Classification user manual. Office For National Statistics, London.

Phillips, L.J., Curry, C., Yung, A.R., Yuen, H.P., Adlard, S., McGorry, P.D., 2002. Cannabis use is not associated with the development of psychosis in an 'ultra' high-risk group. Aust. N. Z. J. Psychiatry 36 (6), 800–806.

Raine, A., 1991. The SPQ: a scale for the assessment of schizotypal personality based on DSM-III-R criteria. Schizophr. Bull. 17 (4), 555–564.

Rutter, M., Bailey, A., Lord, C., 2003. SCQ: Social communication questionnaire. Western Psychological Services, Los Angeles, California.

Swanson, J.M., Kinsbourne, M., Nigg, J., Lanphear, B., Stefanatos, G.A., Volkow, N., et al., 2007. Etiologic subtypes of attention-deficit/hyperactivity disorder: brain imaging, molecular genetic and environmental factors and the dopamine hypothesis. Neuropsychol. Rev. 17 (1), 39–59.

Taylor, E., Shachar, R., Hepstinall, E., 1993. Manual for parental account of childhood symptoms interview. Maudsley Hospital, London.

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van Os, J., Bak, M., Hanssen, M., Bijl, R.V., de Graaf, R., Verdoux, H., 2002. Cannabis use and psychosis: a longitudinal population-based study. Am. J. Epidemiol. 156 (4), 319–327.

Verdoux, H., Gindre, C., Sorbara, F., Tournier, M., Swendsen, J.D., 2003. Effects of cannabis and psychosis vulnerability in daily life: an experience sampling test study. Psychol. Med. 33 (1), 23–32.

Wechsler, D., 1999. The Wechsler Abbreviated Scale of Intelligence. The Psychological Corporation, San Antonio.

W.H.O., 1992. Tenth revision of the international classification of diseases and related health problems (ICD-10). WHO, Geneva.

Wing, J.K., Babor, T., Brugha, T., Burke, J., Cooper, J.E., Giel, R., et al., 1990. SCAN Schedules for clinical assessment in neuropsychiatry. Arch. Gen. Psychiatry 47 (6), 589–593.

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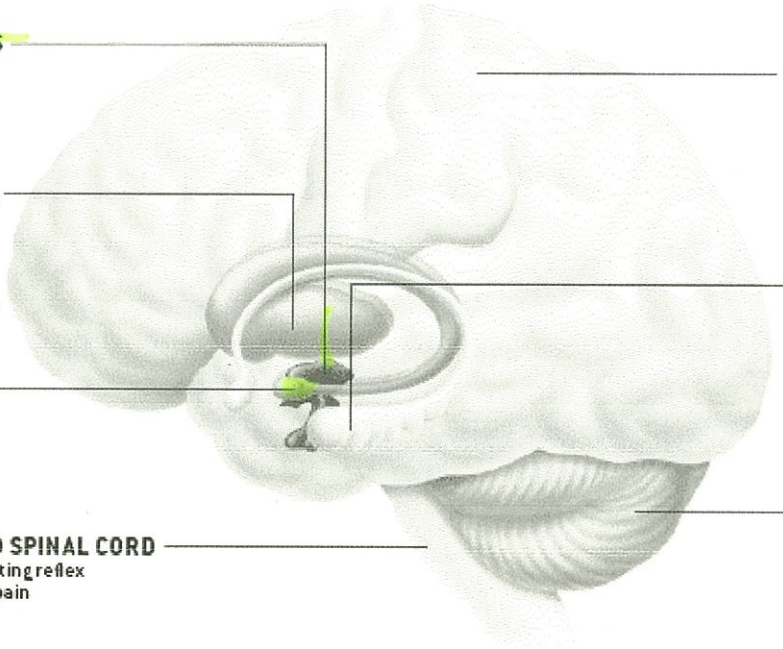
WHERE MARIJUANA ACTS

The drug *Cannabis sativa* binds to the brain's own cannabinoid receptors in many different areas, including those highlighted below. This widespread influence accounts for the diverse effects the drug—and its relatives made by the brain—can have and offers exciting opportunities for devising medications that can specifically target certain sites to control, say, appetite or pain.



HYPOTHALAMUS
Controls appetite, hormonal levels and sexual behavior

BASAL GANGLIA
Involved in motor control and planning, as well as the initiation and termination of action

AMYGDALA
Responsible for anxiety, emotion and fear

BRAIN STEM AND SPINAL CORD
Important in the vomiting reflex and the sensation of pain

NEOCORTEX
Responsible for higher cognitive functions and the integration of sensory information

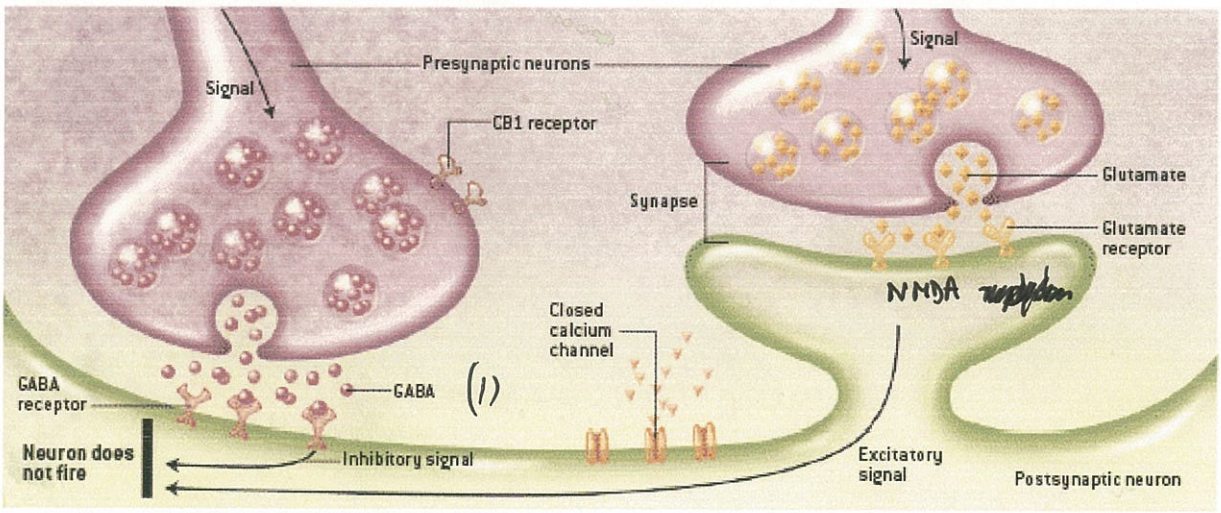
HIPPOCAMPUS
Important for memory and the learning of facts, sequences and places

CEREBELLUM
Center for motor control and coordination

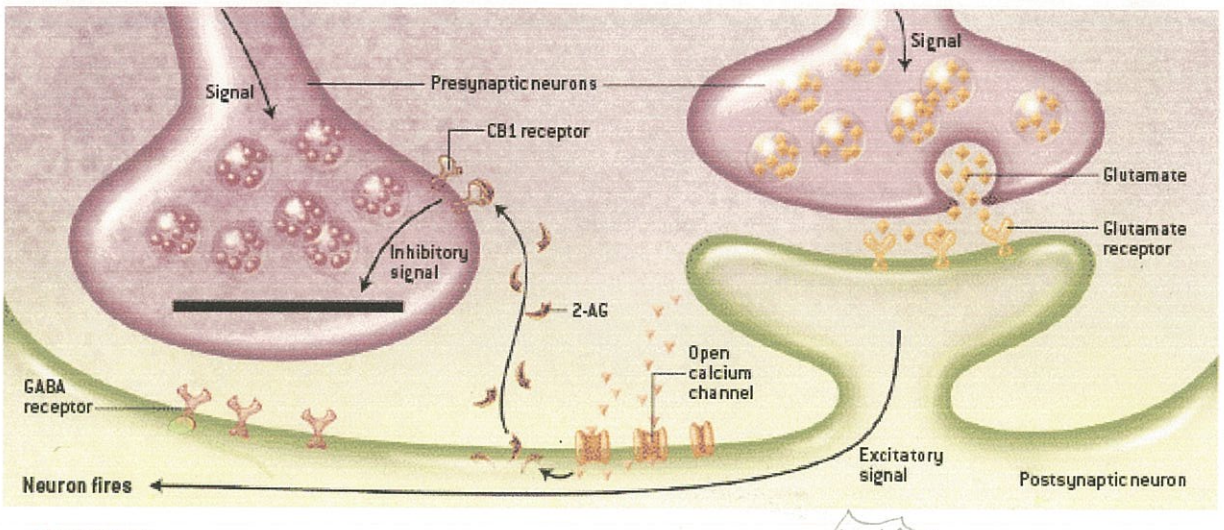
ALICE CHEN

RETROGRADE SIGNALING

Researchers have found that endogenous cannabinoids (endocannabinoids) participate in retrograde signaling, a previously unknown form of communication in the brain. Rather than flowing forward in the usual way from a presynaptic (neurotransmitter-emitting) neuron to a postsynaptic (recipient) one, endocannabinoids work backward, traveling from the postsynaptic cell to the presynaptic one. The endocannabinoid 2-AG released from a postsynaptic cell can, for example, cause a presynaptic cell to decrease its secretion of the inhibitory neurotransmitter GABA onto the postsynaptic cell (*diagrams*).



If GABA from a presynaptic neuron hits a postsynaptic cell at the same time as excitatory signals (such as those carried by the neurotransmitter glutamate) reach the same cell (*above*), the GABA can block the postsynaptic cell from firing. If, however, changes in calcium levels in the postsynaptic neuron trigger the production of 2-AG (*below*), this endocannabinoid will travel back to its receptor (CB1) on the GABA-producing neuron. In a process known as depolarized-induced suppression of inhibition (DSI), it will then prevent the release of GABA and thus allow the excitatory signals to activate the postsynaptic cell.



ALICE CHEN

(1) cholecystokinin ?
|
Cytokine.

GLIA
↓
o Glutamate
↓
Dopamine
↓
noradrenaline.
↓
o Gluten ? hypophyse (dépression).

o alcohol ouvre les canaux

o Alzheimer dépôt cana (la réaction activité) shut time

Scientific American Magazine - December, 2004

The Brain's Own Marijuana

Research into natural chemicals that mimic marijuana's effects in the brain could help to explain--and suggest treatments for--pain, anxiety, eating disorders, phobias and other conditions

By Roger A. Nicoll and Bradley N. Alger

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BACK NEXT

VVG/SCIENCE PHOTO LIBRARY (*neuron*) AND RIC ERGENBRIGHT *Corbis* (*leaves*)

Marijuana is a drug with a mixed history. Mention it to one person, and it will conjure images of potheads lost in a spaced-out stupor. To another, it may represent relaxation, a slowing down of modern madness. To yet another, marijuana means hope for cancer patients suffering from the debilitating nausea of chemotherapy, or it is the promise of relief from chronic pain. The drug is all these things and more, for its history is a long one, spanning millennia and continents. It is also something everyone is familiar with, whether they know it or not. Everyone grows a form of the drug, regardless of their political leanings or recreational proclivities. That is because the brain makes its own marijuana, natural compounds called endocannabinoids (after the plant's formal name, *Cannabis sativa*).

The study of endocannabinoids in recent years has led to exciting discoveries. By examining these substances, researchers have exposed an entirely new signaling system in the brain: a way that nerve cells communicate that no one anticipated even 15 years ago. Fully understanding this signaling system could have far-reaching implications. The details appear to hold a key to devising treatments for anxiety, pain, nausea, obesity, brain injury and many other medical problems. Ultimately such treatments could be tailored precisely so that they would not initiate the unwanted side effects produced by marijuana itself.

A Checkered Past

Marijuana and its various alter egos, such as bhang and hashish, are among the most widely used psychoactive drugs in the world. How the plant has been used varies by culture. The ancient Chinese knew of marijuana's pain-relieving and mind-altering effects, yet it was not widely employed for its psychoactive properties; instead it was cultivated as hemp for the manufacture of rope and fabric. Likewise, the ancient Greeks and Romans used hemp to make rope and sails. In some other places, however, marijuana's intoxicating properties became important. In India, for example, the plant was incorporated into religious rituals. During the Middle Ages, its use was common in Arab lands; in 15th-century Iraq it was used to treat epilepsy; in Egypt it was primarily consumed as an inebriant. After Napoleon's occupation of Egypt, Europeans began using the drug as an intoxicant. During the slave trade, it was transported from Africa to Mexico, the Caribbean and South America.

Marijuana gained a following in the U.S. only relatively recently. During the second half of the 19th century and the beginning of the 20th, cannabis was freely available without a prescription for a wide range of ailments, including migraine and ulcers. Immigrants from Mexico introduced it as a recreational drug to New Orleans and other large cities, where it became popular among jazz musicians. By the 1930s it had fallen into disrepute, and an intense lobbying campaign demonized "reefer madness." In 1937 the U.S. Congress, against the advice of the American Medical Association, passed the Marijuana Tax Act, effectively banning use of the drug by making it expensive and difficult to obtain. Ever since, marijuana has remained one of the most controversial drugs in American society. Despite efforts to change its status, it remains federally classified as a Schedule 1 drug, along with heroin and LSD, considered dangerous and without utility.

Millions of people smoke or ingest marijuana for its intoxicating effects, which are subjective and often described as resembling an alcoholic "high." It is estimated that approximately 30 percent of the U.S. population older than 12 have tried marijuana, but only about 5 percent are current users. Large doses cause hallucinations in some individuals but simply trigger sleep in others. The weed impairs short-term memory and cognition and adversely affects motor coordination, although these setbacks seem to be reversible once the drug has been purged from the body. Smoking marijuana also poses health risks that resemble those of smoking tobacco.

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FURTHER READING

Marijuana and Medicine. Edited by J. E. Joy, S. J. Watson, Jr., and J. A. Benson, Jr. Institute of Medicine, 1999.

Endocannabinoid Signaling in the Brain. R. I. Wilson and R. A. Nicoll in *Science*, Vol. 296, pages 678-682; April 26, 2002.

Retrograde Signaling in the Regulation of Synaptic Transmission: Focus on Endocannabinoids. B. E. Alger in Progress in *Neurobiology*, Vol. 68, No. 4, pages 247-286; November 2002.

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Scientific American Magazine - December, 2004

The Brain's Own Marijuana

Research into natural chemicals that mimic marijuana's effects in the brain could help to explain--and suggest treatments for--pain, anxiety, eating disorders, phobias and other conditions

By Roger A. Nicoll and Bradley N. Alger

On the other hand, the drug has clear medicinal benefits. Marijuana alleviates pain and anxiety. It can prevent the death of injured neurons. It suppresses vomiting and enhances appetite--useful features for patients suffering the severe weight loss that can result from chemotherapy.

Finding the Responsible Agent

Figuring out how the drug exerts these myriad effects has taken a long time. In 1964, after nearly a century of work by many individuals, Raphael Mechoulam of the Hebrew University in Jerusalem identified delta-9-tetrahydrocannabinol (THC) as the compound that accounts for virtually all the pharmacological activity of marijuana. The next step was to identify the receptor or receptors to which THC was binding.

Receptors are small proteins embedded in the membranes of all cells, including neurons, and when specific molecules bind to them--fitting like one puzzle piece into another--changes in the cell occur. Some receptors have water-filled pores or channels that permit chemical ions to pass into or out of the cell. These kinds of receptors work by changing the relative voltage inside and outside the cell. Other receptors are not channels but are coupled to specialized proteins called G-proteins. These G-protein-coupled receptors represent a large family that set in motion a variety of biochemical signaling cascades within cells, often resulting in changes in ion channels.

In 1988 Allyn C. Howlett and her colleagues at St. Louis University attached a radioactive tag to a chemical derivative of THC and watched where the compound went in rats' brains. They discovered that it attached itself to what came to be called the cannabinoid receptor, also known as CB1. Based on this finding and on work by Miles Herkenham of the National Institutes of Health, Lisa Matsuda, also at the NIH, cloned the CB1 receptor. The importance of CB1 in the action of THC was proved when two researchers working independently--Catherine Ledent of the Free University of Brussels and Andreas Zimmer of the Laboratory of Molecular Neurobiology at the University of Bonn--bred mice that lacked this receptor. Both investigators found that THC had virtually no effect when administered to such a mouse: the compound had nowhere to bind and hence could not trigger any activity. (Another cannabinoid receptor, CB2, was later discovered; it operates only outside the brain and spinal cord and is involved with the immune system.)

As researchers continued to study CB1, they learned that it was one of the most abundant G-protein coupled receptors in the brain. It has its highest densities in the cerebral cortex, hippocampus, hypothalamus, cerebellum, basal ganglia, brain stem, spinal cord and amygdala. This distribution explains marijuana's diverse effects. Its psychoactive power comes from its action in the cerebral cortex. Memory impairment is rooted in the hippocampus, a structure essential for memory formation. The drug causes motor dysfunction by acting on movement control centers of the brain. In the brain stem and spinal cord, it brings about the reduction of pain; the brain stem also controls the vomiting reflex. The hypothalamus is involved in appetite, the amygdala in emotional responses. Marijuana clearly does so much because it acts everywhere.

Over time, details about CB1's neuronal location emerged as well. Elegant studies by Tam□s F. Freund of the Institute of Experimental Medicine at the Hungarian Academy of Sciences in Budapest and Kenneth P. Mackie of the University of Washington revealed that the cannabinoid receptor occurred only on certain neurons and in very specific positions on those neurons. It was densely packed on neurons that released GABA (gamma-aminobutyric acid), which is the brain's main inhibitory neurotransmitter (it tells recipient neurons to stop firing). CB1 also sat near the synapse, the contact point between two neurons. This placement suggested that the cannabinoid receptor was somehow involved with signal transmission across GABA-using synapses. But why would the brain's signaling system include a receptor for something produced by a plant?

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FURTHER READING

Marijuana and Medicine. Edited by J. E. Joy, S. J. Watson, Jr., and J. A. Benson, Jr. Institute of Medicine, 1999.

Endocannabinoid Signaling in the Brain. R. I. Wilson and R. A. Nicoll in *Science*, Vol. 296, pages 678-682; April 26, 2002.

Retrograde Signaling in the Regulation of Synaptic Transmission: Focus on Endocannabinoids. B. E. Alger in Progress in *Neurobiology*, Vol. 68, No. 4, pages 247-286; November 2002.

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The Brain's Own Marijuana

Research into natural chemicals that mimic marijuana's effects in the brain could help to explain--and suggest treatments for--pain, anxiety, eating disorders, phobias and other conditions

By Roger A. Nicoll and Bradley N. Alger

The Lesson of Opium

The same question had arisen in the 1970s about morphine, a compound isolated from the poppy and found to bind to so-called opiate receptors in the brain. Scientists finally discovered that people make their own opioids--the enkephalins and endorphins. Morphine simply hijacks the receptors for the brain's opioids.

It seemed likely that something similar was happening with THC and the cannabinoid receptor. In 1992, 28 years after he identified THC, Mechoulam discovered a small fatty acid produced in the brain that binds to CB1 and that mimics all the activities of marijuana. He named it anandamide, after the Sanskrit word ananda, "bliss." Subsequently, Daniele Piomelli and Nephi Stella of the University of California at Irvine discovered that another lipid, 2-arachidonoyl glycerol (2-AG), is even more abundant in certain brain regions than anandamide is. Together the two compounds are considered the major endogenous cannabinoids, or endocannabinoids. (Recently investigators have identified what look like other endogenous cannabinoids, but their roles are uncertain.) The two cannabinoid receptors clearly evolved along with endocannabinoids as part of natural cellular communication systems. Marijuana happens to resemble the endocannabinoids enough to activate cannabinoid receptors.

Conventional neurotransmitters are water-soluble and are stored in high concentrations in little packets, or vesicles, as they wait to be released by a neuron. When a neuron fires, sending an electrical signal down its axon to its tips (presynaptic terminals), neurotransmitters released from vesicles cross a tiny intercellular space (the synaptic cleft) to receptors on the surface of a recipient, or postsynaptic, neuron. In contrast, endocannabinoids are fats and are not stored but rather are rapidly synthesized from components of the cell membrane. They are then released from places all over the cells when levels of calcium rise inside the neuron or when certain G-protein-coupled receptors are activated.

As unconventional neurotransmitters, canna-bin-oids presented a mystery, and for several years no one could figure out what role they played in the brain. Then, in the early 1990s, the answer emerged in a somewhat roundabout fashion. Scientists (including one of us, Alger, and his colleague at the University of Maryland School of Medicine, Thomas A. Pitler) found something unusual when studying pyramidal neurons, the principal cells of the hippocampus. After calcium concentrations inside the cells rose for a short time, incoming inhibitory signals in the form of GABA arriving from other neurons declined.

At the same time, Alain Marty, now at the Laboratory of Brain Physiology at the Ren□ Descartes University in Paris, and his colleagues saw the same action in nerve cells from the cerebellum. These were unexpected observations, because they suggested that receiving cells were somehow affecting transmitting cells and, as far as anyone knew, signals in mature brains flowed across synapses in one way only: from the presynaptic cell to the postsynaptic one.

A New Signaling System

it seemed possible that a new kind of neuronal communication had been discovered, and so researchers set out to understand this phenomenon. They dubbed the new activity DSI, for depolarization-induced suppression of inhibition. For DSI to have occurred, some unknown messenger must have traveled from the postsynaptic cell to the presynaptic GABA-releasing one and somehow shut off the neurotransmitter's release.

Such backward, or "retrograde," signaling was known to occur only during the development of the nervous system. If it were also involved in interactions among adult neurons, that would be an intriguing finding--a sign that perhaps other processes in the brain involved retrograde transmission as well. Retrograde signaling might facilitate types of neuronal information processing that were difficult or impossible to accomplish with conventional synaptic transmission. Therefore, it was important to learn the properties of the retrograde signal. Yet its identity remained elusive. Over the years, countless molecules were proposed. None of them worked as predicted.

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FURTHER READING

Marijuana and Medicine. Edited by J. E. Joy, S. J. Watson, Jr., and J. A. Benson, Jr. Institute of Medicine, 1999.

Endocannabinoid Signaling in the Brain. R. I. Wilson and R. A. Nicoll in *Science*, Vol. 296, pages 678-682, April 26, 2002.

Retrograde Signaling in the Regulation of Synaptic Transmission: Focus on Endocannabinoids. B. E. Alger in Progress in *Neurobiology*, Vol. 68, No. 4, pages 247-286; November 2002.

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The Brain's Own Marijuana

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By Roger A. Nicoll and Bradley N. Alger

Then, in 2001, one of us (Nicoll) and his colleague at the University of California at San Francisco, Rachel I. Wilson--and at the same time, but independently, a group led by Masanobu Kano of Kanazawa University in Japan--reported that an endocannabinoid, probably 2-AG, perfectly fit the criteria for the unknown messenger. Both groups found that a drug blocking cannabinoid receptors on presynaptic cells prevents DSI and, conversely, that drugs activating CB1 mimic DSI. They soon showed, as did others, that mice lacking cannabinoid receptors are incapable of generating DSI. The fact that the receptors are located on the presynaptic terminals of GABA neurons now made perfect sense. The receptors were poised to detect and respond to endocannabinoids released from the membranes of nearby postsynaptic cells.

Over time, DSI proved to be an important aspect of brain activity. Temporarily dampening inhibition enhances a form of learning called long-term potentiation--the process by which information is stored through the strengthening of synapses. Such storage and information transfer often involves small groups of neurons rather than large neuronal populations, and endocannabinoids are well suited to acting on these small assemblages. As fat-soluble molecules, they do not diffuse over great distances in the watery extracellular environment of the brain. Avid uptake and degradation mechanisms help to ensure that they act in a confined space for a limited period. Thus, DSI, which is a short-lived local effect, enables individual neurons to disconnect briefly from their neighbors and encode information.

A host of other findings filled in additional gaps in understanding about the cellular function of endocannabinoids. Researchers showed that when these neurotransmitters lock onto CB1 they can in some cases block presynaptic cells from releasing excitatory neurotransmitters. As Wade G. Regehr of Harvard University and Anatol C. Kreitzer, now at Stanford University, found in the cerebellum, endocannabinoids located on excitatory nerve terminals aid in the regulation of the massive numbers of synapses involved in coordinated motor control and sensory integration. This involvement explains, in part, the slight motor dysfunction and altered sensory perceptions often associated with smoking marijuana.

Recent discoveries have also begun to precisely link the neuronal effects of endocannabinoids to their behavioral and physiological effects. Scientists investigating the basis of anxiety commonly begin by training rodents to associate a particular signal with something that frightens them. They often administer a brief mild shock to the feet at the same time that they generate a sound. After a while the animal will freeze in anticipation of the shock if it hears the sound. If the sound is repeatedly played without the shock, however, the animal stops being afraid when it hears the sound--that is, it unlearns the fear conditioning, a process called extinction. In 2003 Giovanni Marsicano of the Max Planck Institute of Psychiatry in Munich and his co-workers showed that mice lacking normal CB1 readily learn to fear the shock-related sound, but in contrast to animals with intact CB1, they fail to lose their fear of the sound when it stops being coupled with the shock.

The results indicate that endocannabinoids are important in extinguishing the bad feelings and pain triggered by reminders of past experiences. The discoveries raise the possibility that abnormally low numbers of cannabinoid receptors or the faulty release of endogenous cannabinoids are involved in post-traumatic stress syndrome, phobias and certain forms of chronic pain. This suggestion fits with the fact that some people smoke marijuana to decrease their anxiety. It is also conceivable, though far from proved, that chemical mimics of these natural substances could allow us to put the past behind us when signals that we have learned to associate with certain dangers no longer have meaning in the real world.

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FURTHER READING

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Endocannabinoid Signaling in the Brain. R. I. Wilson and R. A. Nicoll in *Science*, Vol. 296, pages 678-682; April 26, 2002.

Retrograde Signaling in the Regulation of Synaptic Transmission: Focus on Endocannabinoids. B. E. Alger in Progress in *Neurobiology*, Vol. 68, No. 4, pages 247-286; November 2002.

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The Brain's Own Marijuana

Research into natural chemicals that mimic marijuana's effects in the brain could help to explain--and suggest treatments for--pain, anxiety, eating disorders, phobias and other conditions

By Roger A. Nicoll and Bradley N. Alger

Devising New Therapies

The repertoire of the brain's own marijuana has not been fully revealed, but the insights about endocannabinoids have begun helping researchers design therapies to harness the medicinal properties of the plant. Several synthetic THC analogues are already commercially available, such as nabilone and dronabinol. They combat the nausea brought on by chemotherapy; dronabinol also stimulates appetite in AIDS patients. Other cannabinoids relieve pain in myriad illnesses and disorders. In addition, a CB1 antagonist--a compound that blocks the receptor and renders it impotent--has worked in some clinical trials to treat obesity. But though promising, these drugs all have multiple effects because they are not specific to the region that needs to be targeted. Instead they go everywhere, causing such adverse reactions as dizziness, sleepiness, problems of concentration and thinking abnormalities.

One way around these problems is to enhance the role of the body's own endocannabinoids. If this strategy is successful, endocannabinoids could be called forth only under the circumstances and in the locations in which they are needed, thus avoiding the risks associated with widespread and indiscriminant activation of cannabinoid receptors. To do this, Piomelli and his colleagues are developing drugs that prevent the endocannabinoid anandamide from being degraded after it is released from cells. Because it is no longer broken down quickly, its anxiety-relieving effects last longer.

Anandamide seems to be the most abundant endocannabinoid in some brain regions, whereas 2-AG dominates in others. A better understanding of the chemical pathways that produce each endocannabinoid could lead to drugs that would affect only one or the other. In addition, we know that endocannabinoids are not produced when neurons fire just once but only when they fire five or even 10 times in a row. Drugs could be developed that would alter the firing rate and hence endocannabinoid release. A precedent for this idea is the class of anticonvulsant agents that suppress the neuronal hyperactivity underlying epileptic seizures but do not affect normal activity.

Finally, indirect approaches could target processes that themselves regulate endocannabinoids. Dopamine is well known as the neurotransmitter lost in Parkinson's disease, but it is also a key player in the brain's reward systems. Many pleasurable or addictive drugs, including nicotine and morphine, produce their effects in part by causing dopamine to be released in several brain centers. It turns out that dopamine can cause the release of endocannabinoids, and various research teams have found that two other neurotransmitters, glutamate and acetylcholine, also initiate endocannabinoid synthesis and release. Indeed, endocannabinoids may be a source of effects previously attributed solely to these neurotransmitters. Rather than targeting the endocannabinoid system directly, drugs could be designed to affect the conventional neurotransmitters. Regional differences in neurotransmitter systems could be exploited to ensure that endocannabinoids would be released only where they were needed and in appropriate amounts.

In a remarkable way, the effects of marijuana have led to the still unfolding story of the endocannabinoids. The receptor CB1 seems to be present in all vertebrate species, suggesting that systems employing the brain's own marijuana have been in existence for about 500 million years. During that time, endocannabinoids have been adapted to serve numerous, often subtle, functions. We have learned that they do not affect the development of fear, but the forgetting of fear; they do not alter the ability to eat, but the desirability of the food, and so on. Their presence in parts of the brain associated with complex motor behavior, cognition, learning and memory implies that much remains to be discovered about the uses to which evolution has put these interesting messengers.

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Endocannabinoïc

1st - Anandamide

2nd 2-AG

FURTHER READING

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Endocannabinoid Signaling in the Brain. R. I. Wilson and R. A. Nicoll in *Science*, Vol. 296, pages 678-682; April 26, 2002.

Retrograde Signaling in the Regulation of Synaptic Transmission: Focus on Endocannabinoids. B. E. Alger in Progress in *Neurobiology*, Vol. 68, No. 4, pages 247-286; November 2002.

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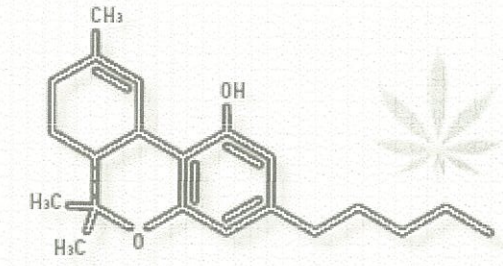
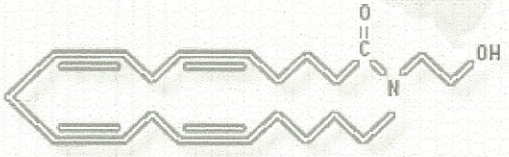


Delta-9-Tetrahydrocannabinol (THC)



Anandamide

2-Arachidonoyl glycerol (2-AG)

DESPITE DIFFERENCES in their structures, THC, produced by the marijuana plant, and the brain chemicals anandamide and 2-AG can all activate the same receptor (CB1) in the brain.

TOMMY MOORMAN

November 22, 2004

Help Could Be on the Way For:

By Roger A. Nicoll and Bradley N. Alger

Anxiety. Experiments suggest that too few endocannabinoid receptors, or insuffi cient release of endocannabinoids themselves, underlie chronic anxiety and post-traumatic stress disorder. To alleviate anxiety, researchers are working to prevent the breakdown of anandamide, thereby increasing the amount available to act on the receptors.

Appetite and obesity. The antinausea medication dronabinal, a cannabinoid-based compound, has been shown to stimulate appetite in immunosuppressed patients, suggesting the possibility that an antagonist--a compound that blocks cannabinoid receptors--could suppress appetite. Clinical trials of one such antagonist have been promising, although many side effects have been recorded.

Nausea. Several drugs already on the market, including dronabinal and nabilone, are similar to the active component of marijuana, THC, and thus reduce the nausea associated with chemotherapy.

Neurological disorders. Dopamine, a neurotransmitter important to pleasure and movement, triggers the release of endocannabinoids. By regulating endocannabinoid activity, researchers hope to help treat Parkinson's disease, drug addiction and other illnesses that involve the dopamine system.

Pain. A wealth of cannabinoid receptors have been observed in several of the brain's pain centers; medicines that acted on those receptors might therefore help ease pain.

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November 22, 2004

Overview/Brain's Marijuana

By Roger A. Nicoll and Bradley N. Alger

Marijuana and related drugs affect behavior by acting on receptors for compounds called endocannabinoids that are produced by the brain.
These endocannabinoids participate in regulating pain, anxiety, hunger and vomiting, among other processes. This wide range of effects explains why the use of marijuana seems to elicit so many different responses.
By developing drugs that can mimic specific beneficial actions of endocannabinoids--without triggering some of the adverse effects of marijuana--researchers hope to find new treatments for diverse problems.

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Scientific American Magazine - December, 2004

Marijuana Research

Current restrictions on marijuana research are absurd

By The Editors

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The human brain naturally produces and processes compounds closely related to those found in *Cannabis sativa*, better known as marijuana [see "The Brain's Own Marijuana," by Roger A. Nicoll and Bradley E. Alger. These compounds are called endogenous cannabinoids or endocannabinoids. As the journal *Nature Medicine* put it in 2003, "the endocannabinoid system has an important role in nearly every paradigm of pain, in memory, in neurodegeneration and in inflammation." The journal goes on to note that cannabinoids' "clinical potential is enormous." That potential may include treatments for pain, nerve injury, the nausea associated with chemotherapy, the wasting related to AIDS and more.

Yet outdated regulations and attitudes thwart legitimate research with marijuana. Indeed, American biomedical researchers can more easily acquire and investigate cocaine. Marijuana is classified as a so-called Schedule 1 drug, alongside LSD and heroin. As such, it is defined as being potentially addictive and having no medical use, which under the circumstances becomes a self-fulfilling prophecy.

Any researcher attempting to study marijuana must obtain it through the National Institute on Drug Abuse (NIDA). The U.S. research crop, grown at a single facility, is regarded as less potent--and therefore less medicinally interesting--than the marijuana often easily available on the street. Thus, the legal supply is a poor vehicle for studying the approximately 60 cannabinoids that might have medical applications.

This system has unintended, almost comic, consequences. For example, it has created a market for research marijuana, with "buyers" trading journal co-authorships to "sellers" who already have a marijuana stockpile or license. The government may also have a stake in a certain kind of result. One scientist tells of a research grant application to study marijuana's potential medical benefits. NIDA turned it down. That scientist rewrote the grant to emphasize finding marijuana's negative effects. The study was funded.

Some may argue that researchers do not need to study the drug--after all, there is Marinol, a synthetic version of marijuana's major active compound, tetrahydrocannabinol, or THC; it relieves nausea and stimulates appetite. But patients are often disappointed with Marinol as compared with marijuana. A 1997 editorial in the *New England Journal of Medicine* noted that "it is difficult to titrate the therapeutic dose of this drug, and it is not widely prescribed. By contrast, smoking marijuana produces a rapid increase in the blood level of the active ingredients and is thus more likely to be therapeutic."

The reasonable course is to make it easier for American researchers to at least examine marijuana for possible medical benefits. Great Britain, no slacker in the war on drugs, takes this approach: the government has authorized a pharmaceutical firm to grow different strains of marijuana for clinical trials.

This call for marijuana research is not a closet campaign for drug legalization--easing research barriers would not require that marijuana be reclassified, nor would it have any bearing on individual states' decisions to approve limited use of medical marijuana. As a 1995 editorial in the *Journal of the American Medical Association* said, "We are not asking readers for immediate agreement with our affirmation that marijuana is medically useful, but we hope they will do more to encourage open and legal exploration of its potential." After almost a decade of little progress, we reiterate that sentiment.

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Review Article

Endocannabinoid System and Synaptic Plasticity: Implications for Emotional Responses

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Recommended by Patrice Venault

The endocannabinoid system has been involved in the regulation of anxiety, and proposed as an inhibitory modulator of neuronal, behavioral and adrenocortical responses to stressful stimuli. Brain regions such as the amygdala, hippocampus and cortex, which are directly involved in the regulation of emotional behavior, contain high densities of cannabinoid CB1 receptors. Mutant mice lacking CB1 receptors show anxiogenic and depressive-like behaviors as well as an altered hypothalamus pituitary adrenal axis activity, whereas enhancement of endocannabinoid signaling produces anxiolytic and antidepressant-like effects. Genetic and pharmacological approaches also support an involvement of endocannabinoids in extinction of aversive memories. Thus, the endocannabinoid system appears to play a pivotal role in the regulation of emotional states. Endocannabinoids have emerged as mediators of short- and long- term synaptic plasticity in diverse brain structures. Despite the fact that most of the studies on this field have been performed using in vitro models, endocannabinoid-mediated plasticity might be considered as a plausible candidate underlying some of the diverse physiological functions of the endogenous cannabinoid system, including developmental, affective and cognitive processes. In this paper, we will focus on the functional relevance of endocannabinoid-mediated plasticity within the framework of emotional responses. Alterations of the endocannabinoid system may constitute an important factor in the aetiology of certain neuropsychiatric disorders, and, in turn, enhancers of endocannabinoid signaling could represent a potential therapeutical tool in the treatment of both anxiety and depressive symptoms.

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1. INTRODUCTION

Fear is an adaptive component of the acute stress response to potentially dangerous stimuli which threaten the integrity of the individual. However, when disproportional in intensity, chronic, irreversible, and/or not associated with any actual risk, it constitutes a maladaptive response and may be symptomatic of an anxiety-related neuropsychiatric disorder such as generalized anxiety, phobia, or post-traumatic stress disorder (PTSD), among others. A diversity of mechanisms, including GABAergic, serotonergic, and noradrenergic systems, appears to be involved in the regulation of anxious states which may contribute to an appropriate emotional response to aversive events [1]. In the recent years, an increasing interest in the endocannabinoid system has arisen as part of the complex circuitry that regulates anxiety and as a crucial mediator of emotional learning. Brain distribution of cannabinoid CB1 receptors is consistent with an involvement of this system in the regulation of emotional reactivity. Indeed, CB1 receptors are highly expressed in brain structures such as the amygdala, hippocampus, anterior cingulate cortex, and prefrontal cortex [2–8], key regions in the regulation of emotional responses. Moreover, the cannabinoid CB1 agonist CP 55,940 increased Fos immunoreactivity in brain structures known to be involved in anxiety and fear-related responses such as the central nucleus of the amygdala, the periaqueductal gray, and the paraventricular nucleus (PVN) of the hypothalamus [9].

Depression is a mood disorder in which the prevailing emotional mood is distorted or inappropriate to the circumstances. There are important links between chronic stress and depression. Upon exposure to acute stressful stimuli, the organism initiates a series of neuroendocrine short-term responses that are beneficial in terms of adaptation. However, exposure to chronic, unavoidable situations of stress may have deleterious consequences, including endocrine, emotional, and cognitive alterations associated with neuropsychiatric disorders such as depression. In this context,

hyperactivity of the hypothalamus-pituitary-adrenal (HPA) axis with increased glucocorticoids levels appears to be linked to major depression [10, 11]. There is evidence for an involvement of the endocannabinoid system in the regulation of neural, behavioral, and endocrine responses to aversive stimuli [12, 13] and it has been suggested that stress-induced dysregulation of specific components of the endocannabinoid system might be associated with deficits in behavioral flexibility that can be manifested in stress-related disorders such as PTSD and depression [14].

Endocannabinoids have been shown to act as retrograde transmitters at the synaptic level. Though the exact role of retrograde endocannabinoid signaling in vivo is not fully clarified yet, it is likely that by this mechanism endocannabinoids play important roles in synaptic transmission and plasticity, including modulation of emotional responses. Indeed, endocannabinoids have recently emerged as one of the most thoroughly investigated, and widely accepted, classes of retrograde messengers in the brain [15]. Cannabinoid-induced neuroplasticity may underlie diverse physiological functions modulated by the endocannabinoid system, that is, pain [16] and memory [17]. Synaptic plasticity within the amygdala appears to play a crucial role in acquisition, storage, and extinction of aversive memories, basic neural processes that serve adaptive behaviors, and the endocannabinoid system has emerged as a crucial mediator of such neuroplasticity-related phenomena. Marsicano et al. [18, 19] proposed that endocannabinoids facilitate extinction of aversive memories through their selective inhibitory effects on local inhibitory networks in the amygdala, providing evidence for a functional role of endocannabinoid release-based synaptic plasticity. Apart from the amygdala, there are some other brain areas that have been postulated as substrates for cannabinoid-induced neural plasticity such as the hippocampus and the hypothalamus where cannabinoid-dependent synaptic plasticity is involved in the regulation of the stress-response system [17, 20]. Pharmacological modulation of the endocannabinoid system has been proposed as a novel potential therapeutical strategy for the treatment of anxiety disorders and depression [21], and therapeutic interventions directed at normalization of the HPA system [11] might potentially include modulation of endocannabinoid signaling.

2. THE ENDOCANNABINOID SYSTEM AND CANNABINOID-RELATED COMPOUNDS

The endocannabinoid system includes the cannabinoid receptors, the endogenous lipid ligands (endocannabinoids), and the enzymatic machinery for their synthesis and inactivation. Endocannabinoids are important neuromodulators that appear to be involved in a plethora of physiological processes such as modulation of nociception, regulation of motor activity, cognitive processes, neuroprotection, immune function and inflammatory responses, antiproliferative actions in tumoral cells, control of cardiovascular system, and neurodevelopment, among others [22–29]. Notably, the endocannabinoid system appears to be critically involved in the maintenance of homeostasis [28, 30]. In this review, we aim to highlight its function as a stress-recovery system.

Endocannabinoids are polyunsaturated fatty acid derivatives. The ethanolamide of arachidonic acid anandamide (AEA) and 2-arachidonoylglycerol (2-AG) are the most studied endocannabinoids and have been implicated in a wide range of physiological and pathological processes. Other molecules such as 2-arachidonyl-glyceryl ether (noladin, 2-AGE), O-arachidonoyl-ethanolamine (virhodamine), and N-arachidonoyl-dopamine (NADA) have been discovered more recently. The anabolic and catabolic pathways for AEA and 2-AG appear to rely on very complex enzymatic cascades and are in the progress of being elucidated. In brief, the enzime N-acylphosphatidylethanolamine-specific phospholipase D (NAPE-PLD) synthesizes AEA from N-arachidonoylphosphatidylethanolamine (NArPE), whereas the diacylglycerol lipase (DAGL) generates 2-AG from diacylglycerol (DAG) substrates. Due to their lipophilic nature, endocannabinoids cannot be stored in vesicles. It is widely accepted that, unlike other mediators, the endocannabinoids are synthesized and released on demand, in response to diverse physiological and pathological stimuli, and appear to exert important actions as retrograde messengers. Endocannabinoid inactivating mechanisms include cellular reuptake and hydrolysis. AEA appears to be taken up by several cell types at least in part via a facilitated transport mechanism, known as the anandamide membrane transporter (AMT), which can also transport 2-AG intracellularly. Though this putative transporter has not been isolated or cloned yet, there are compounds that are considered as inhibitors of cellular uptake. A fatty acid amide hydrolase (FAAH) is the main AEA hydrolase, whereas a monoacylglycerol lipase (MAGL) is critical in degrading 2-AG. It is important to take into consideration that the actions of endocannabinoids are considered to be spatially and temporally restricted. Therefore, the effects of exogenously applied cannabinoids, which lack such selectivity, do not necessarily mimic physiological functions of the endocannabinoid system [26, 28]. Compounds that enhance endocannabinoid signaling by inhibiting endocannabinoid reuptake (e.g., VDM11, OMDM-1, OMDM-2, UCM707) or by degradation (e.g., the FAAH inhibitors URB597, AM374, or N-arachidonoyl-serotonin) are widely used in preclinical studies and appear to have a potential therapeutical interest. A profound discussion of biochemical aspects of the endocannabinoid system is beyond the scope of this paper, but the reader can find comprehensive excellent reviews (e.g., [27, 28, 31–35]) as well as recent papers on specific aspects such as alternative biosynthetic pathways for endocannabinoids [36, 37] and endocannabinoid membrane transport [38].

Cannabinoids mainly exert their pharmacological effects by the activation of specific membrane receptors. Mammalian tissues contain at least two types of cannabinoid receptors, CB1 and CB2, which are metabotropic receptors coupled to G-proteins of the Gi/o type. CB1 receptors are

localized mainly in the central nervous system, but are also present in a variety of peripheral tissues; they are among the most abundant and widely distributed G-protein coupled receptors in the brain. Transduction systems include inhibition of adenylyl cyclase and of certain voltage-sensitive calcium channels (predominately, those found presynaptically) and activation of inwardly-rectifying potassium channels and mitogen-activated protein (MAP) kinase [39]. Autoradiographic and immunohistochemical studies have shown that CB1 receptors are expressed in multiple brain areas, including the olfactory bulb, neocortex, pyriform cortex, hippocampus, amygdala, basal ganglia, thalamic and hypothalamic nuclei, cerebellar cortex and brainstem nuclei. In particular, a high density of CB1 receptors is found in cortical and limbic regions associated with emotional responses. The levels of expression vary among the various brain regions and neuronal subpopulations, and there is apparently no strict correlation between levels of expression and receptor functionality. Thus, the activity of cannabinoids at CB1 receptor depends not only on the relative receptor density but also on other factors such as receptor coupling efficiency [2, 28, 40–43]. It has been widely accepted that cannabinoids regulate GABA release by activation of CB1 receptor type, and the highest levels of CB1 cannabinoid receptors are found on the terminals of cholecystokinin-positive GABAergic interneurons [44, 45]. On the other hand, the expression of CB1 receptor in glutamatergic neurons has been vigorously debated in recent years. In fact, some authors proposed that a novel non-CB1/non-CB2 cannabinoid-sensitive receptor could be responsible for the inhibition of glutamatergic neurotransmission [46, 47]. However, it has been now well established that functional cannabinoid CB1 receptors are present on glutamatergic terminals of the hippocampal formation, colocalizing with vesicular glutamate transporter 1 [48], as well as in other cortical areas (see, e.g., [26, 49, 50]). These evidences do not exclude that a non-CB1 receptor might exist in the brain, but there is to date no molecular evidence for such novel receptor.

Cannabinoid CB2 receptors are mostly peripherally located on immunological tissues, and therefore implicated in immunological functions. However, they have also been found within the central nervous system on neurons and glial cells with their expression mainly related to conditions of inflammation [51–53]. More recent immunohistochemical analyses have revealed immunostaining for CB2 receptors in apparent neuronal and glial processes in diverse rat brain areas, including cerebellum and hippocampus [54, 55]. These results change the classical view of peripherally located CB2 receptors and suggest broader functional roles for these receptors.

It has been shown that some of the effects of anandamide are mediated by the transient receptor potential vanilloid type-1 channel (TRPV1), formerly called vanilloid receptor VR1 [39]. These receptors have been traditionally known for their function in sensory nerves where they mediate perception of inflammatory and thermal pain, but they are also expressed within the brain contributing to other important physiological functions. Co-expression of cannabinoid CB1 and TRPV1 receptors was found by using immunofluorescence techniques in diverse brain areas involved in the regulation of emotional responses. In particular, within the hippocampus, CB1/TRPV1 was detected on cell bodies of many pyramidal neurons throughout the CA1–CA3 subfields and in the molecular layer of dentate gyrus [56]. Interestingly, TRPV1 knockout mice (TRPV1-KO) showed less anxiety-related behavior in the light-dark test and in the elevated plus-maze than their wild-type littermates as well as less freezing to a tone after auditory fear conditioning and stress sensitization. TRPV1-KO also showed impaired hippocampus-dependent contextual fear together with a decrease in long-term potentiation (LTP) in the Schaffer collateral-commissural pathway to CA1 hippocampal neurons. These data provide first evidence for fear-promoting effects of TRPV1 with respect to both innate and conditioned fear and for a decisive role of this receptor in synaptic plasticity [57]. Collectively, these findings open new avenues for the study of possible functional relationships between CB1 and TRPV1 receptors, in particular regarding stress, fear, and anxiety responses.

Recently, an additional G-protein-coupled receptor (GPCR) GPR55 has been proposed as a possible new cannabinoid receptor that might play a physiological role in lipid or vascular biology [58].

3. BASIC PRINCIPLES OF ENDOCANNABINOID-MEDIATED SYNAPTIC PLASTICITY

One of the most salient features of the nervous system is its plasticity, including structural and functional changes in individual neurons and synapses. This characteristic is present both during brain development and in the adult life. Synaptic plasticity allows changes in the strength and number of synaptic connections between neurons. It is considered as one of the major mechanisms underlying learning and memory and appears to mediate several other functions in the central nervous system. The resulting changes in synaptic efficacy are thought to be crucial in experience-dependent modifications of neural function. A closely related concept is behavioral flexibility that allows an organism to adapt to variable environmental demands and produce adaptive responses.

Given the prominent presynaptic localization of cannabinoid CB1 receptors, together with its mainly inhibitory actions, cannabinoids have been proposed as local retrograde modulators, with an important role in modulating essential physiological functions and contributing in diverse synaptic plasticity phenomena [59–62]. The endocannabinoid system seems to affect neuronal excitability participating in the maintenance of homeostatic conditions in the brain [26, 63, 64]. In this respect, data obtained from conditional CB1 mutant mice suggest that the endocannabinoid system may protect neurons against excessive activity, and consequently against excitotoxicity. Marsicano et al. generated conditional mutant mice that lacked expression of the CB1 receptor in principal forebrain neurons but

not in adjacent inhibitory interneurons. In mutant mice, the excitotoxin kainic acid (KA) induced excessive seizures in vivo, and the threshold to KA-induced neuronal excitation in vitro was severely reduced in their hippocampal pyramidal neurons. Moreover, KA administration rapidly raised hippocampal levels of anandamide and induced protective mechanisms in wild-type principal hippocampal neurons, whereas these protective mechanisms could not be triggered in mutant mice. These findings indicate that neural excitability is increased in CB1-deficient mice and that the endocannabinoid system may act as a neuroprotective system against abnormally increased discharge activity [26, 65]. The CB1 receptor-mediated neuroprotective effect in the kainate model is apparently mediated by decrease of excitability of glutamatergic hippocampal neurons [48].

Activation of postsynaptic receptors, at diverse neuronal types, induces the release of endogenous cannabinoid compounds that move backwards across the synapse, until reaching the cannabinoid CB1 receptor, to which they bind, therefore inhibiting further neurotransmitter release. Endocannabinoid-mediated synaptic plasticity can be transient or long lasting and can be found at both excitatory and inhibitory synapses in diverse brain structures. Endocannabinoid-mediated short-term synaptic plasticity includes two electrophysiological phenomena, depolarization-induced suppression of inhibition (DSI), and depolarization-induced suppression of excitation (DSE). DSI is due to a presynaptic action that reduces GABA release, while DSE results from presynaptic inhibition of glutamatergic release. There is also an involvement of the endocannabinoid system in long-term forms of synaptic plasticity. Long-term potentiation (LTP) is a long-lasting increase in the strength of a synapse, while long-term depression (LTD) is a long lasting weakening of synaptic strength. Both are mechanisms of synaptic plasticity that can persist for hours to weeks and have important implications on various forms of learning and memory. Endocannabinoid-induced long-lasting inhibition of neurotransmitter release has been found in diverse brain structures and at both excitatory and inhibitory synapses (for exhaustive discussion of these phenomena, see [15, 26, 64, 66, 67]).

4. EFFECTS OF CANNABINOIDS ON ANXIETY-RELATED RESPONSES

The main feature of the recreational use of cannabis is that it produces a euphoriant effect. This "high" can be accompanied by decreased anxiety and increased sociability. However, cannabis can also produce dysphoric reactions, feelings of anxiety, panic, paranoia, and psychosis [68–72]. It is possible that the reasons for this lie on the bidirectional effects of cannabinoids on anxiety, with low doses having anxiolytic, and high doses having anxiogenic-like effects. The previous history of the individual and the environmental context may also critically influence the induced cannabinoid effects. Data from animal models provide further evidence for the complexity of the scenario. Low doses of several cannabinoid receptor agonists, nabilone [73], CP 55,940 [74, 75], and Δ^9-tetrahydrocannabinol (THC) [76] induced anxiolytic-like effects in both the elevated plus-maze and the light-dark box. In contrast, high doses of the cannabinoid agonist HU-210 produced anxiogenic-like responses in the defensive withdrawal test [77] and enhanced emotional responding to tactile stimulation [78], and mid-high doses of CP 55,940 showed anxiogenic-like effects in the plus-maze [74, 75, 79, 80] and in the social interaction test [81].

It has been shown that exposure to chronic stress enhances the anxiety-like responsiveness to cannabinoids in rats [82], a phenomenon that is also observed in humans. Accordingly, Patel et al. [83] have recently analyzed the interactions between cannabinoids and environmental stress in the regulation of amygdalar activation in mice. The combination of restraint stress and CB1 agonist administration produced robust Fos induction within the central amygdala, indicating a synergistic interaction between environmental stress and CB1 receptor activation. These data suggest that the central amygdala could be an important neural substrate relevant to the context-dependent effects of cannabinoids on emotional/affective responses.

It is worth noting that, in addition to anxiety, there are other behavioral responses, such as motor activity and exploration [75, 80, 81, 84, 85], that are affected by cannabinoid agonists in a biphasic manner. In general, low doses are stimulatory, whereas high doses are inhibitory. Bimodal effects of cannabinoids might be explained by two distinct populations of presynaptic CB1 receptors, with different sensitivities to cannabinoids, particularly WIN 55,212-2 (WIN), located possibly on glutamatergic and GABAergic neurons [26, 86]. The administration of WIN resulted in a biphasic, dose-dependent effect on hippocampal acetylcholine (ACh) release: a low dose and a high dose of the compound induced a transient stimulation and a prolonged inhibition of hippocampal ACh efflux, respectively. These amphidromic responses appeared to involve the same structural entities, Gi-coupled CB1 receptors, but different neuroanatomical sites. The low-dose excitatory effects were mediated in the septum, whereas the high-dose inhibitory effects were mediated locally in hippocampus. Moreover, the stimulatory and the inhibitory effects of the cannabinoid agonist involved activation of dopamine D_1 and D_2 receptors, respectively [7]. Local infusion of cannabinoid compounds in specific brain areas might be instrumental to identify neural pathways and neuroanatomically separated CB1 receptor subpopulations that may play distinct roles and mediate opposing actions of cannabinoids, notably, anxiolytic versus anxiogenic effects [87]. This possibility might further explain why elevation of endocannabinoids levels sometimes has effects that are different from those observed with exogenous cannabinoids [26]. An additional hypothesis which might account for the biphasic effects of cannabinoids is the possible differential implication of Gs and Gi proteins in the stimulatory and inhibitory effects, respectively [88]. It would be interesting to test this hypothesis in vivo, in relation to anxiety-related effects.

5. ROLE OF THE ENDOCANNABINOID SYSTEM IN THE REGULATION OF ANXIETY

5.1. CB1 receptor knockout mice

The development of knockout (KO) mice deficient in CB1(CB1-KO) receptors has provided an excellent tool to evaluate the physiological roles of the endocannabinoid system, and particularly its possible implication in the regulation of anxiety. The CB1-KO mice showed an increase in the aggressive response measured in the resident-intruder test and an anxiogenic-like behavior in the light-dark box, the elevated plus-maze test, and the social interaction test [89, 90]. On the other hand, Marsicano et al. [18] did not find an anxiogenic-like response in the plus-maze in their CB1-KO mice. Discrepancies might be attributed to differences in the genetic background of mutant mice, and also to differences on baseline anxiety levels and to context-dependent stress elicited. In particular, CB1-KO mice exclusively showed an anxiogenic-like behavior under high-stress conditions: light in the plus-maze and unfamiliar environment in the social interaction test [18, 89–91]. An impaired action of anxiolytic drugs, such as bromazepam and buspirone, has been also observed in mutant mice [90]. This latter result suggests that functional integrity of cannabinoid CB1 receptors is necessary to achieve a complete efficacy of anxiolytic drugs, which may have consequences in the treatment of mood-related disorders, including those derived from cannabinoid abuse.

5.2. Pharmacological blockade of CB1 receptors

Evidence for an endogenous anxiolytic cannabinoid tone also comes from certain effects of the CB1 receptor antagonist rimonabant (SR141716A). This drug has anxiogenic effects in adult rats submitted to the defensive withdrawal test and the elevated plus-maze [79, 92]. The cannabinoid receptor agonist CP 55,940 reduced ultrasonic vocalization in rat pups separated from their mother, indicating an anxiolytic effect, and rimonabant not only reversed this effect, but also enhanced pup ultrasonic vocalizations when administered alone [93]. These results further support the view that there is an endogenous regulation of emotional states mediated by the cannabinoid system that might be present since early developmental stages. As for CB1-KO animals, certain results obtained in mice following rimonabant administration showed apparently contradictory results since this compound was found to be anxiolytic in the plus-maze [89]. These data may reflect species differences, but it seems likely that environmental context and baseline anxiety levels critically account for at least some of the discrepancies observed in the literature. The context dependency is indirectly supported by the "one-trial sensitization" phenomenon described by Rodgers et al. [94] in the plus-maze. In these experiments, the CB1 receptor antagonist had no behavioral effects in maze-naïve mice, but induced an anxiolytic-like effect in the second trial of the test.

With respect to recent clinical trials, rimonabant has been tested for its possible therapeutical application in obesity and metabolic disorders, and the most frequent adverse events resulting in discontinuation of the drug included depression and anxiety [95–97].

5.3. Inhibitors of endocannabinoids inactivation

As indicated above (Section 2), the enzyme FAAH catalyzes the hydrolysis of the endogenous cannabinoid anandamide. Pharmacological blockade of this enzyme by URB597 and URB532 produced anxiolytic-like effects in the elevated zero-maze in adult rats and in the isolation-induced ultrasonic emission test in rat pups. These effects were accompanied by augmented brain levels of anandamide and were prevented by CB1 receptor blockade. Moreover, the anxiolytic actions of URB597 were not accompanied by typical cannabinoid signs of intoxication in rodents such as catalepsy or hypothermia. These results indicate that anandamide participates in the modulation of emotional states and point to FAAH inhibition as an innovative approach to antianxiety therapy [98].

A model has been proposed to explain the possible mechanism by which the AEA-CB1 receptor system may participate in the control of anxious states. Endocannabinoids might be generated in the amygdala in response to the anxiety inducing stimulus, and would, therefore, regulate emotional states by influencing amygdala outputs [99]. This view is supported also by the fact that AEA content in the mouse basolateral amygdala rises when the animal is conditioned to expect a foot shock after hearing a tone [18]. Thus, the endocannabinoid system, and AEA in particular, might be activated in response to anxiogenic situations and this activation could be part of a negative feedback system that limits anxiety [99]. In line with this hypothesis, there are data suggesting a role of endocannabinoid signaling as an inhibitory modulator of behavioral and neuronal responses to aversive stimuli [13] and in the inhibition of stress-induced activation of HPA axis [12] (see next section). A recent paper by Patel and Hillard [100] further supports a crucial role for endocannabinoids in the induction of anxiolytic-like effects. The inhibitor of endocannabinoids metabolism, URB597, produced a linear dose-dependent anxiolytic effect. In turn, AM404 that is considered as an inhibitor of endocannabinoids uptake exerted an action that was more similar to that elicited by direct agonists, with low doses producing anxiolytic effects and the highest dose having no effect [98]. The different profiles of AM404 might be due to the fact that in addition to increasing the endocannabinoid-mediated tone, this compound can also activate TRV1 receptors [101] which, as indicated by the study by Marsh et al. quoted above [57], are also involved in the regulation of anxiety.

Collectively, a majority of evidence suggests the existence of an anxiolytic endocannabinoid tone. The modulatory role of the endocannabinoid system against stress is further supported by studies from Patel et al. [12, 13] indicating that endocannabinoids act as inhibitory modulators of both neuronal and behavioral activations during an acute stress and negatively modulate HPA axis activity (see Section 7).

6. CONDITIONED FEAR RESPONSES, AVERSIVE MEMORIES, AND FEAR EXTINCTION

Neurobiological substrates of emotional-based learning have been extensively examined in animal models that allow the study of acquisition, expression, and retention of Pavlovian fear conditioning . In this paradigm, an initially innocuous/neutral stimulus (the to-be conditioned stimulus (CS); e.g., a light, tone, or odor) is paired with an innately aversive unconditioned stimulus (US; e.g., a footshock). Following several pairings, the subject comes to exhibit a conditioned fear response to the CS. Conditioned fear behavioral and physiological responses include changes in heart rate and blood pressure and freezing or cue-induced fear potentiated startle reflex. Excessive fear and anxiety are hallmarks of a variety of disabling neuropsychiatric disorders. Adaptive strategies leading to an appropriate interplay between fear expression and fear extinction are necessary for adequate coping with aversive encounters. In experimental studies like the ones mentioned above, fear inhibition is frequently studied through a procedure in which the previously fear conditioned subject is exposed to the fear-eliciting cue in the absence of any aversive event. This procedure results in a decline in conditioned fear. In other words, repeated presentation of the conditioned stimulus alone leads to extinction of the fearful response. There are clear clinical implications of research on fear extinction. Anxiety- related pathologies such as phobias and post-traumatic stress disorder (PTSD) seem to be disorders of fear dysregulation in which inhibition of fear is absent or insufficient in situations that are patently safe. In the last years, there is an increasing interest in revealing the neural mechanisms of fear inhibition, including the regions in which extinction-related plasticity occurs and the cellular and molecular processes that are implicated in this plasticity-related phenomenon (comprehensive reviews on these mechanisms can be found in [102–105]). In the present section, we will focus on the possible functional implication of the endocannabinoid system.

The use of CB1-KO mice and pharmacological blockade of CB1 receptors have yielded information regarding the involvement of the endocannabinoid system in conditioned fear responses. It has been reported that CB1-KO mice showed strongly impaired short- and long-term extinction in auditory fear-conditioning tests, with unaffected memory acquisition and consolidation. Consistent with this finding, pharmacological blockade of CB1 receptors with rimonabant led to a similar deficit in extinction in wild-type mice [18]. The authors also found that during the extinction protocol (exposure to the tone alone), the levels of endocannabinoids were raised within the basolateral amygdala, a region known to control extinction of aversive memories, both in mutant and normal mice. In subsequent studies, Azad et al. [19] showed that low-frequency stimulation of afferents in the lateral amygdala released endocannabinoids postsynaptically from neurons of the basolateral amygdala of mice, and thereby induced an LTP of inhibitory GABAergic synaptic transmission (LTDi) via a presynaptic mechanism. In turn, lowering inhibitory synaptic transmission significantly increased the amplitude of excitatory synaptic currents in principal neurons of the central nucleus, which is the main output site of the amygdala. LTDi was blocked by rimonabant, abolished in CB1-KO animals, and significantly enhanced in mice lacking FAAH, the anandamide-degrading enzyme [19]. More recently, it has been addressed whether CB1 blockade would similarly disrupt extinction in rats, using fear-potentiated startle as a measure of conditioned fear. The authors further investigated whether pharmacologic augmentation of CB1 activation would lead to enhancements in extinction. The results indicated that rimonabant dose-dependently blocked the extinction of conditioned fear in rats, as it does in mice. Moreover, administration of AM404, an inhibitor of endocannabinoid reuptake, led to a dose-dependent enhancement in extinction and this effect was blocked almost completely by rimonabant, indicating an implication of CB1 receptors. The animals treated with AM404 also showed decreased shock-induced reinstatement of fear, suggesting that this compound may reduce susceptibility to reinstatement of fear [106]. Lin et al. [107] have shown that bilateral infusion of CB1 receptor agonists into the amygdala after memory reactivation blocked reconsolidation of fear memory measured with fear-potentiated startle. These authors proposed that activation of CB1 receptors could facilitate extinction on one hand and block reconsolidation on the other.

Hölter et al. [108] have compared CB1-KO mice with their wild-type controls in an appetitively motivated operant conditioning task including food reward. During the extinction phase, when the positive reinforcement was omitted, control and CB1-KO mice showed a similar decline in accuracy of performance and total number of correct responses, accompanied by an increase in errors of omission [108]. A recent pharmacological study using rimonabant [109] further supports the notion that the cannabinoid CB1 receptor plays a pivotal role in extinction of aversive memories but is not essential for extinction of positively reinforced memories.

It has been claimed that fear conditioning in mice combines both associative and non-associative (sensitization) components and that extinction involves a significant habituation component [110]. In a more recent study, Kamprath et al. [111] have found that CB1-KO mice were severely impaired not only in extinction of the fear response to a tone after fear conditioning, but also in habituation of the fear response to a tone after sensitization with an inescapable footshock. Based on these findings, they have proposed that CB1 receptor might be critically involved in non-associative learning processes (habituation), which would contribute to the decrease in the fear response. A mouse model has been recently proposed that may allow exploring the role of the endocannabinoid system in the associative and non-associative components of fear has been recently proposed [112].

7. CANNABINOIDS AND THE HYPOTHALAMUS-PITUITARY-ADRENAL AXIS

An electrophysiological study by Di et al. [20] has revealed that glucocorticoids elicit a rapid, nongenomic suppression

of glutamate release onto parvocellular neuroendocrine cells of the hypothalamic paraventricular nucleus (PVN) by stimulating the retrograde release of endocannabinoids that would subsequently activate presynaptic cannabinoid CB1 receptors. By this mechanism, endocannabinoids may be involved in the modulation of a number of peptidergic systems, including CRH. Patel et al. [12] have addressed a role of the endocannabinoid system in the modulation of stress-induced adrenocortical activity in vivo. These authors confirmed previous studies showing that rimonabant was able to increase serum corticosterone concentrations under basal conditions. Moreover, the CB1 receptor antagonist potentiated restraint stress-induced HPA axis activation, whereas pretreatment of mice with either a low dose of the CB1 receptor agonist CP 55,940, the endocannabinoid transport inhibitor AM404, or the FAAH inhibitor URB597 significantly decreased or eliminated restraint-induced corticosterone release. Acute restraint-induced corticosterone release was associated with a decrease in hypothalamic 2-AG content, whereas the attenuation of adrenocortical response observed after prolonged stress was associated with an increase in hypothalamic 2-AG content. In view of the above data, the following speculative model can be suggested: during resting (baseline) conditions, the HPA axis would be tonically inhibited by endocannabinoids via CB1 receptors located in the PVN of the hypothalamus. In this way, the endocannabinoid system might keep under control the stress response. Upon an acute stress exposure, that is, when the stress response is needed, a reduction of endocannabinoids signaling would allow the HPA axis to be activated (disinhibition). If the stress becomes chronic, endocannabinoid levels would increase again to restore a normal homeostasis.

With respect to the effects of exogenous cannabinoid agonists, in general the literature indicates that they exert a dose-dependent effect on adrenocortical activity with high doses increasing corticosterone responses [21, 84, 113]. As previously indicated, high doses of cannabinoids are also anxiogenic. However, we have found that, at certain doses, the effects of cannabinoids on anxiety can be dissociated from their effects on adrenocortical activity. Thus a high dose of the cannabinoid agonist CP 55,940 (75 μg/kg) induced both, anxiogenic-like effects in the plus-maze and stimulation of adrenocortical activity [80]. However, a dose of 50 μg/kg induced an anxiogenic-like effect in the same test, without increasing corticosterone concentrations [113].

As in the case of anxiety, literature regarding HPA axis activity supports the general concept that the pharmacological administration of exogenous cannabinoids may lead to a completely different action when compared with the physiological functions of the endocannabinoid system [26, 28, 30].

8. ENDOCANNABINOID SYSTEM AND DEPRESSION

Several lines of evidence suggest that the endocannabinoid system may play a role in the aetiology of depression and could represent a new therapeutic target for its treatment. CB1-KO mice showed altered HPA axis function [90] and a higher sensitivity to exhibit depressive-like responses in the chronic unpredictable mild stress procedure, which suggests an increased susceptibility to develop an anhedonic state [114]. These characteristics together with their heightened anxiety [89, 90] and deficits in extinction of aversive memories [18] have been proposed to be analogous to certain symptoms of melancholic depression [115].

Several cannabinoid compounds have been evaluated in behavioral tests such as the forced swimming test (FST) and the tail-suspension test (TST) that are among the most widely used screening tests of antidepressant potential of novel compounds [116]. In the rat FST, administration of AM404 (endocannabinoid uptake inhibitor) and HU-210, a potent CB1 receptor agonist, induced decreases in immobility (indicative of antidepressant activity) that were blocked by pretreatment with the selective CB1 receptor antagonist AM251. The reduction in immobility induced by the cannabinoid compounds was comparable to that seen with the reference antidepressant desipramine [117]. In turn, the FAAH inhibitor URB597 exerted potent antidepressant-like actions in the mouse TST and the rat FST, and these effects were prevented or attenuated by rimonabant [118].

During the last years, there has been an active investigation on the implications of hippocampal neurogenesis in the pathophysiology and treatment of mood disorders. Preclinical and clinical studies indicate that stress (possibly through the action of elevated glucocorticoids) and depression lead to atrophy and loss of neurons in the adult hippocampus. On the other hand, chronic antidepressant treatment up-regulates hippocampal neurogenesis which could counteract the stress-induced damage [119, 120]. An elegant study by Jiang et al. [121] revealed an important implication of hippocampal neurogenesis in the antidepressant and anxiolytic-like effects of cannabinoid agonists. They showed that both embryonic and adult rat hippocampal neural stem/progenitor cells were immunoreactive for cannabinoid CB1 receptors, indicating that cannabinoids could act on these receptors to regulate neurogenesis. A chronic (but not acute) treatment with the potent synthetic cannabinoid HU210 promoted neurogenesis in the hippocampal dentate gyrus of adult rats and exerted anxiolytic- and antidepressant-like effects. The cannabinoid–induced newborn neurons appeared to be of functional significance, since X-irradiation of the hippocampus blocked both the neurogenic and behavioral effects of chronic HU210 treatment. These evidences strongly suggest that cannabinoid agonists might produce anxiolytic- and antidepressant-like effects by promoting hippocampal neurogenesis. In line with these findings, administration of the endocannabinoids uptake inhibitor AM404 prior to exposure to predator odor stress inhibited both the stress-induced activation of defensive burying and the suppression of cell proliferation in the hippocampus [122], indicating a role for endocannabinoids in the modulation of stress-induced changes in hippocampal cell proliferation.

The efficacy of antidepressants has been linked in part to their ability to reduce the activity of the HPA axis [123]. In view of the above data, it is tempting to speculate that the

endocannabinoid system is somehow involved in the action of currently used antidepressant drugs. In favor of this hypothesis, it has been shown that chronic administration of the tricyclic antidepressant desipramine resulted in a significant increase in the density of the cannabinoid CB1 receptor in both hippocampus and hypothalamus as well as in a reduction in swim stress-induced corticosterone secretion and immediate early *c-fos* gene in the medial dorsal parvocellular region of the PVN of the hypothalamus. Moreover, acute treatment with the CB1 receptor antagonist AM251 before exposure to stress occluded the effects of desipramine on corticosterone secretion and neuronal activation [124].

9. CONCLUDING REMARKS

During the last few years, the increasing interest in the link between the endocannabinoid system and emotional responses has led to a number of interesting data derived from animal studies. These results may contribute to understand the complex scenario of cannabinoid effects in humans, and to clarify the mechanisms underlying associations between cannabis abuse and mental disorders. Results obtained from transgenic mice lacking CB1 receptors and by using CB1 receptors selective antagonists and inhibitors of endocannabinoids inactivation suggest the existence of an intrinsic endocannabinoid tone which contributes to the regulation of stress responses and anxiety. An adequate endocannabinoid function appears to be necessary for adaptive extinction of aversive memories. The endocannabinoid system might play a pivotal role in maintaining homeostasis, notably with regard to physiological and behavioral responses to acute and prolonged stress. Certain forms of endocannabinoid-dependent synaptic plasticity have been proposed as crucial mechanisms subserving these phenomena. Throughout this review, we have focused on the endocannabinoid system as a major player in the modulation of synaptic transmission and plasticity considering solely interneural communication. However, the critical functional role of glial cells in maintaining a correct brain function and their implications in diverse neuropathological conditions are now clearly recognized. The new concept of the tripartite synapse in which the glial cell (notably astrocytes) plays an active role in the modulation of neurotransmission has recently emerged [125]. Expression of cannabinoid CB1 receptors and endocannabinoid synthesis and release have been observed in different types of glial cells [126, 127]. This "glial endocannabinoid system" may have important physiological and pathological implications [128, 129] and it would be interesting to explore a possible role in the expression of synaptic plasticity in limbic and extra-limbic regions related to stress, fear, and anxiety responses.

Disregulation or malfunctioning of the endocannabinoid system might contribute to the aetiology of anxiety-related disorders and to certain symptoms of melancholic depression. In turn, the endocannabinoid system might constitute an interesting pharmacological target for the development of anti-anxiety and antidepressant therapies.

The involvement of the endocannabinoid system in the regulation of anxiety and its participation in the modulation of behavioral and physiological responses to aversive situations have other obvious implications. Cannabis abuse may be one of the causes disrupting the necessary balance for an appropriate function of the system. There are functional interactions between the endocannabinoid system and other monoaminergic and peptidergic systems also involved in the regulation of emotional responses [113, 130]. Thus, the disruption of the endocannabinoid system as a consequence of cannabis abuse may alter these other neurochemical systems contributing to the development of emotional disorders. In addition to acute aversive emotional reactions to cannabis, the chronic use of this addictive drug may result in mental disturbances and neuropsychiatric disorders. In particular, there are data suggesting that exposure to cannabis derivatives is associated with a higher risk of schizophrenia, depression, and anxiety [68–72, 131, 132]. In this review, we have highlighted the importance of endocannabinoid-based neuroplasticity phenomena in the regulation of neuroendocrine and neurochemical systems implicated in the modulation of emotional responses and extinction of perseverative behaviors and inadaptative aversive memories. Consequently, it is likely that impairment of endocannabinoid-mediated synaptic transmission and plasticity contribute to the expression of at least some aspects of these psychiatric illnesses.

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REFERENCES

[1] M. J. Millan, "The neurobiology and control of anxious states," *Progress in Neurobiology*, vol. 70, no. 2, pp. 83–244, 2003.

[2] M. Herkenham, A. B. Lynn, M. D. Little, et al., "Cannabinoid receptor localization in brain," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 87, no. 5, pp. 1932–1936, 1990.

[3] M. Herkenham, A. B. Lynn, M. R. Johnson, L. S. Melvin, B. R. de Costa, and K. C. Rice, "Characterization and localization of cannabinoid receptors in rat brain: a quantitative in vitro autoradiographic study," *The Journal of Neuroscience*, vol. 11, no. 2, pp. 563–583, 1991.

[4] M. Glass, M. Dragunow, and R. L. M. Faull, "Cannabinoid receptors in the human brain: a detailed anatomical and quantitative autoradiographic study in the fetal, neonatal and adult human brain," *Neuroscience*, vol. 77, no. 2, pp. 299–318, 1997.

[5] I. Katona, E. A. Rancz, L. Acsády, et al., "Distribution of CB_1 cannabinoid receptors in the amygdala and their role in the control of GABAergic transmission," *The Journal of Neuroscience*, vol. 21, no. 23, pp. 9506–9518, 2001.

[6] N. Hàjos and T. F. Freund, "Distinct cannabinoid sensitive receptors regulate hippocampal excitation and inhibition," *Chemistry and Physics of Lipids*, vol. 121, no. 1-2, pp. 73–82, 2002.

[7] E. T. Tzavara, M. Wade, and G. G. Nomikos, "Biphasic effects of cannabinoids on acetylcholine release in the hippocampus: site and mechanism of action," *The Journal of Neuroscience*, vol. 23, no. 28, pp. 9374–9384, 2003.

[8] M. Pistis, S. Perra, G. Pillolla, M. Melis, G. L. Gessa, and A. L. Muntoni, "Cannabinoids modulate neuronal firing in the rat basolateral amygdala: evidence for CB_1- and non-CB_1-mediated actions," *Neuropharmacology*, vol. 46, no. 1, pp. 115–125, 2004.

[9] J. C. Arnold, A. N. Topple, P. E. Mallet, G. E. Hunt, and I. S. McGregor, "The distribution of cannabinoid-induced Fos expression in rat brain: differences between the Lewis and Wistar strain," *Brain Research*, vol. 921, no. 1-2, pp. 240–255, 2001.

[10] B. S. McEwen, "Mood disorders and allostatic load," *Biological Psychiatry*, vol. 54, no. 3, pp. 200–207, 2003.

[11] G. E. Tafet and R. Bernardini, "Psychoneuroendocrinological links between chronic stress and depression," *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, vol. 27, no. 6, pp. 893–903, 2003.

[12] S. Patel, C. T. Roelke, D. J. Rademacher, W. E. Cullinan, and C. J. Hillard, "Endocannabinoid signaling negatively modulates stress-induced activation of the hypothalamic-pituitary-adrenal axis," *Endocrinology*, vol. 145, no. 12, pp. 5431–5438, 2004.

[13] S. Patel, C. T. Roelke, D. J. Rademacher, and C. J. Hillard, "Inhibition of restraint stress-induced neural and behavioural activation by endogenous cannabinoid signalling," *European Journal of Neuroscience*, vol. 21, no. 4, pp. 1057–1069, 2005.

[14] M. N. Hill, S. Patel, E. J. Carrier, et al., "Downregulation of endocannabinoid signaling in the hippocampus following chronic unpredictable stress," *Neuropsychopharmacology*, vol. 30, no. 3, pp. 508–515, 2005.

[15] B. E. Alger, "Retrograde signaling in the regulation of synaptic transmission: focus on endocannabinoids," *Progress in Neurobiology*, vol. 68, no. 4, pp. 247–286, 2002.

[16] S. C. Azad, V. Huge, P. Schöps, et al., "Endogenous cannabinoid system. Effect on neuronal plasticity and pain memory," *Der Schmerz*, vol. 19, no. 6, pp. 521–527, 2005.

[17] G. Riedel and S. N. Davies, "Cannabinoid function in learning, memory and plasticity," in *Handbook of Experimental Pharmacology*, vol. 168, pp. 445–477, Springer, Berlin, Germany, 2005.

[18] G. Marsicano, C. T. Wotjak, S. C. Azad, et al., "The endogenous cannabinoid system controls extinction of aversive memories," *Nature*, vol. 418, no. 6897, pp. 530–534, 2002.

[19] S. C. Azad, K. Monory, G. Marsicano, et al., "Circuitry for associative plasticity in the amygdala involves endocannabinoid signaling," *The Journal of Neuroscience*, vol. 24, no. 44, pp. 9953–9961, 2004.

[20] S. Di, R. Malcher-Lópes, K. C. Halmos, and J. G. Tasker, "Nongenomic glucocorticoid inhibition via endocannabinoid release in the hypothalamus: a fast feedback mechanism," *The Journal of Neuroscience*, vol. 23, no. 12, pp. 4850–4857, 2003.

[21] M.-P. Viveros and E.-M. Marco, "Cannabinoids, anxiety and depression," in *Chronic and Common Diseases—II*, J. N. Govil and V. K. Singh, Eds., vol. 18 of *Recent Progress in Medicinal Plants*, Stadium Press, Houston, Tex, USA, 2006.

[22] D. M. Lambert and C. J. Fowler, "The endocannabinoid system: drug targets, lead compounds, and potential therapeutic applications," *Journal of Medicinal Chemistry*, vol. 48, no. 16, pp. 5059–5087, 2005.

[23] C. T. Wotjak, "Role of endogenous cannabinoids in cognition and emotionality," *Mini-Reviews in Medicinal Chemistry*, vol. 5, no. 7, pp. 659–670, 2005.

[24] T. C. Kirkham and S. A. Tucci, "Endocannabinoids in appetite control and the treatment of obesity," *CNS & Neurological Disorders - Drug Targets*, vol. 5, no. 3, pp. 272–292, 2006.

[25] K. Mackie, "Cannabinoid receptors as therapeutic targets," *Annual Review of Pharmacology and Toxicology*, vol. 46, pp. 101–122, 2006.

[26] G. Marsicano and B. Lutz, "Neuromodulatory functions of the endocannabinoid system," *Journal of Endocrinological Investigation*, vol. 29, no. 3 supplement, pp. 27–46, 2006.

[27] P. Pacher, S. Bátkai, and G. Kunos, "The endocannabinoid system as an emerging target of pharmacotherapy," *Pharmacological Reviews*, vol. 58, no. 3, pp. 389–462, 2006.

[28] U. Pagotto, G. Marsicano, D. Cota, B. Lutz, and R. Pasquali, "The emerging role of the endocannabinoid system in endocrine regulation and energy balance," *Endocrine Reviews*, vol. 27, no. 1, pp. 73–100, 2006.

[29] J. Fernández-Ruiz, J. Romero, G. Velasco, R. M. Tolón, J. A. Ramos, and M. Guzmán, "Cannabinoid CB_2 receptor: a new target for controlling neural cell survival?" *Trends in Pharmacological Sciences*, vol. 28, no. 1, pp. 39–45, 2007.

[30] J. G. Tasker, "Rapid glucocorticoid actions in the hypothalamus as a mechanism of homeostatic integration," *Obesity*, vol. 14, no. 5, pp. 259S–265S, 2006.

[31] L. De Petrocellis, M. G. Cascio, and V. Di Marzo, "The endocannabinoid system: a general view and latest additions," *British Journal of Pharmacology*, vol. 141, no. 5, pp. 765–774, 2004.

[32] T. Bisogno, A. Ligresti, and V. Di Marzo, "The endocannabinoid signalling system: biochemical aspects," *Pharmacology Biochemistry and Behavior*, vol. 81, no. 2, pp. 224–238, 2005.

[33] A. Ligresti, M. G. Cascio, and V. Di Marzo, "Endocannabinoid metabolic pathways and enzymes," *CNS & Neurological Disorders - Drug Targets*, vol. 4, no. 6, pp. 615–623, 2005.

[34] M. Bari, N. Battista, F. Fezza, V. Gasperi, and M. Maccarrone, "New insights into endocannabinoid degradation and its therapeutic potential," *Mini-Reviews in Medicinal Chemistry*, vol. 6, no. 3, pp. 257–268, 2006.

[35] I. Matias and V. Di Marzo, "Endocannabinoid synthesis and degradation, and their regulation in the framework of energy balance," *Journal of Endocrinological Investigation*, vol. 29, no. 3 supplement, pp. 15–26, 2006.

[36] J. Liu, L. Wang, J. Harvey-White, et al., "A biosynthetic pathway for anandamide," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 103, no. 36, pp. 13345–13350, 2006.

[37] G. M. Simon and B. F. Cravatt, "Endocannabinoid biosynthesis proceeding through glycerophospho-*N*-acyl ethanolamine and a role for α/β-hydrolase 4 in this pathway," *Journal of Biological Chemistry*, vol. 281, no. 36, pp. 26465–26472, 2006.

[38] A. Hermann, M. Kaczocha, and D. G. Deutsch, "2-arachidonoylglycerol (2-AG) membrane transport: history and outlook," *The AAPS Journal*, vol. 8, no. 2, pp. E409–E412, 2006.

[39] A. C. Howlett, F. Barth, T. I. Bonner, et al., "International Union of Pharmacology. XXVII. Classification of cannabinoid receptors," *Pharmacological Reviews*, vol. 54, no. 2, pp. 161–202, 2002.

[40] P. Mailleux and J.-J. Vanderhaeghen, "Distribution of neuronal cannabinoid receptor in the adult rat brain: a comparative receptor binding radioautography and in situ hybridization histochemistry," *Neuroscience*, vol. 48, no. 3, pp. 655–668, 1992.

[41] K. Tsou, S. Brown, M.C. Sañudo-Peña, K. Mackie, and J. M. Walker, "Immunohistochemical distribution of cannabinoid CB_1 receptors in the rat central nervous system," *Neuroscience*, vol. 83, no. 2, pp. 393–411, 1998.

[42] K. Mackie, "Distribution of cannabinoid receptors in the central and peripheral nervous system," in *Handbook of Experimental Pharmacology*, R. G. Pertewee, Ed., vol. 168, pp. 299–325, Springer, Berlin, Germany, 2005.

[43] A. Straiker and K. Mackie, "Cannabinoids, electrophysiology, and retrograde messengers: challenges for the next 5 years," *The AAPS Journal*, vol. 8, no. 2, pp. E272–E276, 2006.

[44] I. Katona, B. Sperlágh, A. Sík, et al., "Presynaptically located CB_1 cannabinoid receptors regulate GABA release from axon terminals of specific hippocampal interneurons," *The Journal of Neuroscience*, vol. 19, no. 11, pp. 4544–4558, 1999.

[45] K. Tsou, K. Mackie, M.C. Sañudo-Peña, and J. M. Walker, "Cannabinoid CB_1 receptors are localized primarily on cholecystokinin-containing GABAergic interneurons in the rat hippocampal formation," *Neuroscience*, vol. 93, no. 3, pp. 969–975, 1999.

[46] N. Hájos, C. Ledent, and T. F. Freund, "Novel cannabinoid-sensitive receptor mediates inhibition of glutamatergic synaptic transmission in the hippocampus," *Neuroscience*, vol. 106, no. 1, pp. 1–4, 2001.

[47] T. F. Freund, I. Katona, and D. Piomelli, "Role of endogenous cannabinoids in synaptic signaling," *Physiological Reviews*, vol. 83, no. 3, pp. 1017–1066, 2003.

[48] K. Monory, F. Massa, M. Egertová, et al., "The endocannabinoid system controls key epileptogenic circuits in the hippocampus," *Neuron*, vol. 51, no. 4, pp. 455–466, 2006.

[49] M. R. Domenici, S. C. Azad, G. Marsicano, et al., "Cannabinoid receptor type 1 located on presynaptic terminals of principal neurons in the forebrain controls glutamatergic synaptic transmission," *The Journal of Neuroscience*, vol. 26, no. 21, pp. 5794–5799, 2006.

[50] E. L. Hill, T. Gallopin, I. Férézou, et al., "Functional CB_1 receptors are broadly expressed in neocortical GABAergic and glutamatergic neurons," *Journal of Neurophysiology*, vol. 97, no. 4, pp. 2580–2589, 2007.

[51] S. Galiegue, S. Mary, J. Marchand, et al., "Expression of central and peripheral cannabinoid receptors in human immune tissues and leukocyte subpopulations," *European Journal of Biochemistry*, vol. 232, no. 1, pp. 54–61, 1995.

[52] A. R. Schatz, M. Lee, R. B. Condie, J. T. Pulaski, and N. E. Kaminski, "Cannabinoid receptors CB_1 and CB_2: a characterization of expression and adenylate cyclase modulation within the immune system," *Toxicology and Applied Pharmacology*, vol. 142, no. 2, pp. 278–287, 1997.

[53] M. Begg, P. Pacher, S. Bátkai, et al., "Evidence for novel cannabinoid receptors," *Pharmacology and Therapeutics*, vol. 106, no. 2, pp. 133–145, 2005.

[54] M. D. Van Sickle, M. Duncan, P. J. Kingsley, et al., "Identification and functional characterization of brainstem cannabinoid CB_2 receptors," *Science*, vol. 310, no. 5746, pp. 329–332, 2005.

[55] E. S. Onaivi, H. Ishiguro, J.-P. Gong, et al., "Discovery of the presence and functional expression of cannabinoid CB_2 receptors in brain," *Annals of the New York Academy of Sciences*, vol. 1074, pp. 514–536, 2006.

[56] L. Cristino, L. de Petrocellis, G. Pryce, D. Baker, V. Guglielmotti, and V. Di Marzo, "Immunohistochemical localization of cannabinoid type 1 and vanilloid transient receptor potential vanilloid type 1 receptors in the mouse brain," *Neuroscience*, vol. 139, no. 4, pp. 1405–1415, 2006.

[57] R. Marsch, E. Foeller, G. Rammes, et al., "Reduced anxiety, conditioned fear, and hippocampal long-term potentiation in transient receptor potential vanilloid type 1 receptor-deficient mice," *The Journal of Neuroscience*, vol. 27, no. 4, pp. 832–839, 2007.

[58] D. Baker, G. Pryce, W. L. Davies, and C. R. Hiley, "In silico patent searching reveals a new cannabinoid receptor," *Trends in Pharmacological Sciences*, vol. 27, no. 1, pp. 1–4, 2006.

[59] T. Ohno-Shosaku, T. Maejima, and M. Kano, "Endogenous cannabinoids mediate retrograde signals from depolarized postsynaptic neurons to presynaptic terminals," *Neuron*, vol. 29, no. 3, pp. 729–738, 2001.

[60] R. I. Wilson and R. A. Nicoll, "Endogenous cannabinoids mediate retrograde signalling at hippocampal synapses," *Nature*, vol. 410, no. 6828, pp. 588–592, 2001.

[61] A. C. Kreitzer and W. G. Regehr, "Retrograde signaling by endocannabinoids," *Current Opinion in Neurobiology*, vol. 12, no. 3, pp. 324–330, 2002.

[62] D. Piomelli, "The molecular logic of endocannabinoid signalling," *Nature Reviews Neuroscience*, vol. 4, no. 11, pp. 873–884, 2003.

[63] F. Grotenhermen, "Cannabinoids and the endocannabinoid system," *Cannabinoids*, vol. 1, no. 1, pp. 10–14, 2006.

[64] V. Chevaleyre, K. A. Takahashi, and P. E. Castillo, "Endocannabinoid-mediated synaptic plasticity in the CNS," *Annual Review of Neuroscience*, vol. 29, pp. 37–76, 2006.

[65] G. Marsicano, S. Goodenough, K. Monory, et al., "CB_1 cannabinoid receptors and on-demand defense against excitotoxicity," *Science*, vol. 302, no. 5642, pp. 84–88, 2003.

[66] G. L. Gerdeman and D. M. Lovinger, "Emerging roles for endocannabinoids in long-term synaptic plasticity," *British Journal of Pharmacology*, vol. 140, no. 5, pp. 781–789, 2003.

[67] M. A. Diana and A. Marty, "Endocannabinoid-mediated short-term synaptic plasticity: depolarization-induced suppression of inhibition (DSI) and depolarization-induced suppression of excitation (DSE)," *British Journal of Pharmacology*, vol. 142, no. 1, pp. 9–19, 2004.

[68] W. Hall and N. Solowij, "Adverse effects of cannabis," *The Lancet*, vol. 352, no. 9140, pp. 1611–1616, 1998.

[69] C. H. Ashton, "Pharmacology and effects of cannabis: a brief review," *The British Journal of Psychiatry*, vol. 178, pp. 101–106, 2001.

[70] G. C. Patton, C. Coffey, J. B. Carlin, L. Degenhardt, M. Lynskey, and W. Hall, "Cannabis use and mental health in young people: cohort study," *British Medical Journal*, vol. 325, no. 7374, pp. 1195–1198, 2002.

[71] M. Tournier, F. Sorbara, C. Gindre, J. D. Swendsen, and H. Verdoux, "Cannabis use and anxiety in daily life: a naturalistic investigation in a non-clinical population," *Psychiatry Research*, vol. 118, no. 1, pp. 1–8, 2003.

[72] P. N. Dannon, K. Lowengrub, R. Amiaz, L. Grunhaus, and M. Kotler, "Comorbid cannabis use and panic disorder: short term and long term follow-up study," *Human Psychopharmacology*, vol. 19, no. 2, pp. 97–101, 2004.

[73] E. S. Onaivi, M. R. Green, and B. R. Martin, "Pharmacological characterization of cannabinoids in the elevated plus maze," *Journal of Pharmacology and Experimental Therapeutics*, vol. 253, no. 3, pp. 1002–1009, 1990.

[74] R. F. Genn, S. Tucci, E.-M. Marco, M.-P. Viveros, and S. E. File, "Anxiolytic and anxiogenic effects of the cannabinoid agonist CP 55,940 in animal tests of anxiety," *Journal of Psychopharmacology*, vol. 17, p. A27, 2003.

[75] E.-M. Marco, L. Pérez-Alvarez, E. Borcel, et al., "Involvement of 5-HT_{1A} receptors in behavioural effects of the cannabinoid receptor agonist CP 55,940 in male rats," *Behavioural Pharmacology*, vol. 15, no. 1, pp. 21–27, 2004.

[76] F. Berrendero and R. Maldonado, "Involvement of the opioid system in the anxiolytic-like effects induced by Δ^9-tetrahydrocannabinol," *Psychopharmacology*, vol. 163, no. 1, pp. 111–117, 2002.

[77] F. Rodriguez de Fonseca, P. Rubio, F. Menzaghi, et al., "Corticotropin-releasing factor (CRF) antagonist [D-Phe12,Nle21,38,CαMeLeu37]CRF attenuates the acute actions of the highly potent cannabinoid receptor agonist HU-210 on defensive-withdrawal behavior in rats," *Journal of Pharmacology and Experimental Therapeutics*, vol. 276, no. 1, pp. 56–64, 1996.

[78] D. Giuliani, F. Ferrari, and A. Ottani, "The cannabinoid agonist HU 210 modifies rat behavioural responses to novelty and stress," *Pharmacological Research*, vol. 41, no. 1, pp. 47–53, 2000.

[79] C. Arévalo, R. De Miguel, and R. Hernández-Tristán, "Cannabinoid effects on anxiety-related behaviours and hypothalamic neurotransmitters," *Pharmacology Biochemistry and Behavior*, vol. 70, no. 1, pp. 123–131, 2001.

[80] S. Marín, E.-M. Marco, M. Biscaia, et al., "Involvement of the κ-opioid receptor in the anxiogenic-like effect of CP 55,940 in male rats," *Pharmacology Biochemistry and Behavior*, vol. 74, no. 3, pp. 649–656, 2003.

[81] R. F. Genn, S. Tucci, E.-M. Marco, M.-P. Viveros, and S. E. File, "Unconditioned and conditioned anxiogenic effects of the cannabinoid receptor agonist CP 55,940 in the social interaction test," *Pharmacology Biochemistry and Behavior*, vol. 77, no. 3, pp. 567–573, 2004.

[82] M. N. Hill and B. B. Gorzalka, "Enhancement of anxiety-like responsiveness to the cannabinoid CB_1 receptor agonist HU-210 following chronic stress," *European Journal of Pharmacology*, vol. 499, no. 3, pp. 291–295, 2004.

[83] S. Patel, B. F. Cravatt, and C. J. Hillard, "Synergistic interactions between cannabinoids and environmental stress in the activation of the central amygdala," *Neuropsychopharmacology*, vol. 30, no. 3, pp. 497–507, 2005.

[84] F. Chaperon and M.-H. Thiébot, "Behavioral effects of cannabinoid agents in animals," *Critical Reviews in Neurobiology*, vol. 13, no. 3, pp. 243–281, 1999.

[85] I. S. McGregor, C. N. Issakidis, and G. Prior, "Aversive effects of the synthetic cannabinoid CP 55,940 in rats," *Pharmacology Biochemistry and Behavior*, vol. 53, no. 3, pp. 657–664, 1996.

[86] S. Maione, T. Bisogno, V. de Novellis, et al., "Elevation of endocannabinoid levels in the ventrolateral periaqueductal grey through inhibition of fatty acid amide hydrolase affects descending nociceptive pathways via both cannabinoid receptor type 1 and transient receptor potential vanilloid type-1 receptors," *Journal of Pharmacology and Experimental Therapeutics*, vol. 316, no. 3, pp. 969–982, 2006.

[87] F. A. Moreira, D. C. Aguiar, and F. S. Guimarães, "Anxiolytic-like effect of cannabinoids injected into the rat dorsolateral periaqueductal gray," *Neuropharmacology*, vol. 52, no. 3, pp. 958–965, 2007.

[88] E. Sulcova, R. Mechoulam, and E. Fride, "Biphasic effects of anandamide," *Pharmacology Biochemistry and Behavior*, vol. 59, no. 2, pp. 347–352, 1998.

[89] J. Haller, N. Bakos, M. Szirmay, C. Ledent, and T. F. Freund, "The effects of genetic and pharmacological blockade of the CB_1 cannabinoid receptor on anxiety," *European Journal of Neuroscience*, vol. 16, no. 7, pp. 1395–1398, 2002.

[90] L. Urigüen, S. Pérez-Rial, C. Ledent, T. Palomo, and J. Manzanares, "Impaired action of anxiolytic drugs in mice deficient in cannabinoid CB_1 receptors," *Neuropharmacology*, vol. 46, no. 7, pp. 966–973, 2004.

[91] J. Haller, B. Varga, C. Ledent, I. Barna, and T. F. Freund, "Context-dependent effects of CB_1 cannabinoid gene disruption on anxiety-like and social behaviour in mice," *European Journal of Neuroscience*, vol. 19, no. 7, pp. 1906–1912, 2004.

[92] M. Navarro, E. Hernández, R. M. Muñoz, et al., "Acute administration of the CB_1 cannabinoid receptor antagonist SR 141716A induces anxiety-like responses in the rat," *NeuroReport*, vol. 8, no. 2, pp. 491–496, 1997.

[93] I. S. McGregor, F. N. Dastur, R. A. McLellan, and R. E. Brown, "Cannabinoid modulation of rat pup ultrasonic vocalizations," *European Journal of Pharmacology*, vol. 313, no. 1-2, pp. 43–49, 1996.

[94] R. J. Rodgers, J. Haller, J. Halasz, and E. Mikics, "'One-trial sensitization' to the anxiolytic-like effects of cannabinoid receptor antagonist SR141716A in the mouse elevated plus-maze," *European Journal of Neuroscience*, vol. 17, no. 6, pp. 1279–1286, 2003.

[95] J.-P. Després, A. Golay, L. Sjöström, et al., "Effects of rimonabant on metabolic risk factors in overweight patients with dyslipidemia," *The New England Journal of Medicine*, vol. 353, no. 20, pp. 2121–2134, 2005.

[96] L. F. Van Gaal, A. M. Rissanen, A. J. Scheen, et al., "Effects of the cannabinoid-1 receptor blocker rimonabant on weight reduction and cardiovascular risk factors in overweight patients: 1-year experience from the RIO-Europe study," *The Lancet*, vol. 365, no. 9468, pp. 1389–1397, 2005.

[97] F. X. Pi-Sunyer, L. J. Aronne, H. M. Heshmati, et al., "Effect of rimonabant, a cannabinoid-1 receptor blocker, on weight and cardiometabolic risk factors in overweight or obese patients. RIO-North America: a randomized controlled trial," *Journal of the American Medical Association*, vol. 295, no. 7, pp. 761–775, 2006.

[98] S. Kathuria, S. Gaetani, D. Fegley, et al., "Modulation of anxiety through blockade of anandamide hydrolysis," *Nature Medicine*, vol. 9, no. 1, pp. 76–81, 2003.

[99] S. Gaetani, V. Cuomo, and D. Piomelli, "Anandamide hydrolysis: a new target for anti-anxiety drugs?" *Trends in Molecular Medicine*, vol. 9, no. 11, pp. 474–478, 2003.

[100] S. Patel and C. J. Hillard, "Pharmacological evaluation of cannabinoid receptor ligands in a mouse model of anxiety: further evidence for an anxiolytic role for endogenous cannabinoid signaling," *Journal of Pharmacology and Experimental Therapeutics*, vol. 318, no. 1, pp. 304–311, 2006.

[101] S. M. Rawls, Z. Ding, and A. Cowan, "Role of TRPV1 and cannabinoid CB_1 receptors in AM 404-evoked hypothermia in rats," *Pharmacology Biochemistry and Behavior*, vol. 83, no. 4, pp. 508–516, 2006.

[102] A. O. Hamm and A. I. Weike, "The neuropsychology of fear learning and fear regulation," *International Journal of Psychophysiology*, vol. 57, no. 1, pp. 5–14, 2005.

[103] F. Sotres-Bayon, C. K. Cain, and J. E. LeDoux, "Brain mechanisms of fear extinction: historical perspectives on the contribution of prefrontal cortex," *Biological Psychiatry*, vol. 60, no. 4, pp. 329–336, 2006.

[104] K. M. Myers and M. Davis, "Mechanisms of fear extinction," *Molecular Psychiatry*, vol. 12, no. 2, pp. 120–150, 2007.

[105] M. Davis, K. M. Myers, J. P. Chhatwal, and K. J. Ressler, "Pharmacological treatments that facilitate extinction of fear: relevance to psychotherapy," *NeuroRx*, vol. 3, no. 1, pp. 82–96, 2006.

[106] J. P. Chhatwal, M. Davis, K. A. Maguschak, and K. J. Ressler, "Enhancing cannabinoid neurotransmission augments the extinction of conditioned fear," *Neuropsychopharmacology*, vol. 30, no. 3, pp. 516–524, 2005.

[107] H.-C. Lin, S.-C. Mao, and P.-W. Gean, "Effects of intra-amygdala infusion of CB_1 receptor agonists on the reconsolidation of fear-potentiated startle," *Learning and Memory*, vol. 13, no. 3, pp. 316–321, 2006.

[108] S. M. Hölter, M. Kallnik, W. Wurst, G. Marsicano, B. Lutz, and C. T. Wotjak, "Cannabinoid CB_1 receptor is dispensable for memory extinction in an appetitively-motivated learning task," *European Journal of Pharmacology*, vol. 510, no. 1-2, pp. 69–74, 2005.

[109] F. Niyuhire, S. A. Varvel, A. J. Thorpe, R. J. Stokes, J. L. Wiley, and A. H. Lichtman, "The disruptive effects of the CB_1 receptor antagonist rimonabant on extinction learning in mice are task-specific," *Psychopharmacology*, vol. 191, no. 2, pp. 223–231, 2007.

[110] K. Kamprath and C. T. Wotjak, "Nonassociative learning processes determine expression and extinction of conditioned fear in mice," *Learning and Memory*, vol. 11, no. 6, pp. 770–786, 2004.

[111] K. Kamprath, G. Marsicano, J. Tang, et al., "Cannabinoid CB_1 receptor mediates fear extinction via habituation-like processes," *The Journal of Neuroscience*, vol. 26, no. 25, pp. 6677–6686, 2006.

[112] A. Siegmund and C. T. Wotjak, "A mouse model of posttraumatic stress disorder that distinguishes between conditioned and sensitised fear," *Journal of Psychiatric Research*, vol. 41, no. 10, pp. 848–860, 2007.

[113] M.-P. Viveros, E.-M. Marco, and S. E. File, "Endocannabinoid system and stress and anxiety responses," *Pharmacology Biochemistry and Behavior*, vol. 81, no. 2, pp. 331–342, 2005.

[114] M. Martin, C. Ledent, M. Parmentier, R. Maldonado, and O. Valverde, "Involvement of CB_1 cannabinoid receptors in emotional behaviour," *Psychopharmacology*, vol. 159, no. 4, pp. 379–387, 2002.

[115] M. N. Hill and B. B. Gorzalka, "Is there a role for the endocannabinoid system in the etiology and treatment of melancholic depression?" *Behavioural Pharmacology*, vol. 16, no. 5-6, pp. 333–352, 2005.

[116] R. McArthur and F. Borsini, "Animal models of depression in drug discovery: a historical perspective," *Pharmacology Biochemistry and Behavior*, vol. 84, no. 3, pp. 436–452, 2006.

[117] M. N. Hill and B. B. Gorzalka, "Pharmacological enhancement of cannabinoid CB_1 receptor activity elicits an antidepressant-like response in the rat forced swim test," *European Neuropsychopharmacology*, vol. 15, no. 6, pp. 593–599, 2005.

[118] G. Gobbi, F. R. Bambico, R. Mangieri, et al., "Antidepressant-like activity and modulation of brain monoaminergic transmission by blockade of anandamide hydrolysis," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 102, no. 51, pp. 18620–18625, 2005.

[119] G. A. Elder, R. De Gasperi, and M. A. Gama Sosa, "Research update: neurogenesis in adult brain and neuropsychiatric disorders," *Mount Sinai Journal of Medicine*, vol. 73, no. 7, pp. 931–940, 2006.

[120] J. L. Warner-Schmidt and R. S. Duman, "Hippocampal neurogenesis: opposing effects of stress and antidepressant treatment," *Hippocampus*, vol. 16, no. 3, pp. 239–249, 2006.

[121] W. Jiang, Y. Zhang, L. Xiao, et al., "Cannabinoids promote embryonic and adult hippocampus neurogenesis and produce anxiolytic- and antidepressant-like effects," *Journal of Clinical Investigation*, vol. 115, no. 11, pp. 3104–3116, 2005.

[122] M. N. Hill, J. S. Kambo, J. C. Sun, B. B. Gorzalka, and L. A. M. Galea, "Endocannabinoids modulate stress-induced suppression of hippocampal cell proliferation and activation of defensive behaviours," *European Journal of Neuroscience*, vol. 24, no. 7, pp. 1845–1849, 2006.

[123] B. L. Mason and C. M. Pariante, "The effects of antidepressants on the hypothalamic-pituitary-adrenal axis," *Drug News & Perspectives*, vol. 19, no. 10, pp. 603–608, 2006.

[124] M. N. Hill, W.-S. V. Ho, K. J. Sinopoli, V. Viau, C. J. Hillard, and B. B. Gorzalka, "Involvement of the endocannabinoid system in the ability of long-term tricyclic antidepressant treatment to suppress stress-induced activation of the hypothalamic-pituitary-adrenal axis," *Neuropsychopharmacology*, vol. 31, no. 12, pp. 2591–2599, 2006.

[125] G. Perea and A. Araque, "Glial calcium signaling and neuron-glia communication," *Cell Calcium*, vol. 38, no. 3-4, pp. 375–382, 2005.

[126] N. Stella, "Cannabinoid signaling in glial cells," *GLIA*, vol. 48, no. 4, pp. 267–277, 2004.

[127] M. R. Pazos, E. Núñez, C. Benito, R. M. Tolón, and J. Romero, "Functional neuroanatomy of the endocannabinoid system," *Pharmacology Biochemistry and Behavior*, vol. 81, no. 2, pp. 239–247, 2005.

[128] C. Benito, W.-K. Kim, I. Chavarría, et al., "A glial endogenous cannabinoid system is upregulated in the brains of macaques with simian immunodeficiency virus-induced encephalitis," *The Journal of Neuroscience*, vol. 25, no. 10, pp. 2530–2536, 2005.

[129] I. Galve-Roperh, T. Aguado, D. Rueda, G. Velasco, and M. Guzmán, "Endocannabinoids: a new family of lipid mediators involved in the regulation of neural cell development," *Current Pharmaceutical Design*, vol. 12, no. 18, pp. 2319–2325, 2006.

[130] M. van der Stelt and V. Di Marzo, "The endocannabinoid system in the basal ganglia and in the mesolimbic reward system: implications for neurological and psychiatric disorders," *European Journal of Pharmacology*, vol. 480, no. 1–3, pp. 133–150, 2003.

[131] M.-P. Viveros, R. Llorente, E. Moreno, and E.-M. Marco, "Behavioural and neuroendocrine effects of cannabinoids in critical developmental periods," *Behavioural Pharmacology*, vol. 16, no. 5-6, pp. 353–362, 2005.

[132] L. Arseneault, M. Cannon, J. Witton, and R. M. Murray, "Causal association between cannabis and psychosis: examination of the evidence," *The British Journal of Psychiatry*, vol. 184, pp. 110–117, 2004.

Dr David Bearman 52

ADHD and the Endocannabinoid System

Several physicians who recommend/approve medicinal cannabis as an important part of their medical practice have seen/noticed an increasing the number of patients coming in who have been diagnosed with Attention Deficit Disorder (ADD) or Attention Deficit with Hyperactivity Disorder (ADHD) or who they diagnose as ADD/ADHD.

What is meant by these diagnoses.

• What is ADHD?

These diagnoses don't correspond to any recognized pathology. For example, if we say somebody has appendicitis, we can look at the removed appendix under the microscope and see some specific changes like a lot of a certain type of cells that create an inflammatory response. Somebody with asthma will have certain easily identifiable changes in their lungs, etc. A person with ADD/ADHD does not have any such changes as far as we know. What ADD/ADHD have is a certain group of symptoms, like difficulty concentrating, hyperactivity, behavior issues, etc. also appears to be a deficiency in free dopamine.

The cause of ADHD is unknown, although scientists feel that at least 50% of cases have a genetic component. Also studies show that people with ADHD have 70% more dopamine transporters, thereby decreasing the availability of free dopamine.

Attention Deficit Hyperactivity Disorder (ADHD), was formerly called hyperkinesis or minimal brain dysfunction. It is described as a chronic, neurologically based syndrome characterized by any or all of three types of behavior: hyperactivity, distractibility, and impulsivity. Hyperactivity refers to feelings of restlessness, fidgeting, or inappropriate activity (running, wandering) when one is expected to be quiet; distractibility to heightened distraction by irrelevant sights and sounds or carelessness and inability to carry simple tasks to completion; and impulsivity to socially inappropriate speech (e.g., blurting out something without thinking) or striking out. Unlike similar behaviors caused by emotional problems or anxiety, ADHD does not fluctuate with emotional states. While the three typical behaviors occur in nearly everyone from time to time, in those with ADHD they are excessive, long-term, and pervasive and create difficulties in school, at home, or at work. ADHD is usually diagnosed before age seven. It is often accompanied by a learning disability. More recently there has also been described adult ADHD.

• Treatment

While we strongly suspect that there is an important role for dopamine dysfunction (probably to little but a few researchers say too much), we don't really know the specific cause of the ADD/ADHD symptoms, and there is no routine test for dopamine levels, so the diagnosis becomes what we call a "diagnosis of exclusion." That is, we make sure the person does not have some other identifiable condition, such as depression, learning

disabilities or a physical problem. If they don't, and have a certain number of symptoms from a predefined list, we label them with ADD/ADHD and give a drug that tends to make them a bit more manageable. The accepted drug treatments tell that Ritalin cures nothing but in many cases many make the patient more manageable.

Conventional ADD treatment usually includes behavioral therapy and emotional counseling combined with sympathominetic medications (drugs that stimulate the sympathetic nervous system), such as methyphenidate hydrochloride (Ritalin) or dextroamphetamine (Dexedrine), Atomoxetine (Strattera), Amphetamine mixture (Adderal) or long-acting methylphenidate (e.g., Metadate LD, Concerta, Ritalin LA), that in many cases make the patient more manageable. They also have many unacceptable side effects.

Cannabis Safety

The first dictum of medicine is "first, do no harm." Another bedrock principal is that a prescriber must balance off the side effects of the treatment with the benefits. With ADD, the use of Ritalin and other stimulants has been routinely and repeatedly criticized because of their side effects profile. Not surprising all of my patients with ADD have been critical of either the side effects of these drugs, the lack of effectiveness or both. They have found cannabis to be both more effective and have far fewer side effects.

No less an authority than DEA Administrative Law Judge, Francis L. Young, in 1988, after a two-year hearing to reschedule cannabis said:

> "Nearly all medicines have toxic, potentially lethal effects. But marijuana is not such a substance. There is no record in the extensive medical literature describing a proven, documented cannabis-induced fatality ... Simply stated, researchers have been unable to give animals enough marijuana to induce death .. In practical terms, marijuana cannot induce a lethal response as a result of drug-related toxicity ... In strict medical terms marijuana is far safer than many foods we commonly consume ... Marijuana, in its natural form, is one of the safest therapeutically active substances known to man."

When a medication gives you a symptom that you did not want, we call that symptom a "side effect." When it comes to treatment of ADHD for many, cannabinoids have far fewer and less annoying side effects than the stimulants that are often used to treat AD/HD and other conditions. The most common stimulants are methyphenidate (Ritalin, Concerta, Metadate-ER) and amphetamine (Dexedrine, Dexedrine Spansules, Adderall). Some individuals who take stimulants experience mild problems, some much more significant, unpleasant side effects. Some are simply unable to tolerate stimulants. Many people simply stop their prescribed stimulant medication instead of working with their physician to find a way to decrease side effects. Cannabinoids offer another viable option.

What is Association? What is Case and What is Self-Treatment?
A study reported in Clinician Reviews in 2000 entitled "Treating ADHD May Prevent Substance Abuse" found that:

> "... untreated ADHD presents a significant risk factor for Substance Use Disorder (SUD) in adolescence, whereas treating ADHD may reduce this risk." (NOTE: I have no idea why substance use - as opposed to substance abuse- is a disorder.) At any rate, the authors "point to previous studies in which they found ADHD-SUD associations in adults with ADHD who had never been diagnosed or treated as children. Further examination is necessary in order to evaluate the risk factors for girls and nonwhite boys. However, these findings may reduce apprehension in treating children who have ADHD and promote earlier intervention. This, in turn, may prevent the academic, psychiatric, and interpersonal complications of ADHD in adolescents, and subsequently, in adults.

In some cases where Ritalin is ineffective or unacceptable, cannabis has been found to be helpful.

• Cannabis Studies

Much of the evidence about the medicinal use of cannabis is anecdotal, however that is changing. On 11/19/2000 Daniel Q. Haney of the Associated Press wrote:

> "Maybe the smoke is about to clear in the debate over medical marijuana. Few ideas, it seems, are so firmly held by the public and so doubted by the medical profession as the healing powers of pot. But at last, researchers are tiptoeing into this field, hoping to prove once and for all whether marijuana really is good medicine.
> "To believers, marijuana's benefits are already beyond discussion: Pot eases pain, settles the stomach, builds weight and steadies spastic muscles. "And that's hardly the beginning. They speak of relief from MS, glaucoma, itching, insomnia, arthritis, depression, childbirth, attention deficit disorder and ringing in the ears.
>
> Marijuana is a powerful and needed medicine, they say, tragically withheld by misplaced phobia about drug addiction."

In his article, Haney points out that some are not impressed with these anecdotal reports stretching over centuries funding. However the State of California has founded the Center for Medicinal Cannabis Research, headquartered at UCSD has several million dollars to scientifically study cannabis' medical efficacy. There are 18 FDA-approved studies at UCI, UCSF, UC Davis, and UCSD School of Medicine. In Haney's words:

> "Pot has many effects on the body, including some that are probably worthwhile. But does it substantially relieve human suffering, they ask? And if so, is it any better than medicines already in drugstores?
>
> "For the first time in at least two decades, marijuana the medicine is being put to the test. Scientists say they will try to hold marijuana to the same standard as any other drug, to settle whether its benefits match its mystique.
>
> "One way to buff up a pharmaceuticals' raffish image -- especially one that's a drug in more than one sense of the word -- is to call it something else. When the University of California at San Diego started the country's first institute to study the medical uses of marijuana this year, they named it the Center for Medicinal Cannabis Research. Cannabis is the botanical term for pot.
>
> "We talked about it a lot," says Dr. Igor Grant, the psychiatrist who heads the new center. "Marijuana is such a polarizing name. We don't want this institute to be caught in the crossfire between proponents and antagonists. Ultimately, if cannabis drugs become medicine, they will almost certainly be known by that name, not marijuana."

The Center for Medicinal Cannabis Research appears to be living up to its expectations. It was authorized to give out $9 million to California researchers over the three years from 2000-2003. This has been enough funding to underwrite 18 NIDA/FDA-approved studies. At least one or two are looking at cannabis and ADHD.

Research Implications
Here's my take on the implication of the brain studies related to cannabinoids.

• Cannabinoid Receptors
There are two cannabdinoid receptors in the body – CB_1 located in the brain, and CB_2 in the periphery. For CB_1 there are two natural ligands in the body anandamide (arachidonyl ethanolamide) and 2-AG (2 arachidonyl glyceride). For CB_2 receptors, palmitylethanolamide is the natural ligand.

• Cannabis/Tetrahydrocannabinol
There are over 400 different chemicals in marijuana, about 66 of which are known as cannabinoids. These chemicals are found nowhere else in nature. The most pharmacologically important cannabinoid in marijuana is known as delta-9-tetrahydrocannabinol (9 THC). It binds to CB_1 receptors (G-protein-coupled receptors) that are present on presynaptic membranes in several parts of the brain. THC is the main psychoactive (mind-altering) ingredient in marijuana. It is clear though that several other cannabinoids and possibly other chemical components of the plant have therapeutic value. These plant cannabinoids can stimulate the body's endocannabinoid system.

• Endocannabinoid System

Cannabinoid CB(1) receptors are highly localized in the central nervous system. A 2000 report of the work of Martin, Ledent, et.al. in the February 2002 issue of Psycho Pharmacology concluded that endogenous cannabinoids, through the activation of CB1 receptors, are implicated in the control of emotional behavior and participate in the physiological processes of learning and memory.

The highest concentration of CB1 THC receptors in the brain are found in the hippocampus (where memory is formed), cerebellum (deals with coordinating movements and balance), the striatum, amygdala (emotion), cerebral cortex (higher centers of reasoning) and the basal ganglia. An important class of neurons that express high levels of CB(1) receptors are GABAergic interneurons in the hippocampus, amygdala and cerebral cortex. They may act as retrograde synaptic mediators (inhibitors of neuronal transmission). This phenomena is caused by the depolarization-induced suppression of inhibition or excitation in hippocampus and cerebellum. In plain English, cannabinoids may help treat ADHD by decreasing both the amount and speed of sensory input to the midbrain and forebrain. The endocannabinoid system represents a mechanism by which neurons can communicate backwards across synapses to modulate/decrease their inputs. Increased free dopamine released by cannabinoids stimulates increased dopamine in the intasynaptic cleft. This anandamide stimulation can release dopamine and from the synaptic endplate. This can depolarize that nerve, making it harder to stimulate the nerves stops the brain from being inundated from both internal and external stimuli.

Cannabinoid receptors are co-localized with dopamine receptors suggesting that cannabinoids influence the dopaminergic processes. They probably can release dopamine which influences neurons to depolarize thereby making it harder to stimulate them. With fewer inputs to the higher centers of thinking and with the neural input being slower, it makes it that much easier for the patient to focus and process these thoughts.

• Cannabinoids

It is possible that, in part at least, cannabis' effects are due to the cannabinoids, a major nonpsychotropic constituent of cannabis. It was recently discovered that the cannabinoids (as opposed to THC) effect the inhibition of anandamide uptake.

• Prefrontal Cortex (PFC) and Midbrain

The prefrontal cortex (PFC) is essential for control of attention, organization and planning. Lesions to the PFC in humans can produce distractibility, hyperactivity, and impulsivity (Stuss, Eskes, & Foster, 1994). The PFC projects to many subcortical (e.g., lower down in the brain) regions, including the dorsal and ventral striatum, thalamus, amygdala, substantia nigra, and ventral tegmental area (Alexander, DeLong, & Strick, 1986) all areas with high concentration of THC receptors. The motor dysregulation characteristic of ADHD and neuroimaging data suggest that dysfunction in the striatum or

in the cortical regulation of striatum is involved in the pathophysiology of ADHD. This dysregulation may be associated with lower than normal levels of free dopamine.

• Dopamine

The power for the getting neural impulses around the brain are the neurotransmittors. Neurotransmittors cross the synapse and stimulate receptors in the next neuron causing transmission of nerve impulses. Neurotransmitters include norepinephrine, serutonin, acetylcholine, dopamine and anandamide (the naturally occurring cannabinoid).

Catecholamines include Dopamine, Norepinephrine, and Epinephrine (adrenalin). They control the so-called adrenergic systems. Some of these neurons radiate from the limbic system and discharge neurotransmitters in a diffuse manner into the frontal cortex, i.e. into broad areas of brain tissue as opposed to delivering the chemical to specific synapses. They thus account for "global vigilance" (staying awake), mood, fight or flight response, etc. Dopamine also contributes to the feelings of bliss and regulates feeling of pain in the body. Chocolate, coffee, nicotine, THC and stress all have been found to increase Dopamine. Thorazine and Haldol block Dopamine action (less learning, remembering and motivation).

Attention-Deficit Disorder (ADD) and Attention-Deficit Hyperactivity Disorder (ADHD) patients probably have less free synaptic dopamine available than the norm. They may produce less dopamine than those who do not have this condition. Also a preliminary study reveals that adults with ADD/ADHD have 70 percent more dopamine transporters in their brains than normal subjects. These transporters tie up dopamine leaving less free dopamine available for neuro stimulation.

• Low Dopamine

In 2000 Grace proposed a model of dopaminergic dysfunction in ADHD at the cellular level that explain many of the symptoms of ADHD. He suggests that, possibly because of reduced stimulation from PFC, children with ADHD have low tonic dopaminergic activity. Low tonic stimulation of inhibitory autoreceptors produces high phasic activity in the nucleus accumbens, and possibly other subcortial sites as well, that may result in dysregulated motor and impulse control,

Dopamine receptor oversensitivity. I must confess I do not know what that is, also may cause the body to decrease the amount of dopamine being produced. A shortage of dopamine in the frontal lobe can contribute to poor working memory.

• Stimulants May Inhibit Dopamine Breakdown

There is strong evidence that the catecholamines dopamine and norepinephrine are important in the pathophysiology of ADHD, as well in the mechanism of therapeutic action of stimulant drugs. Because of the known effects of exogenous stimulants in blocking reuptake of catecholamines and (in the case of d-amphetamine) facilitating their release, it has traditionally been believed that the stimulants compensate for catecholamine deficiency in ADHD.

Hyperactivity, and possibly poor motor impulse control, in ADHD results from dopaminergic dysfunction in the limbic system. Some believe the cause is excessive dopaminergic activity. In this theory, stimulation of the presynaptic autoreceptors reduces phasic dopamine release. The striatum and/or nucleus accumbens are possibilities for places in the limbic system so affected. Stimulant drugs may reduce hyperactivity by reducing activation of the striatum, possibly through a mechanism that involves stimulation of inhibitory pre-synaptic autoreceptors.

By blocking dopamine reuptake, stimulants increase levels of dopamine in the extracellular space, thus increasing the stimulation of impulse-regulating presynaptic autoreceptors. This acts as an inhibitor of subsequent stimulation of these neurons. The increase in intrasynaptic dopamine thereby reduces the ability to stimulant neurons in the limbic system. This then can decrease hyperactivity and increase focus.

• Cannabinoids Regulate Neural Traffic

There are essentially two kinds of brain cells, according to Stanford University neuroscientist Dan Madison. There are the principal cells that make up what he likened to a superhighway system of long-range information movement, and there are "interneurons," which are like traffic signals along that highway.

"Cannabinoids are a way for the principal cells to regulate the traffic lights," Madison said. After two years of laboratory study and frustrating dead ends, Wilson and Nicoll found that the role of the brain's cannabis is to make the feedback system work. Harvard researchers, working independently, found an essentially identical role for endogenous cannabinoids in another part of the brain, called the cerebellum, which helps to control motor function.

"It's a way for a nerve cell to adjust the gain or intensity of the information coming into it," Nicoll said. "It turns up the amplifier, in a way, and allows more input to get through." Conversely of course one can turn down the gain and decrease the input which gets through.

These adjustments seem to have an important role in the brain's uncanny ability to synchronize the firing of nerve cells scattered throughout the brain linking behavior with mood and memory with vision or hearing. Thousands of signals thus become molded into vast oscillations, helping the brain bind together different aspects of perception into coherent state of mind -- a feeling of being in love, perhaps, when we look at someone.

• Retrograde Messenger System/Synaptic Inhibition

Experiments in the last few years have shown that in any neural circuits where *endocannabinoids* are present these endocannabinoids *may participate in a retrograde messenger system whose goal is presynaptic inhibition*. Endocannabinoids serve as the messengers in this system, and CB1 serves as the receptor that initiates the inhibition. This is especially important in signaling between neurons in the hippocampus, where

strengthening and weakening of neural connections, thereby reorganizing neural circuits, is thought to be a cellular correlate of learning and memory.

Cannabis appears to treat ADD and ADHD by increasing the availability of dopamine. This then has the same effect but is a different mechanism of action than stimulants like Ritalin (methylphenidate) and dexedrine (1) amphetamine which act by binding to the dopamine and interfering with the metabolic breakdown of dopamine. Cannabis (THC) is an anandamide agonist, that is it stimulates the anandamide (CB1) receptor sites.

Researchers working on Tourette's Syndrome (TS) favorable response to Δ^9-THC said: "neuroanatomical structures which are probably involved in TS pathology are heavily associated with the CB_1 receptor system. Considering an involvement of the dopamine system in TS pathophysiology it can be speculated that tic improvement might be caused by an interaction between cannabinoid and dopamine mechanisms. I believe that this is true for the rather closely related ADHD.

• Research

What does research say about cannabinoids controlling ADD or hyperactivity. An animal model study, published in the May 2000 issue of the Journal of Neuroscience, reports that synthetic compounds developed to block the way anandamide – the body's own cannabis-like compound or cannabinoid – is inactivated or broken down could correct forms of hyperactivity, such as attention deficit disorder.

Research by Dr. Daniel Piomelli at UCI also suggests a possible mechanism of action for cannabis in treating ADD. Dr. Piomelli, professor of pharmacology, led a team that found that a chemical called AM404 reversed the normal inactivation of a naturally occurring chemical in the brain called anandamide, which is related to marijuana's active ingredient and opposes or counteracts the actions of dopamine. According to Reuters article, Piomelli's study showed that: A chemical that boosts a marijuana-like substance in the brain may insure new treatments for brain disorders such as schizophrenia, Parkinson's disease, and attention-deficit/hyperactivity disorder (ADHD). (Note: Arachidonoyl ethanolamide (AEA) was the first endogenous cannabinoid to be isolated and characterized as an agonist acting on the same receptors (CB_1 and CB_2) as tetrahydrocannabinols (THC). This means that stimulating the anandamide receptors could effectively treat ADD.

Piomelli and his colleagues found that AM404 targeted nerves that produced unusually high levels of dopamine and caused exaggerated movements and other problems in rats. Instead of directly encouraging the production of dopamine-curbing anandamide, AM404 was found to discourage the disintegration of existing anandamide. More anandamide was then available to bind to receptors on nerve cells and reduce the stimulation of nerve cells by dopamine.

What I believe is happening is two things. One is that release of anandamide slows down the rate of neurotransmission. A second action from stimulating anandamide receptor

sites may be the stimulation Renshaw cells. Renshaw cells are in the midbrain and their neurons go downward in the brain. Their function is to turn off some of the cells which provide sensory input.

Piomelli's studies showed that by reversing the inactivation of anandamide, AM404 is able to gently curb the exaggerated movements and other disorders caused by too much dopamine activity in nerve cells. This position that too much dopamine is the problem is held by a minority of researchers in this field. Piomelli believes that in the case of Parkinson's disease, patients have too little dopamine, while people with ADHD, schizophrenia or Tourette's syndrome may have too much. His hope is that AM404 will lay the groundwork for a new class of drugs that either boost or block dopamine, without the side effects linked to current treatments.

Piomelli told Reuters Health in an interview. "Our results are interesting," he said, "because they show that you can modulate dopamine without acting on the dopamine system." Instead of directly encouraging the production of dopamine-curbing anandamide, AM404 was found to discourage the disintegration of existing anandamide. More anandamide was then available to bind to receptors on nerve cells and reduce the stimulation of nerve cells by dopamine.

Piomelli and his colleagues showed for the first time that in rats, anandamide naturally counters dopamine. Usually, though, anandamide is inactive in the brain. The California team's latest experiments in rats reveal that AM404 stops anandamide from being "drained from the brain," which allows it to suppress dopamine.

A UCI news release of May 1, 2000 states that:
"If further research proves successful, the chemical could be used to treat schizophrenia, Tourette's, Parkinson's, autism and attention-deficit disorder, all of which are currently treated by drugs that attack the dopamine system in the brain." Piomelli's research shows "you can modulate dopamine without acting on the dopamine system." These conditions are treated with drugs that affect the dopamine system. Piomelli points out that these existing treatments have side effects such as lethargy and impaired sexual activity. The potential for anandamide-boosting drugs to work against these disorders has some anecdotal backing. Anandamide's counterpart, marijuana, is used by many schizophrenics who report that it relieves their symptoms, Piomelli noted.

"But," he said, "we are not implying that marijuana is use for these conditions."

Piomelli is quoted by USA Today that, "Marijuana has a lot of pharmaceutical and pharmacological potential. The potential now is becoming very, very clear." Many decades-old prejudices are being lifted and that is reflected by the considerable funding that the federal government is giving to research marijuana.

Marijuana, according to Piomelli, is far less selective than anandamide in activating brain cells. Because pot smoking overstimulates the brain, he said, cells eventually become

desensitized to any benefits the drug initially brings. SOURCE: Journal of Neuroscience May 2000.

"I would be very surprised that if in the next 10 years there isn't an important new medicine developed from our better understanding of the cannabis system in the body," says Piomelli.

While Piomelli himself has discounted the use of cannabis for these disorders, this research clearly lays out a potential mechanism of action. An article by UCI staff writer Andreas Von Bubroff states that: "Anandamide is similar to marijuana's active ingredient, THC and belongs to a class of neurotransmitters called endogenous cannabinoids since it is naturally produced by some of the brain's nerve cells." It is known that cannabis is neuroprotective and in practice it has shown to provide relief for some epilepsy, Tourette's and some ADD sufferers. I myself have had several patients who have benefited from cannabis for ADD and with far fewer side effects than Ritalin.

Lastly, there is a six (6) page paper by Kurt E. Patterson discussing marijuana and ADD. In it he states that "There is some evidence available that medical marijuana has been found to be an effective medication for some types of ADD by other researchers in the field. (1) Unfortunately, ADD encompasses such a variety of conditions that the limited amount of research in the field leaves many of the effective therapeutic mechanisms under-investigated. Considering the regulatory difficulties in researching the effects of medical marijuana, it isn't surprising that the information regarding medical marijuana and ADD is largely anecdotal(2)."

• What does Proposition 215 mean?

It is difficult to parse the language where on the one hand the preamble talks about "serious illnesses" and on the other Proposition 215 gives a list of conditions including nausea, glaucoma, migraine, pain and then adds for "any other condition for which a physician feels that cannabis may be useful." An argument was made for a broad interpretation of 215 approvals and recommendations by no less authorities than the California DA's Association, California Sheriffs Association, and California Narcotics Officer's Association in their ballot argument against 215. They argued that if 215 passed, cannabis could be recommended for anything. A somewhat narrower reading is argued for by the brief of the CMA and concurred in by the 9th circuit court of appeals, arguing that 215 protects a physician's first amendment right to communication with his patient and the patient's right to hear what medications, be they prescription, herbal, vitamins or alternatives and complementing medicine may be helpful for their medical condition.

ADD has been termed a serious enough condition that hundreds of thousands of school children are treated with (some would say subjected to) the not so benign medication Ritalin. ADD as well as ADHD and adult ADD or ADHD have been shown to be very disruptive on people's lives – their self-esteem, their ability to succeed in life, their ability to do well in school. There are numerous websites on ADD and ADHD which assert that

this is a serious condition. ADD and ADHD then qualify under a reasonable interpretation of either a narrow or broad interpretation of 215.

Several physicians in California who regularly made 215 recommendation (Dr. Frank Lucido, Dr. Tom O'Connell, Dr. Tod Mikuriya) have indicated that they have made many recommendations for the medical use of cannabis to treat ADD. Dr. Lester Grinspoon, Emeritus Professor of Psychiatry at Harvard School of Medicine and author of Marijuana, The Forbidden Medicine, has a website which lists anecdotal reports of the medicinal benefits of cannabis. Out of a sample of 25 displayed anecdotes to his website 3, or 12% were describing cannabis' benefits for treatment of ADD. A March 5, 2002, a 48-Hours TV program chronicled the effectiveness of a medical recommendation for the treatment of ADD in an eight year old child. A California court determined that this constituted an appropriate treatment for this child. The book Jeffries Journey discusses this case.

There is overwhelming anecdotal evidence regarding the benefit in treating ADD and ADHD with cannabinoids but also that many view ADD as a serious condition. Further, it is clear that the FDA, at least in the person of Administrative Law Judge Francis Young, has found that marijuana is safer than Ritalin.

Chapter 6

The Role of the Endocannabinoid System in the Central Nervous System

Pivotal studies over the past 2 decades firmly established the presence and distribution of cannabinoid CB_1 receptors, endocannabinoids, and their metabolic enzymes in the brain,[1-7] thereby confirming the presence of the endocannabinoid system (ECS) in the central nervous system (CNS). In 1988, Howlett et al[8] described the presence of high-affinity binding sites for cannabinoids in rat brain membranes. Shortly afterwards, Herkenham et al[9] performed autoradiographic mapping studies of cannabinoid binding sites in rat, human, rhesus monkey, dog, and guinea pig brain.[9] Matsuda et al cloned the CB_1 receptor[10] and determined its distribution by in situ hybridization studies in rat brain.[11] Determining the location of CB_1 receptors in the brain has provided significant insight into the function of the ECS in the CNS (Table 1).[12]

Table 1. CB_1 Receptors in the Central Nervous System

Structure	Function
Hippocampus	Cognition and encoding memory
Cerebellum	Coordination of motor function, posture, balance
Basal ganglia	Motor function, reinforcement behaviors
Hypothalamus	Thermal regulation, neuroendocrine regulation, appetite
Spinal cord	Nociception
Brain stem	Nausea and emesis, appetite
Cerebral cortex	Cognition, emesis
Prefrontal cortex	Executive function, reinforcement

Adapted from Croxford, 2003.[12]

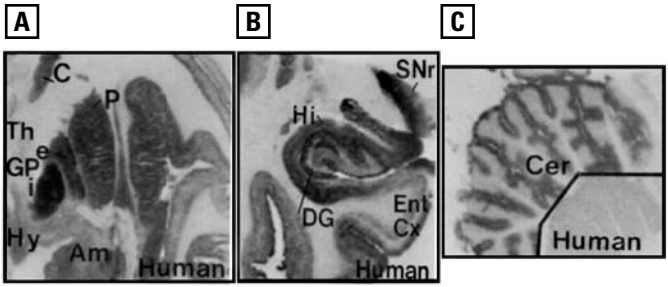

Figure 1. Coronal sections of human brain showing autoradiography of [^{3}H]CP-55940 binding. [^{3}H]CP-55940 is a radiolabeled synthetic cannabinoid used to visualize the distribution of cannabinoid receptors (predominantly CB_1 receptors in the brain). Gray levels represent relative levels of receptor densities. (A) C, caudate; P, putamen; TH, thalamus; GP, caudate-putamen (e, external; i, internal); Hy, hypothalamus; Am, amygdala (x 1.3 magnification). (B) SNr, substantia nigra pars reticulata; Hi, hippocampus; DG, dentate gyrus; Ent Cx, entorhinal cortex (x 1.7 magnification). (C) Cer, cerebellum (x 2.6 magnification). Reproduced with permission.[9]

Cell Biology

CB_1 receptors are among the most abundant receptors in the brain.[13] In the brain areas with the highest levels of CB_1 receptors, their density is similar to levels of γ-aminobutyric acid- (GABA) and glutamate-gated ion channels.[14] [^{35}S]GTPγS autoradiography studies in rat brain demonstrate that the distribution of cannabinoid-activated G proteins, in general, parallels CB_1 receptor mRNA expression and binding.[14] [^{3}H]CP-55940 autoradiography studies demonstrate the presence of CB_1 receptors in human brain (Figure 1). The extent of CB_2 expression in brain is controversial. However, expression of CB_2 receptor mRNA and protein were recently demonstrated in rat and ferret brainstem neurons.[15] Determining the extent of CB_2 receptors in the brain is an active area of investigation. An interesting aspect of CB_2 receptors is that in many cases their levels strongly increase following a pathological insult. For example, CB_2 receptor expression is induced in brain microglial cells during inflammation.[16]

An important role for CB_1 receptors in the brain is to mediate retrograde signaling. This is defined as the communication by signaling molecules (in this case, endocannabinoids) derived from postsynaptic and delivered to presynaptic structures (opposite to the direction of travel of conventional neurotransmitters) (Figure 2).[17-19] Substantial evidence demonstrates that retrograde signaling underlies a variety of short- and long-term changes in synaptic efficacy.[18]

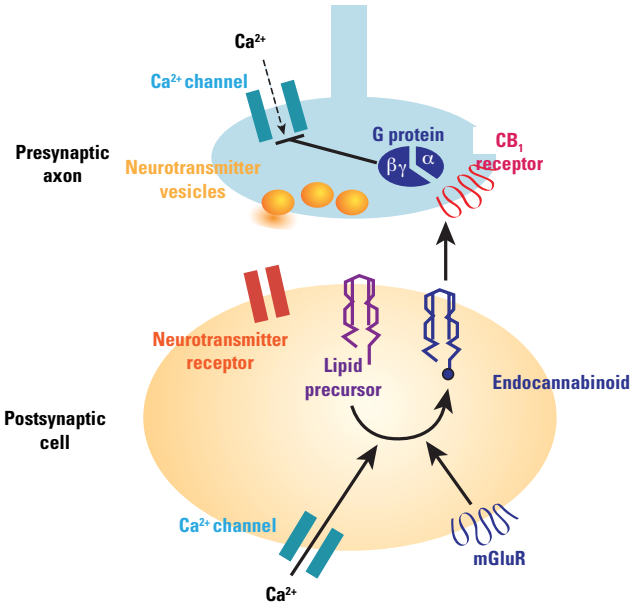

Figure 2. Retrograde signaling by endocannabinoids in the brain. Postsynaptic depolarization opens voltage-dependent Ca^{2+} channels. The influx of Ca^{2+} through these channels activates the enzymes that synthesize endocannabinoids from arachidonic acid-containing membrane phospholipids. Activation of postsynaptic group I metabotropic glutamate receptors (mGluRs) can also generate endocannabinoids. Endocannabinoids then leave the postsynaptic cell and activate CB_1 receptors located in the presynaptic cell membrane. G-protein activation liberates Gbg which inhibits Ca^{2+} influx. This decreases the probability of release of a vesicle of neurotransmitter. Reproduced with permission.[13]

CB_1 receptors are localized mainly on axons and axon terminals where their stimulation is directly coupled to inhibition of certain voltage-activated Ca^{2+} channels.[20,21] Endocannabinoid activation of CB_1 receptors also opens somatic potassium (K^+) channels, and the resulting hyperpolarization inhibits neuronal firing.[22] Thus, inhibition of Ca^{2+} channels and stimulation of K^+ channels both contribute to inhibition of neuronal excitability and suppression of neurotransmitter release.[20] CB_1 receptor activation attenuates GABA and glutamate release from CB_1 receptor–containing nerve terminals.[21] Endocannabinoid-mediated activation of CB_1 receptors on nerve terminals is widespread in the brain. It inhibits neurotransmitter release in many brain regions including the striatum, hippocampus, cerebellum, cortex, hypothalamus, and nucleus accumbens.[23] In addition to inhibiting glutamate and GABA release, CB_1 receptor activation also inhibits serotonin and acetylcholine release at other synapses and inhibits release of neuropeptides.[21,24]

It is likely that some of the CNS effects of anandamide occur through a complex interplay with other systems (see Chapter 2). For example, high concentrations of anandamide activate the transient receptor potential vanilloid (TRPV1) receptor (ion channel found on sensory neurons) causing Ca^{2+} influx and subsequent neurotransmitter release.[25]

Roles of the ECS in the Central Nervous System

Appetite

Ample preclinical data suggest that CB_1 receptor stimulation in the CNS facilitates feeding. For example, short-term food deprivation in rats leads to elevated levels of brain endocannabinoids (see Chapter 3, Figure 1),[26] and administration of 2-AG into the shell subregion of the nucleus accumbens (a limbic forebrain area implicated in eating motivation) induces short-term hyperphagia (abnormally increased consumption of and appetite for food).[26]

CB_1 receptor signaling is intimately involved in several forms of neuronal plasticity; that is, the ability of nerve cells to change their properties for

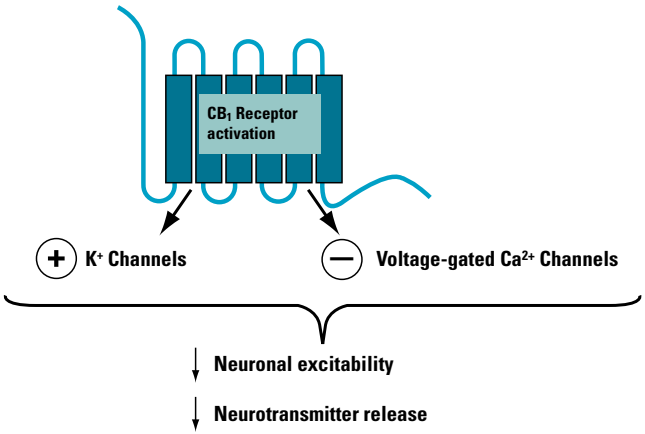

Figure 3. Inhibition of Ca^{2+} channels and stimulation of K^+ channels both contribute to inhibition of neuronal excitability and suppression of neurotransmitter release.[20] CB_1 receptor activation leads to inhibition of voltage-gated Ca^{2+} channels, which may be involved in decreasing Ca^{2+} influx, glutamate release, and subsequent excitotoxic progression. CB_1 receptor activation also inhibits adenylyl cyclase activity and its downstream signaling pathways (see Chapter 2), which may play a role in the therapeutic effects of cannabinoid agonists. K^+ channel-induced hyperpolarization of the cell with the subsequent decrease neurotransmitter release may also be involved in neuroprotection.[22] Adapted from Karanian and Bahr.[80]

example by making new synapses or altering the strength of existing synapses. Energy balance is regulated by a complex interaction of neural signals emanating from the brain, as well as hormones from peripheral organs that act on the brain and other tissues (see Chapter 3). For example, the adipocyte-derived protein, leptin, reduces food intake by activating leptin receptors in the hypothalamus.[27] One influence of leptin on endocannabinoid levels appeared to occur in the hypothalamus, as levels of hypothalamic anandamide and 2-AG decreased in normal rats treated with intravenous leptin (vs rats treated with control solution). Hypothalamic levels of anandamide and 2-AG increased in mice with disrupted leptin signaling (*db/db* mice).[28] In contrast, levels of endocannabinoids in the cerebellum did not differ between *db/db* mice and wild-type mice.[28]

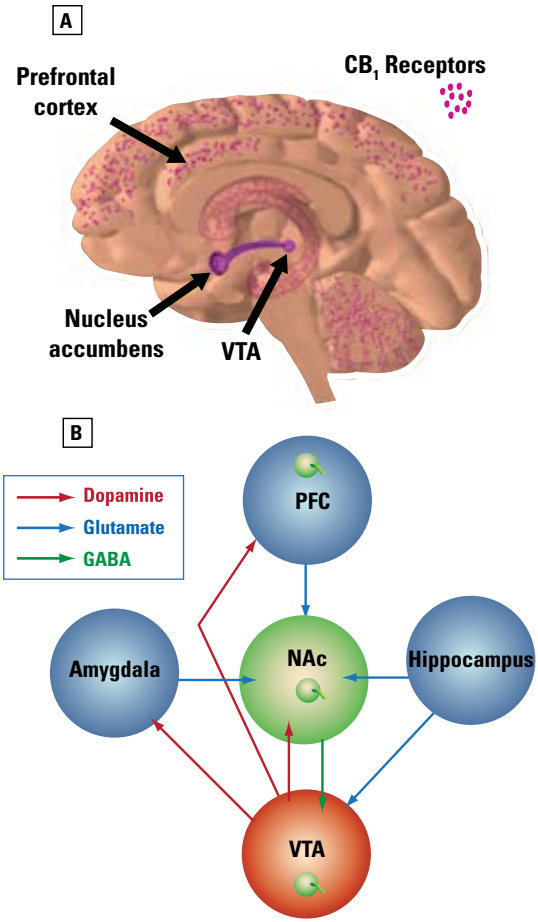

Figure 4. (A) A simplified schematic of the brain reward system: the ventral tegmental area (VTA), the nucleus accumbens (NAc), and the prefrontal cortex (PFC). Dopaminergic axons project from the VTA to the NAc and the PFC. This pathway is activated by the expectation of rewarding stimuli. Expression of CB_1 receptors is represented by pink dots, based on THC-binding studies. (B) Neurotransmitter pathways that CB_1 receptors might modulate in cue- and drug-induced reinstatement of drug-seeking. CB_1 receptor expression is high on neurons impinging on mesolimbic dopamine reward circuits, where perceptions associated with

pleasure/palatability and appetite/incentive stimuli are processed.[119] Functional interactions have been reported between CB_1 receptors and the dopamine, GABA, and glutamate systems. For example, CB_1 receptor activation enhances the release of dopamine from VTA-originating neurons by disinhibition of GABA-containing interneurons in this area. At the level of the NAc, the release of glutamate from neurons originating in several cortical and subcortical areas that are known to be involved in relapse is modulated by CB_1 receptors. Stimulation of NAc CB_1 receptors may suppress glutamatergic activity, with consequent inhibition of GABAergic neurons that normally inhibit VTA dopamine neurons. The basolateral amygdala and hippocampus have an important role in mediating discrete and contextual cue-induced relapse. The anatomical sites where CB_1 receptors exert their modulatory action on drug-seeking behavior are still unknown.

Neurons in the lateral hypothalamus appear to play a role in the motivational aspects of food intake. Jo et al[29] showed that leptin attenuates the CB_1 receptor-mediated suppression of inhibitory postsynaptic currents in perifornical lateral hypothalamic neurons (see Chapter 4, Figure 1). Presumably, this is due to a suppression of endocannabinoid formation by leptin.[29] In addition, hormones from the gastrointestinal tract act in concert with central mechanisms to regulate food intake.[30] Moreover, hormones and metabolites, including insulin and fatty acids, cross the blood–brain barrier in the hindbrain and hypothalamus, thereby modulating hunger and satiety.[31]

Learning and Memory

Studies in rodents indicate that acute and chronic treatment with CB_1 receptor agonists disrupts working memory (memory of new/recent information).[32-35] However, reference memory (recall of previously learned information) does not appear to be similarly affected.[36] Animal studies also demonstrate that endocannabinoids facilitate selective extinction of aversive memories.[37,38] Electrophysiological data in mice suggest that endocannabinoids can both produce long-term depression and enhance long-term potentiation (both cellular correlates of learning),[39] providing a clue to the cellular mechanisms by which the ECS may influence learning. Selective CB_1 receptor blockade can enhance mnemonic processes in rats, mice, and birds[40-42] and enhance memory in rodents.[43-45] Moreover, CB_1 receptor knockout mice exhibited enhanced memory.[41] In humans, acute

administration of the cannabinoid THC transiently impairs immediate and delayed free recall of information presented while under the influence of the drug.[46] While the ECS appears to be intimately involved in several forms of cognition, additional studies are needed to fully define this relationship.

Emotionality

There are ample—albeit discordant—data on the ECS and emotionality, likely reflecting the model system used in the various studies. Animal studies suggest that altered ECS activity can affect anxiety- and depression-like behavior.[47] Both high and low levels of ECS activity have been linked with mood disorders.[48-50] Preclinical and human studies are equivocal with regard to the effect of CB_1 receptor blockade and emotional responses to stress.[51-54]

Haller et al[55] observed increased anxiety-like behavior in CB_1 receptor knockout mice compared with wild-type mice when the animals were exposed to a stressful environment. Other data suggest an antidepressant effect of CB_1 receptor blockade. For example, Shearman et al[47] treated wild-type mice with the cannabinoid receptor antagonist AM251 and subjected them to the tail-suspension test (TST) and forced-swim test (FST), in which antidepressant activity is determined by increased mobility. Similar to the antidepressant desipramine, AM251 significantly reduced immobility in both the TST and FST. AM251 induced antidepressant-like effects appeared to be mediated by CB_1 receptors. This is supported by the observations that: 1) co-administration of the CB_1 receptor agonist CP55940, at a dose that did not induce motor impairment or profound hypothermia, reversed effects of AM251 in the TST; and 2) effects of AM251 in the FST were absent in CB_1 receptor knockout mice.

Human studies suggest a relationship between CB_1 receptor activity and affective disorders, and support an antidepressant-like potential for CB_1 receptor blockade (reviewed in Witkin et al).[51] However, the role of the ECS in depressive disorders is complex, and there is scientific debate on

the role of CB_1 receptor antagonism and agonism in major depressive and anxiety disorders.[51-54,56] Clinical studies are needed to determine the potential effects of CB_1 receptor blockade treatment on emotionality in different patient populations. Rimonabant (a CB_1 receptor antagonist) was approved by the European Medicines Agency (EMEA) in June 2006 for the treatment of obesity; however, the EMEA recommended that rimonabant be contraindicated in patients with ongoing major depression and in patients being treated with antidepressants following the FDA's advisory committee decision to vote against recommending the approval of rimonabant in the US due in part to lack of safety data in people with depression.[57,58] Although depression and anxiety are manageable disorders, there is a need for prospective analysis of emotionality in clinical trials with CB_1 receptor antagonists.

Nausea and Emesis

Preparations of cannabis were used for the treatment of nausea 3000 years ago. Current studies suggest that the ECS may play an important role in the physiology of nausea and emesis, common side effects of many drugs (eg, cancer chemotherapy) and diseases (eg, irritable bowel syndrome and migraine).

Nausea

Synthetic cannabinoids (Δ^9-THC) are FDA approved for the treatment of nausea and vomiting associated with cancer chemotherapy (ie, nabilone and drobinol).[59,60] Anticipatory nausea and vomiting appear to be best explained by classical conditioning, where a previously neutral stimulus (eg, smells of the chemotherapy environment) elicits a conditioned response (eg, anticipatory nausea and vomiting) after a number of prior pairings.[61] Cancer chemotherapy may be "paired" with a variety of other neutral, environmental stimuli (eg, smells of the setting, oncology nurse, chemotherapy room).[61] These previously neutral stimuli may then elicit anticipatory nausea and vomiting in future chemotherapy cycles. Many patients report anticipatory nausea and vomiting upon re-exposure to the

cues previously associated with treatment.[60] In a rat model of anticipatory nausea, a gaping reaction (a marker for nausea) is induced during exposure to a context previously paired with lithium chloride–induced illness.[62] Pretreatment with THC suppressed the lithium-induced nausea.[62] Conversely, CB_1 receptor blockade with SR141716 or AM251 was shown to potentiate lithium-induced nausea in rats in other studies[63,64] but not when the CB_1 receptor antagonist AM4113 was used.[65] Taken together, these preclinical data may explain how activating CB_1 receptors can be beneficial in treating anticipatory nausea associated with chemotherapy, and suggest that CB_1 receptor antagonists may accentuate the nausea associated with chemotherapy. In clinical trials, the CB_1 receptor antagonist rimonabant has been shown to induce nausea in 11.2%-12.9% of subjects receiving 20 mg of rimonabant compared with 3.2%-6.0% of subjects receiving placebo.[66-69] In a 12-week weight-loss study, CB_1 receptor blockade with taranabant induced nausea in 10.4%-31.4% of subjects receiving 0.5-6 mg of taranabant compared with 6.7% of subjects receiving placebo.[70]

Emesis

ECS stimulation with THC produces anti-emetic effects.[71] In animal models, cisplatin-induced emesis in shrew was blocked by THC and synthetic cannabinoid agonists.[73] The anti-emetic effects may involve both central and peripheral mechanisms.[71] A study by Van Sickle et al[15] suggests that brainstem CB_2 receptors play a role in the inhibition of emesis. In this study, the anti-emetic effects of 2-AG and the endocannabinoid reuptake inhibitor VDM11 were reversed when ferrets were cotreated with the CB_2 receptor antagonist AM630.[15] Other studies in ferrets showed that the CB_1 receptor mediates the anti-emetic action of cannabinoids in the dorsal vagal complex.[73,74] More recently, data from the ferret model demonstrated the presence of an endogenous tone on CB_1 and TRPV1 receptors that inhibits emesis.[75]

Motor Function

CB_1 receptor agonists typically reduce locomotor activity. This is manifested by both a decrease in spontaneous activity and rigid immobility (at higher doses).[76] Preclinical data suggest that signaling by anandamide acting on TRPV1 receptors may modulate spontaneous and L-3,4-dihydroxyphenylalanine (L-DOPA)–induced locomotion in rats,[77] and that endocannabinoid signaling through the CB_1 receptor may be required for cerebellum-dependent discrete motor learning in mice.[78] At low concentrations (<1 µM) in vitro and low doses (<3 mg/kg) in vivo, the CB_1 receptor antagonists SR141716 and AM251 appear to be selective for the CB_1 receptor. However, studies carried out at high antagonist doses may uncover interactions with non-CB_1 receptors, which might explain the hyperactivity observed with SR141716 at <10 mg/kg.[79]

Neuroprotection

The ECS has been implicated in protection against human diseases of the CNS in several animal models (reviewed in Karanian and Bahr).[80] Cannabinoid agonists and cannabinoid antagonists may hold therapeutic potential (both for symptom management and for disease progression) for various CNS disorders, such as multiple sclerosis, Parkinson's disease, and Huntington's disease (reviewed in Pacher, Bátkai, and Kunos, 2006).[81] This therapeutic potential is under investigation.

The ECS, including both CB_1 and CB_2 receptors, may play a role in the pathophysiology of various neuropathologies (reviewed in Howlett et al[82]). Thus, manipulation of ECS activity can be protective or toxic to the CNS.

Examination of human postmortem brains showed that levels of FAAH and CB_2 receptors were increased in Alzheimer's disease.[83] It has been speculated that endocannabinoids might be protective during inflammatory neurodegeneration. For example, enhancing brain 2-AG levels with an inhibitor of endocannabinoid reuptake (VDM11) seems to protect against β-amyloid

neurotoxicity in rats.[84] Thus, modulating ECS activity might be a future therapeutic target for the treatment of neurodegenerative diseases.[85]

Neuroexcitability

Excessive activity of excitatory (ie, glutamatergic) systems could lead to the pathological processes of excitotoxicity,[86] and ECS activation may serve to protect neurons against acute excitotoxicity.[84] This effect may involve specific cellular signaling pathways, including decreased Ca^{2+} influx and decreased glutamate release (Figure 3).[87,88]

It is important to consider the relative protective vs excitotoxic CNS effects of ECS activity in the context of the particular animal neurological model, and with regard to acute injury or chronic repair. Indeed, the impact of CB_1 receptor blockade on outcome following various models of brain injury is conflicting. For example, CB_1 receptor blockade with SR141716 produced protective effects in some ischemic models of brain, despite the fact that CB_1 agonists also exhibited the same protective effect in other models.[89-92] The CB_1 receptor antagonists SR141716 and AM251 increased neurogenesis by approximately 50% in the mouse dentate gyrus and subventricular zone.[93] However, this increase in neurogenesis was observed in CB_1 receptor knockout mice, but not in vanilloid TRPV1 receptor knockout mice, suggesting that the effect involved TRPV1 receptors and not CB_1 receptors.[93] Thus, ECS activity is associated with both neuroprotection[94-96] and neurotoxicity[97] in various experimental models. Additional studies are needed to determine the therapeutic potential of interventions via the ECS for various neuropathologies.

The ECS has also been implicated in other conditions in which neuronal excitotoxicity has been suggested to play a role, such as epilepsy.

Cannabis has been used historically to treat epilepsy.[81] CB_1 receptor activation is typically anticonvulsant and appears to play a role in regulating seizure duration and frequency.[98] CB_1 receptor blockade with SR141716 or AM251 causes status epilepticus-like activity in a hippocampal neuronal culture

model of acquired epilepsy.[99] Moreover, CB_1 receptor blockade with SR141716 was shown to induce seizures in pilocarpine-treated rats.[100] These effects may be explained by ECS modulation of excitability in CNS neurons, and the ECS may protect against acute excitotoxicity in CNS neurons.[85] Stimulation of nucleus accumbens CB_1 receptors suppresses glutamatergic activity, with consequent inhibition of GABAergic neurons.[101,102] However, data from animal studies are very dependent on the models used; some of which hold very little relevance to human epilepsy. Thus, additional studies are needed to determine the therapeutic potential for targeting the ECS for the treatment of epilepsy.[81]

Pain Perception

The ECS has a well-established role in modulating pain and represents a validated clinical target. Cannabinoid agonists are effective in animal models of acute and chronic pain.[103-105] Animal studies have revealed an important role of the CB_1 receptor, and likely the CB_2 receptor, in modulating pain perception. Some of these effects are counterintuitive. For example, CB_1 receptor blockade suppressed the antinociceptive effects of paracetamol[106] and THC[107] can produce hyperalgesia.[108]

Genetic deletion of the FAAH gene in mice elevates brain anandamide levels and unmasks the anti-nociceptive effects of this compound. In addition, FAAH knockout mice have attenuated inflammatory responses and exhibit enhanced CB_1 receptor–mediated analgesia.[109] Likewise, pharmacological blockade of FAAH activity reduces nocifensive behavior in animal models of acute and inflammatory pain. In a mouse chronic constriction injury (CCI) model of neuropathic pain, oral administration of the selective FAAH inhibitor URB597 produced a dose-dependent reduction in nocifensive responses to thermal and mechanical stimuli, which was prevented by the CB_1 receptor blockade with SR141716.[110] The antihyperalgesic effects of URB597 were accompanied by a reduction in plasma extravasation induced by CCI.[110] This effect was prevented by CB_1 receptor blockade and attenuated

by the CB_2 receptor antagonist SR144528, further supporting a role for both CB_1 and CB_2 receptors in the nociceptive effects of endocannabinoids.[110] Both the non-selective cannabinoid agonist HU210 and the selective CB_2 receptor agonist JWH-133 attenuated established inflammatory hypersensitivity and swelling in the carrageenan model of inflammatory hyperalgesia in rats.[111] Recently, Agarwal et al[105] studied the analgesic effects of cannabinoids in mice that lacked functional CB_1 receptors specifically in nociceptive neurons in the peripheral nervous system. These mice exhibited reduced antinociceptive effects from local and systemic, but not intrathecal, administration of cannabinoids. These data suggest that CB_1 receptors in peripheral sensory neurons have a prominent role in cannabinoid-mediated analgesia.

Substantial human studies support efficacy of cannabinoid agonists for treating neuropathic pain,[112-114] For example, the analgesic properties of whole plant extracts of *Cannabis sativa* (GW-1000-02) were demonstrated to be effective in a randomized, double-blind, placebo-controlled study in patients with neuropathic pain of peripheral origin.[113] Taken together, these data support a potential role for cannabinoid-based drugs in the treatment of chronic inflammatory pain and neuropathic pain.

Reinforcement

Reinforcement, which occurs when a stimulus is temporally paired with a response that increases the frequency of subsequent responses, plays an important role in the development, maintenance, and recovery from addiction.[115,116] THC self-administration in squirrel monkeys was shown to be CB_1 receptor mediated,[117] and CB_1 receptor blockade with SR141716 reduced cue-induced reinstatement of drug-seeking behavior.[118,119] The ECS likely plays an important role in brain reward processes through its interactions with the mesolimbic dopaminergic system,[120] primarily by modulating neurotransmitter release (Figure 4).[21,101] Specifically, CB_1 receptors appear to mediate the effects of endocannabinoids in the brain reward process.

Endocannabinoids can increase extracellular levels of, dopamine,[120] and CB_1 and dopamine receptors have been shown to interact in rat and monkey striatum.[121] Thus, the ECS may be involved in the reinforcing properties of several drugs of abuse including nicotine, ethanol, and opiates. There is evidence to suggest that stimulation of nucleus accumbens CB_1 receptors suppresses glutamatergic activity, with consequent inhibition of GABAergic neurons that normally inhibit ventral tegmental area dopamine neurons.[101] The therapeutic potential of CB_1 receptor antagonists in the treatment of addiction is being explored with great interest (reviewed in Mackie[122] and Howlett et al[14]).

Summary

The role of the ECS in depressive disorders, epilepsy, motor function, and neuronal function is complex. ECS stimulation is associated with both neuroprotection and neurotoxicity in various experimental models. The effects of acute and chronic CB_1 receptor blockade are often opposite, and this needs to be considered when evaluating the preclinical data. Additional studies are needed to determine potential therapeutic interventions via the ECS for various neuropathologies. Tissue-specific CB_1 receptor knockout mice will facilitate these studies. Both cannabinoid agonists and cannabinoid antagonists may hold therapeutic potential for various disorders of the CNS.

References

1. Fride E. Endocannabinoids in the central nervous system: from neuronal networks to behavior. *Curr Drug Targets CNS Neurol Disord*. 2005;4:633-642.

2. Di Marzo V, Fontana A, Cadas H, et al. Formation and inactivation of endogenous cannabinoid anandamide in central neurons. *Nature*. 1994;372:686-691.

3. Fegley D, Kathuria S, Mercier R, et al. Anandamide transport is independent of fatty-acid amide hydrolase activity and is blocked by the hydrolysis-resistant inhibitor AM1172. *Proc Natl Acad Sci USA*. 2004;101:8756-8761.

4. Hillard CJ, Wilkison DM, Edgemond WS, Campbell WB. Charac-

terization of the kinetics and distribution of N-arachidonylethanolamine (anandamide) hydrolysis by rat brain. *Biochim Biophys Acta*. 1995;1257:249-256.

5. Matias I, Bisogno T, Di Marzo V. Endogenous cannabinoids in the brain and peripheral tissues: regulation of their levels and control of food intake. *Int J Obes* (Lond). 2006;30(Suppl 1):S7-S12.

6. Mulder AM, Cravatt BF. Endocannabinoid metabolism in the absence of fatty acid amide hydrolase (FAAH): discovery of phosphorylcholine derivatives of N-acyl ethanolamines. *Biochemistry*. 2006;45:11267-11277.

7. Witting A, Walter L, Wacker J, Moller T, Stella N. P2X7 receptors control 2-arachidonoylglycerol production by microglial cells. *Proc Natl Acad Sci USA*. 2004;101:3214-3219.

8. Devane WA, Dysarz FA, 3rd, Johnson MR, Melvin LS, Howlett AC. Determination and characterization of a cannabinoid receptor in rat brain. *Mol Pharmacol*. 1988;34:605-613.

9. Herkenham M, Lynn AB, Little MD, et al. Cannabinoid receptor localization in brain. *Proc Natl Acad Sci U S A*. 1990;87:1932-1936.

10. Matsuda LA, Lolait SJ, Brownstein MJ, Young AC, Bonner TI. Structure of a cannabinoid receptor and functional expression of the cloned cDNA. *Nature*. 1990;346:561-564.

11. Matsuda LA, Bonner TI, Lolait SJ. Localization of cannabinoid receptor mRNA in rat brain. *J Comp Neurol*. 1993;327:535-550.

12. Croxford JL. Therapeutic potential of cannabinoids in CNS disease. *CNS Drugs*. 2003;17:179-202.

13. Wilson RI, Nicoll RA. Endocannabinoid signaling in the brain. *Science*. 2002;296:678-682.

14. Howlett AC, Breivogel CS, Childers SR, Deadwyler SA, Hampson RE, Porrino LJ. Cannabinoid physiology and pharmacology: 30 years of progress. *Neuropharmacology*. 2004;47 Suppl 1:345-358.

15. Van Sickle MD, Duncan M, Kingsley PJ, et al. Identification and functional characterization of brainstem cannabinoid CB_2 receptors. *Science*. 2005;310:329-332.

16. Nunez E, Benito C, Pazos MR, et al. Cannabinoid CB_2 receptors are expressed by perivascular microglial cells in the human brain: an immunohistochemical study. *Synapse*. 2004;53:208-213.

17. Wilson RI, Nicoll RA. Endogenous cannabinoids mediate retrograde

signalling at hippocampal synapses. *Nature*. 2001;410:588-592.

18. Piomelli D. The molecular logic of endocannabinoid signalling. *Nat Rev Neurosci*. 2003;4:873-884.

19. Ohno-Shosaku T, Maejima T, Kano M. Endogenous cannabinoids mediate retrograde signals from depolarized postsynaptic neurons to presynaptic terminals. *Neuron*. 2001;29:729-738.

20. Di Marzo V, Bifulco M, De Petrocellis L. The endocannabinoid system and its therapeutic exploitation. *Nat Rev Drug Discov*. 2004;3:771-784.

21. Mackie K. Mechanisms of CB1 receptor signaling: endocannabinoid modulation of synaptic strength. *Int J Obes* (Lond). 2006;30 Suppl 1:S19-23.

22. Bacci A, Huguenard JR, Prince DA. Long-lasting self-inhibition of neocortical interneurons mediated by endocannabinoids. *Nature*. 2004;431:312-316.

23. Kawamura Y, Fukaya M, Maejima T, et al. The CB1 cannabinoid receptor is the major cannabinoid receptor at excitatory presynaptic sites in the hippocampus and cerebellum. *J Neurosci*. 2006;26:2991-3001.

24. Tzavara ET, Davis RJ, Perry KW, et al. The CB1 receptor antagonist SR141716A selectively increases monoaminergic neurotransmission in the medial prefrontal cortex: implications for therapeutic actions. *Br J Pharmacol*. 2003;138:544-553.

25. Demuth DG, Molleman A. Cannabinoid signalling. *Life Sci*. 2006;78:549-563.

26. Kirkham TC, Williams CM, Fezza F, Di Marzo V. Endocannabinoid levels in rat limbic forebrain and hypothalamus in relation to fasting, feeding and satiation: stimulation of eating by 2-arachidonoyl glycerol. *Br J Pharmacol*. 2002;136:550-557.

27. Rosenbaum M, Leibel RL. The role of leptin in human physiology. *N Engl J Med*. 1999;341:913-915.

28. Di Marzo V, Goparaju SK, Wang L, et al. Leptin-regulated endocannabinoids are involved in maintaining food intake. *Nature*. 2001;410:822-825.

29. Jo YH, Chen YJ, Chua SC, Jr., Talmage DA, Role LW. Integration of endocannabinoid and leptin signaling in an appetite-related neural circuit. *Neuron*. 2005;48:1055-1066.

30. Badman MK, Flier JS. The gut and energy balance: visceral allies in the obesity wars. *Science.* 2005;307:1909-1914.

31. Cota D, Woods S. The role of the endocannabinoid system in the regulation of energy homeostasis. *Curr Opin Endocrinol Diabetes.* 2005;12:338-351.

32. Hampson RE, Deadwyler SA. Role of cannabinoid receptors in memory storage. *Neurobiol Dis.* 1998;5:474-482.

33. Varvel SA, Hamm RJ, Martin BR, Lichtman AH. Differential effects of delta 9-THC on spatial reference and working memory in mice. *Psychopharmacology (Berl).* 2001;157:142-150.

34. Hampson RE, Simeral JD, Kelly EJ, Deadwyler SA. Tolerance to the memory disruptive effects of cannabinoids involves adaptation by hippocampal neurons. *Hippocampus.* 2003;13:543-556.

35. Nakamura EM, da Silva EA, Concilio GV, Wilkinson DA, Masur J. Reversible effects of acute and long-term administration of delta-9-tetrahydrocannabinol (THC) on memory in the rat. *Drug Alcohol Depend.* 1991;28:167-175.

36. Robinson L, McKillop-Smith S, Ross NL, et al. Hippocampal endocannabinoids inhibit spatial learning and limit spatial memory in rats. *Psychopharmacology (Berl).* 2007.

37. Marsicano G, Wotjak CT, Azad SC, et al. The endogenous cannabinoid system controls extinction of aversive memories. *Nature.* 2002;418:530-534.

38. Varvel SA, Lichtman AH. Evaluation of CB_1 receptor knockout mice in the Morris water maze. *J Pharmacol Exp Ther.* 2002;301:915-924.

39. Carlson G, Wang Y, Alger BE. Endocannabinoids facilitate the induction of LTP in the hippocampus. *Nat Neurosci.* 2002;5:723-724.

40. Wise LE, Iredale PA, Stokes RJ, Lichtman AH. Combination of Rimonabant and Donepezil Prolongs Spatial Memory Duration. *Neuropsychopharmacology.* 2007.

41. Reibaud M, Obinu MC, Ledent C, Parmentier M, Bohme GA, Imperato A. Enhancement of memory in cannabinoid CB_1 receptor knockout mice. *Eur J Pharmacol.* 1999;379:R1-2.

42. Shiflett MW, Rankin AZ, Tomaszycki ML, DeVoogd TJ. Cannabinoid inhibition improves memory in food-storing birds, but with a cost. *Proc Biol Sci.* 2004;271:2043-2048.

43. Terranova JP, Storme JJ, Lafon N, et al. Improvement of memory in rodents by the selective CB1 cannabinoid receptor antagonist, SR 141716. *Psychopharmacology (Berl).* 1996;126:165-172.

44. Lichtman AH. SR 141716A enhances spatial memory as assessed in a radial-arm maze task in rats. *Eur J Pharmacol.* 2000;404:175-179.

45. Wolff MC, Leander JD. SR141716A, a cannabinoid CB_1 receptor antagonist, improves memory in a delayed radial maze task. *Eur J Pharmacol.* 2003;477:213-217.

46. Ranganathan M, D'Souza DC. The acute effects of cannabinoids on memory in humans: a review. *Psychopharmacology (Berl).* 2006;188:425-444.

47. Shearman LP, Rosko KM, Fleischer R, et al. Antidepressant-like and anorectic effects of the cannabinoid CB1 receptor inverse agonist AM251 in mice. *Behav Pharmacol.* 2003;14:573-582.

48. Vinod KY, Hungund BL. Role of the endocannabinoid system in depression and suicide. *Trends Pharmacol Sci.* 2006;27:539-545.

49. Vinod KY, Hungund BL. Cannabinoid-1 receptor: a novel target for the treatment of neuropsychiatric disorders. *Expert Opin Ther Targets.* 2006;10:203-210.

50. Bortolato M, Campolongo P, Mangieri RA, et al. Anxiolytic-like properties of the anandamide transport inhibitor AM404. *Neuropsychopharmacology.* 2006;31:2652-2659.

51. Witkin JM, Tzavara ET, Nomikos GG. A role for cannabinoid CB_1 receptors in mood and anxiety disorders. *Behav Pharmacol.* 2005;16:315-331.

52. Ashton CH, Moore PB, Gallagher P, Young AH. Cannabinoids in bipolar affective disorder: a review and discussion of their therapeutic potential. *J Psychopharmacol.* 2005;19:293-300.

53. Gambi F, De Berardis D, Sepede G, et al. Cannabinoid receptors and their relationships with neuropsychiatric disorders. *Int J Immunopathol Pharmacol.* 2005;18:15-19.

54. Koethe D, Llenos IC, Dulay JR, et al. Expression of CB_1 cannabinoid receptor in the anterior cingulate cortex in schizophrenia, bipolar disorder, and major depression. *J Neural Transm.* 2007;114:1055-1063.

55. Haller J, Varga B, Ledent C, Barna I, Freund TF. Context-dependent effects of CB1 cannabinoid gene disruption on anxiety-like and social behaviour in mice. *Eur J Neurosci.* 2004;19:1906-1912.

56. Hungund BL, Vinod KY, Kassir SA, et al. Upregulation of CB_1 receptors and agonist-stimulated [^{35}S]GTPgammaS binding in the prefrontal cortex of depressed suicide victims. *Mol Psychiatry*. 2004;9:184-190.

57. Food and Drug Administration. FDA Briefing document. NDA 21-888. Zimulti (rimonabant) Tablets, 20 mg. Sanofi Aventis. Advisory Committee–June 13, 2007. http://www.fda.gov/ohrms/dockets/AC/07/briefing/2007-4306b1-fda-backgrounder.pdf. Accessed August 9, 2007.

58. European medicines agency recommends Accomplia must not be used in patients on antidepressants or with major depression. Medical News Today, 21 July 2007. www.medicalnewstoday.com.

59. Slatkin NE. Cannabinoids in the treatment of chemotherapy-induced nausea and vomiting: beyond prevention of acute emesis. *J Support Oncol.* 2007;5:(5 Suppl 3):1-9.

60. Weddington WW, Jr., Miller NJ, Sweet DL. Anticipatory nausea and vomiting associated with cancer chemotherapy. *N Engl J Med*. 1982;307:825-826.

61. National Cancer Institute. US National Institutes of Health. Anticipatory nausea and vomiting. http://www.cancer.gov/cancertopics/pdq/supportivecare/nausea/HealthProfessional/page5. Accessed January 10, 2007.

62. Limebeer CL, Hall G, Parker LA. Exposure to a lithium-paired context elicits gaping in rats: A model of anticipatory nausea. *Physiol Behav.* 2006;88:398-403.

63. Cross-Mellor SK, Ossenkopp KP, Piomelli D, Parker LA. Effects of the FAAH inhibitor, URB597, and anandamide on lithium-induced taste reactivity responses: a measure of nausea in the rat. *Psychopharmacology (Berl)*. 2007;190:135-143.

64. Parker LA, Mechoulam R, Schlievert C, Abbott L, Fudge ML, Burton P. Effects of cannabinoids on lithium-induced conditioned rejection reactions in a rat model of nausea. *Psychopharmacology (Berl)*. 2003;166:156-162.

65. Sink KS, McLaughlin PJ, Wood JA, et al. The novel cannabinoid CB_1 receptor neutral antagonist AM4113 suppresses food intake and food-reinforced behavior but does not induce signs of nausea in rats. *Neuropsychopharmacology*. 2007:1-10.

66. Van Gaal LF, Rissanen AM, Scheen AJ, Ziegler O, Rossner S. Effects of the cannabinoid-1 receptor blocker rimonabant on weight reduction and cardiovascular risk factors in overweight patients: 1-year

experience from the RIO-Europe study. *Lancet*. 2005;365:1389-1397.

67. Després JP, Golay A, Sjöström L. Effects of rimonabant on metabolic risk factors in overweight patients with dyslipidemia. *N Engl J Med*. 2005;353:2121-2134.

68. Pi-Sunyer FX, Aronne LJ, Heshmati HM, Devin J, Rosenstock J. Effect of rimonabant, a cannabinoid-1 receptor blocker, on weight and cardiometabolic risk factors in overweight or obese patients: RIO-North America: a randomized controlled trial. *JAMA*. 2006;295:761-775.

69. Scheen AJ, Finer N, Hollander P, Jensen MD, Van Gaal LF. Efficacy and tolerability of rimonabant in overweight or obese patients with type 2 diabetes: a randomised controlled study. *Lancet*. 2006;368:1660-1672.

70. Addy C, Wright H, Van Laere K, et al. The acyclic CB1R inverse agonist taranabant mediates weight loss by increasing energy expenditure and decreasing caloric intake. *Cell Metab*. 2008;7:68–78.

71. Darmani NA, Johnson JC. Central and peripheral mechanisms contribute to the antiemetic actions of delta-9-tetrahydrocannabinol against 5-hydroxytryptophan-induced emesis. *Eur J Pharmacol*. 2004;488:201-212.

72. Darmani NA, Janoyan JJ, Crim J, Ramirez J. Receptor mechanism and antiemetic activity of structurally-diverse cannabinoids against radiation-induced emesis in the least shrew. *Eur J Pharmacol*. 2007;563:187-196.

73. Van Sickle MD, Oland LD, Ho W, et al. Cannabinoids inhibit emesis through CB1 receptors in the brainstem of the ferret. *Gastroenterology*. 2001;121:767-774.

74. Van Sickle MD, Oland LD, Mackie K, Davison JS, Sharkey KA. Δ^9-tetrahydrocannabinol selectively acts on CB_1 receptors in specific regions of dorsal vagal complex to inhibit emesis in ferrets. *Am J Physiol Gastrointest Liver Physiol*. 2003;285:G566-576.

75. Sharkey KA, Cristino L, Oland LD, et al. Arvanil, anandamide and N-arachidonoyl-dopamine (NADA) inhibit emesis through cannabinoid CB1 and vanilloid TRPV1 receptors in the ferret. *Eur J Neurosci*. 2007;25:2773-2782.

76. Fernandez-Ruiz J, Gonzales S. Cannabinoid control of motor function at the basal ganglia. *Handb Exp Pharmacol*. 2005:479-507.

77. Lee J, Di Marzo V, Brotchie JM. A role for vanilloid receptor 1 (TRPV1) and endocannabinnoid signalling in the regulation of spontaneous and L-DOPA induced locomotion in normal and reserpine-treated rats. *Neuropharmacology.* 2006.

78. Kishimoto Y, Kano M. Endogenous cannabinoid signaling through the CB_1 receptor is essential for cerebellum-dependent discrete motor learning. *J Neurosci.* 2006;26:8829-8837.

79. Bass CE, Griffin G, Grier M, Mahadevan A, Razdan RK, Martin BR. SR-141716A-induced stimulation of locomotor activity. A structure-activity relationship study. *Pharmacol Biochem Behav.* 2002;74:31-40.

80. Karanian DA, Bahr BA. Cannabinoid drugs and enhancement of endocannabinoid responses: strategies for a wide array of disease states. *Curr Mol Med.* 2006;6:677-684.

81. Pacher P, Batkai S, Kunos G. The endocannabinoid system as an emerging target of pharmacotherapy. *Pharmacol Rev.* 2006;58:389-462.

82. Howlett AC, Mukhopadhyay S, Norford DC. Endocannabinoids and reactive nitrogen and oxygen species in neuropathologies. Journal of Neuroimmune *Pharmacology.* 2006;1:305-316.

83. Benito C, Nunez E, Tolon RM, et al. Cannabinoid CB_2 receptors and fatty acid amide hydrolase are selectively overexpressed in neuritic plaque-associated glia in Alzheimer's disease brains. *J Neurosci.* 2003;23:11136-11141.

84. van der Stelt M, Mazzola C, Esposito G, et al. Endocannabinoids and beta-amyloid-induced neurotoxicity in vivo: effect of pharmacological elevation of endocannabinoid levels. *Cell Mol Life Sci.* 2006;63:1410-1424.

85. Marsicano G, Goodenough S, Monory K, et al. CB1 cannabinoid receptors and on-demand defense against excitotoxicity. *Science.* 2003;302:84-88.

86. Ben-Ari Y, Cossart R. Kainate, a double agent that generates seizures: two decades of progress. *Trends Neurosci.* 2000;23:580-587.

87. Shen M, Piser TM, Seybold VS, Thayer SA. Cannabinoid receptor agonists inhibit glutamatergic synaptic transmission in rat hippocampal cultures. *J Neurosci.* 1996;16:4322-4334.

88. Shen M, Thayer SA. Cannabinoid receptor agonists protect cultured rat hippocampal neurons from excitotoxicity. *Mol Pharmacol.* 1998;54:459-462.

89. Sommer C, Schomacher M, Berger C, et al. Neuroprotective cannabinoid receptor antagonist SR141716A prevents downregulation of excitotoxic NMDA receptors in the ischemic penumbra. *Acta Neuropathol (Berl)*. 2006;112:277-286.

90. Muthian S, Rademacher DJ, Roelke CT, Gross GJ, Hillard CJ. Anandamide content is increased and CB_1 cannabinoid receptor blockade is protective during transient, focal cerebral ischemia. *Neuroscience*. 2004;129:743-750.

91. Berger C, Schmid PC, Schabitz WR, Wolf M, Schwab S, Schmid HH. Massive accumulation of N-acylethanolamines after stroke. Cell signalling in acute cerebral ischemia? *J Neurochem*. 2004;88:1159-1167.

92. Pegorini S, Zani A, Braida D, Guerini-Rocco C, Sala M. Vanilloid VR1 receptor is involved in rimonabant-induced neuroprotection. *Br J Pharmacol*. 2006;147:552-559.

93. Jin K, Xie L, Kim SH, et al. Defective adult neurogenesis in CB1 cannabinoid receptor knockout mice. *Mol Pharmacol*. 2004;66:204-208.

94. Hayakawa K, Mishima K, Nozako M, et al. Delayed treatment with cannabidiol has a cerebroprotective action via a cannabinoid receptor-independent myeloperoxidase-inhibiting mechanism. *J Neurochem*. 2007;102:1488-1496.

95. Esposito G, De Filippis D, Steardo L, et al. CB_1 receptor selective activation inhibits beta-amyloid-induced iNOS protein expression in C6 cells and subsequently blunts tau protein hyperphosphorylation in co-cultured neurons. *Neurosci Lett*. 2006;404:342-346.

96. Ramirez BG, Blazquez C, Gomez del Pulgar T, Guzman M, de Ceballos ML. Prevention of Alzheimer's disease pathology by cannabinoids: neuroprotection mediated by blockade of microglial activation. *J Neurosci*. 2005;25:1904-1913.

97. Sarne Y, Mechoulam R. Cannabinoids: between neuroprotection and neurotoxicity. *Curr Drug Targets CNS Neurol Disord*. 2005;4:677-684.

98. Wallace MJ, Martin BR, DeLorenzo RJ. Evidence for a physiological role of endocannabinoids in the modulation of seizure threshold and severity. *Eur J Pharmacol*. 2002;452:295-301.

99. Deshpande LS, Sombati S, Blair RE, Carter DS, Martin BR, DeLorenzo RJ. Cannabinoid CB1 receptor antagonists cause status epilepticus-like activity in the hippocampal neuronal culture model of acquired epilepsy. *Neurosci Lett*. 2007;411:11-16.

100. Wallace MJ, Blair RE, Falenski KW, Martin BR, DeLorenzo RJ. The endogenous cannabinoid system regulates seizure frequency and duration in a model of temporal lobe epilepsy. *J Pharmacol Exp* Ther. 2003;307:129-137.

101. Gerdeman GL, Partridge JG, Lupica CR, Lovinger DM. It could be habit forming: drugs of abuse and striatal synaptic plasticity. *Trends Neurosci.* 2003;26:184-192.

102. Robbe D, Alonso G, Duchamp F, Bockaert J, Manzoni OJ. Localization and mechanisms of action of cannabinoid receptors at the glutamatergic synapses of the mouse nucleus accumbens. *J Neurosci.* 2001;21:109-116.

103. Howlett AC, Bidaut-Russell M, Devane WA, Melvin LS, Johnson MR, Herkenham M. The cannabinoid receptor: biochemical, anatomical and behavioral characterization. *Trends Neurosci.* 1990;13:420-423.

104. Jhaveri MD, Richardson D, Chapman V. Endocannabinoid metabolism and uptake: novel targets for neuropathic and inflammatory pain. *Br J Pharmacol.* 2007.

105. Agarwal N, Pacher P, Tegeder I, et al. Cannabinoids mediate analgesia largely via peripheral type 1 cannabinoid receptors in nociceptors. *Nat Neurosci.* 2007;10:870-879.

106. Ottani A, Leone S, Sandrini M, Ferrari A, Bertolini A. The analgesic activity of paracetamol is prevented by the blockade of cannabinoid CB_1 receptors. *Eur J Pharmacol.* 2006;531:280-281.

107. Carta G, Gessa GL, Nava F. Dopamine D(2) receptor antagonists prevent delta(9)-tetrahydrocannabinol-induced antinociception in rats. *Eur J Pharmacol.* 1999;384:153-156.

108. Richardson JD, Aanonsen L, Hargreaves KM. SR 141716A, a cannabinoid receptor antagonist, produces hyperalgesia in untreated mice. *Eur J Pharmacol. 1997;319:R3-4.*

109. Lichtman AH, Shelton CC, Advani T, Cravatt BF. Mice lacking fatty acid amide hydrolase exhibit a cannabinoid receptor-mediated phenotypic hypoalgesia. *Pain.* 2004;109:319-327.

110. Russo R, Loverme J, La Rana G, et al. The fatty acid amide hydrolase inhibitor URB597 (cyclohexylcarbamic acid 3'-carbamoylbiphenyl-3-yl ester) reduces neuropathic pain after oral administration in mice. *J Pharmacol Exp Ther.* 2007;322:236-242.

111. Elmes SJ, Winyard LA, Medhurst SJ, et al. Activation of CB_1 and CB_2 receptors attenuates the induction and maintenance of inflammatory pain in the rat. *Pain.* 2005;118:327-335.

112. Rog DJ, Nurmikko TJ, Young CA. Oromucosal Delta9-tetrahydrocan-

nabinol/cannabidiol for neuropathic pain associated with multiple sclerosis: an uncontrolled, open-label, 2-year extension trial. *Clin Ther*. 2007;29:2068-2079.

113. Nurmikko TJ, Serpell MG, Hoggart B, Toomey PJ, Morlion BJ, Haines D. Sativex successfully treats neuropathic pain characterised by allodynia: A randomised, double-blind, placebo-controlled clinical trial. *Pain*. 2007;133:210-220.

114. Berman JS, Symonds C, Birch R. Efficacy of two cannabis based medicinal extracts for relief of central neuropathic pain from brachial plexus avulsion: results of a randomised controlled trial. *Pain*. 2004;112:299-306.

115. Higgins ST, Heil SH, Lussier JP. Clinical implications of reinforcement as a determinant of substance use disorders. *Annu Rev Psychol*. 2004;55:431-461.

116. Self DW, Nestler EJ. Molecular mechanisms of drug reinforcement and addiction. *Annu Rev Neurosci*. 1995;18:463-495.

117. Tanda G, Munzar P, Goldberg SR. Self-administration behavior is maintained by the psychoactive ingredient of marijuana in squirrel monkeys. *Nat Neurosci*. 2000;3:1073-1074.

118. De Vries TJ, de Vries W, Janssen MC, Schoffelmeer AN. Suppression of conditioned nicotine and sucrose seeking by the cannabinoid-1 receptor antagonist SR141716A. *Behav Brain Res*. 2005;161:164-168.

119. De Vries TJ, Schoffelmeer AN. Cannabinoid CB_1 receptors control conditioned drug seeking. *Trends Pharmacol Sci*. 2005;26:420-426.

120. Solinas M, Justinova Z, Goldberg SR, Tanda G. Anandamide administration alone and after inhibition of fatty acid amide hydrolase (FAAH) increases dopamine levels in the nucleus accumbens shell in rats. *J Neurochem*. 2006;98:408-419

121. Meschler JP, Howlett AC. Signal transduction interactions between CB_1 cannabinoid and dopamine receptors in the rat and monkey striatum. *Neuropharmacology*. 2001;40:918-926.

122. Mackie K. Cannabinoid receptors as therapeutic targets. *Annu Rev Pharmacol Toxicol*. 2006;46:101-122.

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Chapter 6:

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18th Annual Symposium on the Cannabinoids, Burlington, Vermont, International Research Society, p171.

DEGRADATION OF ANANDAMIDE IN ADHD AND ITS RELATIONSHIP WITH DOPAMINE SIGNALING IN THE STRIATUM

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Abnormal dopamine (DA) transmission in the striatum has been proposed to play a role in attention-deficit/hyperactivity disorder (ADHD). Since the modulation of the endocannabinoid system is a major effect of DA receptor stimulation, the present study was aimed at investigating the metabolism of the endocannabinoid anandamide (AEA) in ADHD patients. We found a selective reduction of AEA degradation in the peripheral blood of ADHD patients, an alteration that was replicated in the mouse striatum following the stimulation of dopamine D2-class, but not of D1-class receptors. In addition, we provided neurophysiological evidence that the biochemical defect of AEA degradation found in ADHD patients selectively altered glutamate synaptic transmission but not GABA transmission in the striatum, indicating that ADHD symptoms may rely, at least in part, on differential dysregulation of excitatory and inhibitory synaptic transmission in this bran area. On the basis of our results, it can be proposed that pharmacological modulation of the endocannabinoid system might be useful for the treatment of ADHD patients.

ENDOCANNABINOIDS FINE TUNE SLOW OSCILLATORY ACTIVITY OF VENTRAL TEGMENTAL AREA DOPAMINE NEURONS

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Endogenous cannabinoids (eCBs) play a role in the regulation of neuronal circuitry dynamics underpinning high-order brain functions, including reward, motivation, learning, and memory. Among the neural structures involved in this complex neuronal network, ventral tegmental area (VTA) dopamine (DA) neurons are particularly susceptible to eCBs influence.
Short and long term eCBs actions finely shape DA synaptic inputs to generate pattern of electrical activity that control DA release and functional effects on target cells. Along with single spiking and bursting pattern, DA cells exhibit robust oscillatory activities characterized by repetition of clusters of action potentials at low frequencies (slow oscillation, SO 0.5-1.5 Hz).
Administration of the exogenous CB1 agonist WIN 55212-2 in anesthetized rats enhances DA neuronal activity and SO. To ascertain the physiological relevance of CB1-mediated increase of SO in DA neurons, eCBs levels were enhanced by the administration of URB597 (URB), a selective inhibitor of the enzyme fatty-acid amide hydrolase. DA neurons recorded from URB pre-treated rats (0.1 mg/kg i.v.) showed slightly, but not significant, differences in electrophysiological parameters such as frequency and percent of burst firing. On the other hand, spectral analysis revealed an enhanced expression of SO activity in URB pre-treated rats *versus* naïve rats (URB $P_{0,5\text{-}1,5}$ 0.5 ±0.09 n=15; controls $P_{0,5\text{-}1,5}$ 0.5 ±0.09 n=15 $P<0.001$ Anova and Tukey *post hoc* test). The CB1 receptor antagonist SR141716A (0.5 mg/kg i.v.), ineffective *per se*, was able to prevent URB-induced actions (URB $P_{0,5\text{-}1,5}$ 0.5 ±0.09 n=15; SR $P_{0,5\text{-}1,5}$ 0.3 ±0.06 n=9 $P<0.001$ Anova and Tukey *post hoc* test 0.3). These results point towards an involvement of eCB and CB1 receptors in SO of VTA DA neurons.
Recent experimental evidence ascribes to prefrontal cortex (PFC) a pivotal role in DA cells SO. In particular, oscillation of DA neurons reflects excitatory and inhibitory PFC-VTA pathway activity, providing both temporal and spatial codes relevant in information processing. Tuning of DA neuron SO by the eCBs system may bear relevance in several neuropsychiatric disorders (such as schizophrenia and ADHD) in which dysfunctions of cortical and sub-cortical DA transmission are characterized by alterations in oscillatory activity.

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ENDOCANNABINOID-MEDIATED RETROGRADE SIGNAL TRANSMISSION BETWEEN NEURONS

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Anatomical and functional studies have shown that CB_1 cannabinoid receptors are ubiquitously distributed in the central nervous system and that they are preferentially localized on presynaptic axon terminals, where their activation leads to inhibition of neurotransmitter release from the terminals. However, the physiological role of the CB_1 receptors was, at first, not known. This was changed by the discovery of the roles of endocannabinoids and CB_1 receptors in retrograde synaptic signaling. During usual synaptic transmission (orthograde transmission), the presynaptic axon terminal releases neurotransmitter and the neurotransmitter affects the function of the postsynaptic neuron. The opposite occurs during retrograde synaptic transmission: a signaling molecule released by the postsynaptic neuron affects the function of the presynaptic axon terminal. Endocannabinoids are frequently the signaling molecules during retrograde signaling (see Figure 1), and endocannabinoid-mediated retrograde signaling is the basis of several forms of short- and long-term synaptic plasticity.

It has been shown that endocannabinoids are involved in the short-term (lasting from milliseconds to 3 minutes) plasticity of glutamatergic and GABAergic synapses in the cerebellum, ventral tegmental area, hippocampus, caudate-putamen, amygdala and cortex. Endocannabinoids are also involved in long-term (lasting more than 20 minutes) plasticity of synapses in the amygdala, nucleus accumbens, hippocampus, caudate-putamen and cortex.

Much effort has been undertaken to determine the chemical identity of the released endocannabinoid. Inhibition of the 2-arachidonoylglycerol-producing enzyme diacylglycerol lipase abolished retrograde signaling in most brain regions. In contrast, inhibition of the 2-arachidonoylglycerol-degrading enzyme monoglyceride lipase potentiated retrograde signaling. Thus, it is very likely that 2-arachidonoylglycerol is more important for retrograde signaling than the other major endocannabinoid, anandamide.

The therapeutical possibilities and consequences arising from the operation of endocannabinoid-mediated synaptic plasticity are manifold. On the one hand, it is possible to deliberately influence synaptic plasticity, and thus memory and learning processes, by modulating endocannabinoid production and degradation. On the other hand, activation or blockade of CB_1 receptors for other purposes (for example, for analgesia and appetite reduction) may lead to unwanted interference with endocannabinoid-mediated memory and learning processes.

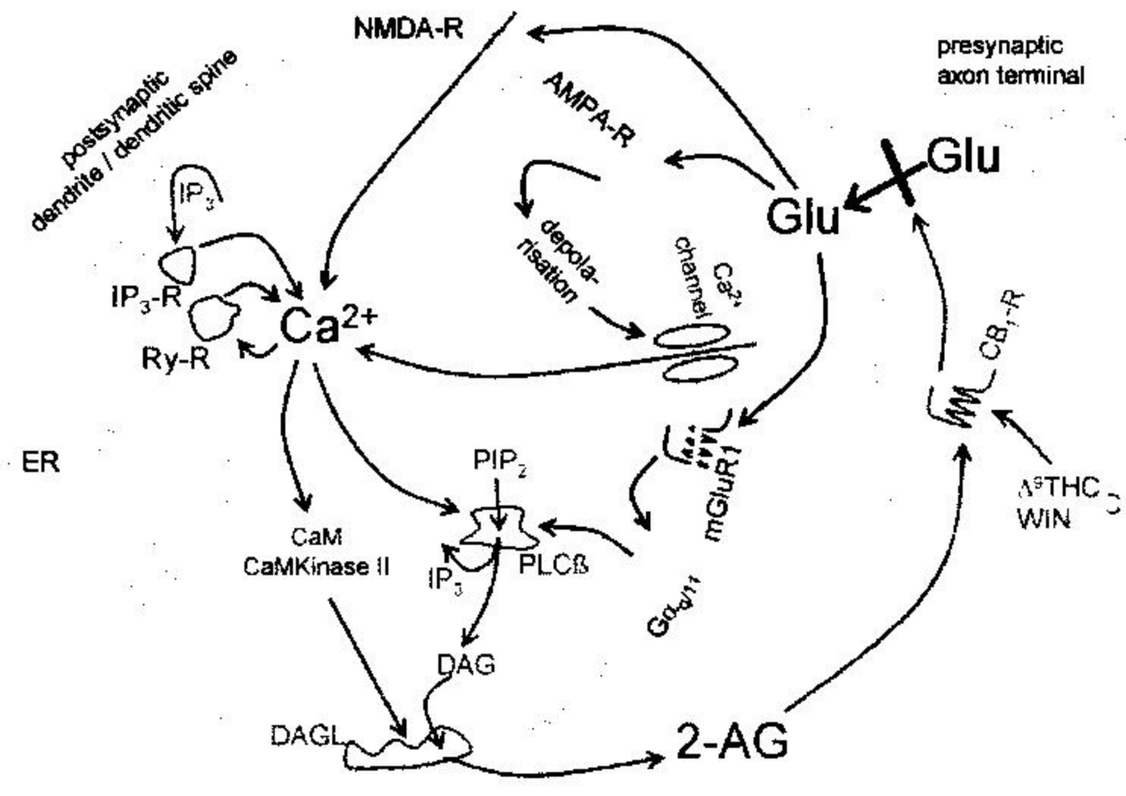

Figure 1. Endocannabinoid-mediated retrograde signaling. Two major triggers can elicit 2-arachidonoylglycerol (2-AG) production in a postsynaptic neuron: an increase in intracellular calcium concentration and activation of $G\alpha_{q/11}$ protein-coupled receptors. Calcium flows into the neuron via voltage-gated calcium channels opened by the depolarizing glutamatergic synaptic input. Glutamate also activates $G\alpha_{q/11}$ protein-coupled metabotropic mGluR1 receptors.

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Role of Endogenous Cannabinoids in Synaptic Signaling

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Freund, Tamás F., István Katona, and Daniele Piomelli. Role of Endogenous Cannabinoids in Synaptic Signaling. *Physiol Rev* 83: 1017–1066, 2003; 10.1152/physrev.00004.2003.—Research of cannabinoid actions was boosted in the 1990s by remarkable discoveries including identification of endogenous compounds with cannabimimetic activity (endocannabinoids) and the cloning of their molecular targets, the CB_1 and CB_2 receptors. Although the existence of an endogenous cannabinoid signaling system has been established for a decade, its physiological roles have just begun to unfold. In addition, the behavioral effects of exogenous cannabinoids such as delta-9-tetrahydrocannabinol, the major active compound of hashish and marijuana, await explanation at the cellular and network levels. Recent physiological, pharmacological, and high-resolution anatomical studies provided evidence that the major physiological effect of cannabinoids is the regulation of neurotransmitter release via activation of presynaptic CB_1 receptors located on distinct types of axon terminals throughout the brain. Subsequent discoveries shed light on the functional consequences of this localization by demonstrating the involvement of endocannabinoids in retrograde signaling at GABAergic and glutamatergic synapses. In this review, we aim to synthesize recent progress in our understanding of the physiological roles of endocannabinoids in the brain. First, the synthetic pathways of endocannabinoids are discussed, along with the putative mechanisms of their release, uptake, and degradation. The fine-grain anatomical distribution of the neuronal cannabinoid receptor CB_1 is described in most brain areas, emphasizing its general presynaptic localization and role in controlling neurotransmitter release. Finally, the possible functions of endocannabinoids as retrograde synaptic signal molecules are discussed in relation to synaptic plasticity and network activity patterns.

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I. INTRODUCTION

Descriptions of the *Cannabis sativa* plant and its medicinal properties were already accessible to Greek and Roman physicians in the first century AD, when Dioscorides included the plant in his classic textbook of pharmacology, entitled *Materia Medica* ("The Materials of Medicine"). Ancient Indian and Chinese medical writers were even more accurate than their European colleagues in describing the remarkable physiological and psychological effects of this plant (for review, see Ref. 241). We know now that these effects, which in humans include a variable combination of euphoria, relaxation, reflex tachycardia, and hypothermia, are primarily produced by the dibenzopyrane derivative, delta-9-tetrahydrocannabinol (delta-9-THC), present in the yellow resin that covers the leaves and flower clusters of the ripe female plant. The chemical structure of delta-9-THC was elucidated by the pioneering studies of R. Adams (6) and Gaoni and Mechoulam (114). Unlike morphine, cocaine, and other alkaloids of plant origin, delta-9-THC is a highly hydrophobic compound, a property that, curiously enough, has slowed the progress on the mode of action of this compound for nearly three decades. The affinity of delta-9-THC for lipid membranes erroneously suggested, indeed, that the drug's main effect was to modify in a nonselective manner the fluidity of cell membranes rather than to activate a selective cell-surface receptor (157, 207).

Two series of events contributed to a radical change of this view. First, motivated by the potential therapeutic applications of cannabis-like ("cannabimimetic") molecules, laboratories in academia and the pharmaceutical industry began to develop families of synthetic analogs of delta-9-THC. These agents exerted pharmacological effects that were qualitatively similar to those of delta-9-THC but displayed both greater potency and stereoselectivity. The latter feature cannot be reconciled with nonspecific membrane interactions, providing the first evidence that delta-9-THC exerts its effects by combining with a selective receptor. Second, as a result of these synthetic efforts, it became possible to explore directly the existence of cannabinoid receptors by using standard radioligand binding techniques. In 1988, Howlett and her co-workers (84, 167) described the presence of high-affinity binding sites for cannabinoid agents in brain membranes and showed that these sites are coupled to inhibition of adenylyl cyclase activity. Conclusively supporting these findings, in 1990 Matsuda et al. (236) serendipitously came across a complementary DNA encoding for the first G protein-coupled cannabinoid receptor, now known as CB_1.

In heterologous expression systems, CB_1 receptors were found to be functionally coupled to multiple intracellular signaling pathways, including inhibition of adenylyl cyclase activity, inhibition of voltage-activated calcium channels, and activation of potassium channels (56, 148, 221, 222, 236, 239). In situ hybridization and immunohistochemical studies have demonstrated that CB_1 receptors are abundantly expressed in discrete regions and cell types of the central nervous system (CNS) (see also sect. III) but are also present at significant densities in a variety of peripheral organs and tissues (41, 225, 226, 235, 345). The selective distribution of CB_1 receptors in the CNS provides a clear anatomical correlate for the cognitive, affective, and motor effects of cannabimimetic drugs.

The cloning and characterization of CB_1 receptors left several important problems unsolved. Since antiquity, it has been known that the actions of *Cannabis* and delta-9-THC are not restricted to the CNS, but include effects on nonneural tissues such as reduction of inflammation, lowering of intraocular pressure associated with glaucoma, and relief of muscle spasms. Are these peripheral effects all produced by activation of CB_1 receptors? An initial answer to this question was provided by the discovery of a second cannabinoid receptor exquisitely expressed in cells of immune origin (260). This receptor, called CB_2, only shares ~44% sequence identity with its brain counterpart, implying that the two subtypes diverged long ago in evolution. The intracellular coupling of the CB_2 receptor resembles, however, that of the CB_1 receptor; for example, in transfected cells, CB_2 receptor activation is linked to the inhibition of adenylyl cyclase activity (113).

The experience with opioid receptors and the enkephalins has accustomed scientists to the idea that whenever a receptor is present in the body, endogenous factor(s) that activate this receptor also exist. Not surprisingly, therefore, as soon as cannabinoid receptors were described, a search began to identify their naturally occurring ligand(s). One way to tackle this problem was based on the premise that, like other neurotransmitters and neuromodulators, an endogenous cannabinoid substance should be released from brain tissue in a calcium-dependent manner. Taking this route, Howlett and co-workers incubated rat brain slices in the presence of a calcium ionophore and determined whether the media from these incubations contained a factor that displaced the binding of labeled CP-55940, a cannabinoid agonist, to brain membranes. These studies demonstrated that a cannabinoid-like activity was indeed released from stimulated slices, but the minute amounts of this factor did not allow the elucidation of its chemical structure (97, 98).

Devane, Mechoulam, and co-workers (85, 243), at the Hebrew University in Jerusalem, adopted a different strategy. Reasoning that endogenous cannabinoids may be as hydrophobic as delta-9-THC, they subjected porcine brains to organic solvent extraction and fractionated the lipid extract by chromatographic techniques while measuring cannabinoid binding activity. This approach turned

out to be highly successful, and the researchers were able to isolate a lipid cannabinoid-like component, which they characterized by mass spectrometry and nuclear magnetic resonance spectroscopy as the ethanolamide of arachidonic acid. They named this novel compound "anandamide" after the sanskrit "ananda," inner bliss.

The chemical synthesis of anandamide confirmed this structural identification and allowed the characterization of its pharmacological properties (112). In vitro and in vivo tests showed a great similarity of actions between anandamide and cannabinoid drugs. Anandamide reduced the electrogenic contraction of mouse vas deferens and closely mimicked the behavioral responses produced by delta-9-THC in vivo; in the rat, the compound was found to produce analgesia, hypothermia, and hypomotility. However, these effects may not be exclusively due to cannabinoid receptor activation, as anandamide is readily metabolized to arachidonic acid, which can be converted in turn to a variety of biologically active eicosanoid compounds. Subsequent studies demonstrated that anandamide is released from brain neurons in an activity-dependent manner (89, 126) and elucidated the unique biochemical routes of anandamide formation and inactivation in the CNS (25, 44, 45, 69, 89). Thus anandamide fulfills all key criteria that define an endogenous cannabinoid (endocannabinoid) substance.

In their 1992 study, Devane, Mechoulam, and coworkers (242) reported that several lipid fractions from the rat brain contained cannabinoid-binding activity, in addition to anandamide's. In characterizing these fractions, they discovered that some of them were composed of polyunsaturated fatty acid ethanolamides similar to anandamide (e.g., eicosatrienoylethanolamide), but others were instead constituted of a distinct lipid component, *sn*-2-arachidonoyl-glycerol (2-AG) (242). Sugiura et al. (330) arrived independently to the same conclusion. That polyunsaturated fatty acid ethanolamides should mimic anandamide, to which they are structurally very similar, does not come as a great surprise. Moreover, the pharmacological properties of these fatty acid ethanolamides, essentially indistinguishable from those of anandamide, and their scarcity in brain relegate them, at least for the moment, to a position secondary to anandamide's. We cannot say the same for 2-AG. This lipid, considered until now a mere intermediate in glycerophospholipid turnover (see sect. II), is present in the brain at concentrations that are ~170-fold greater than those of anandamide and possesses two pharmacological properties that make it crucially different from the latter: it binds to both CB_1 and CB_2 cannabinoid receptors with similar affinities, and it activates CB_1 receptors as a full agonist, whereas anandamide acts as a partial agonist.

Research of endocannabinoids begs for a conjunction of in situ biochemistry and physiology. We have learned much over the past 10 years on the behavioral effects of these molecules, on how these lipid mediators are produced physiologically, and on the functional roles that they may serve. A major step was the discovery that depolarization-induced suppression of inhibition (DSI; or excitation, DSE), a type of short-term synaptic plasticity originally discovered in the cerebellum and the hippocampus (214, 288), is mediated by endocannabinoids (199, 200, 271, 375). This discovery allowed the results of over a decade of research on retrograde synaptic signaling in these networks to be considered as functional characteristics of endocannabinoid signaling. The substrate of retrograde signaling and DSI is the predominantly presynaptic distribution of CB_1 receptors on axon terminals in the hippocampus (188), as well as throughout the brain, where activation of CB_1 by endocannabinoids can efficiently veto neurotransmitter release in many distinct types of synapses (see sect. IV). The conditions of synthesis, release, distance of diffusion, duration of effect, and site of action were all extensively characterized for the mediator of DSI (for review, see Ref. 10) that turned out to be an endocannabinoid (271, 375). The fact that neurons are able to control the efficacy of their own synaptic input in an activity-dependent manner (a phenomenon called retrograde synaptic signaling) is functionally very important, since this mechanism may subserve several functions in information processing by neuronal networks from temporal coding and oscillations to group selection and the fine tuning of signal-to-noise ratio. The crucial involvement of endocannabinoids in these functions just began to emerge from recent studies, which are reviewed in section V. Due to the exceptionally rapid expansion of this field in recent years (and to our special interest in neuronal signaling in complex integrative centres of the brain), we decided to focus the present review on questions related to the composition of the endocannabinoid system and its physiological roles in controlling brain activity at the regional and cellular levels as synaptic signal molecules. We did not aim to provide detailed accounts of studies dealing with other, similarly important, aspects of cannabinoid research, which have been dealt with in excellent recent reviews, e.g., about the relation of the endocannabinoid system to pain modulation (281, 366), the immune system (194), neuroprotection (136), and addiction (228).

The final message of the present review is that to understand the possible physiological functions of the endogenous cannabinoids, their roles in normal and pathological brain activity, pharmacological agents targeting the cascade of anandamide and 2-AG formation, release, uptake, and degradation will have to be developed. Such drugs, which undoubtedly will become invaluable research tools to study the potential functions listed above, may also provide novel therapeutic approaches to diseases whose clinical, biochemical, and pharmacologi-

cal features suggest a link with the endogenous cannabinoid system.

II. THE LIFE CYCLE OF THE ENDOCANNABINOIDS

A. Introduction

A basic principle that has emerged from the last two decades of research on cellular signaling is that simple phospholipids such as phosphatidylcholine or phosphatidylinositol should be regarded not only as structural components of the cell membrane, but also as precursors for transmembrane signaling molecules. Intracellular second messengers like 1,2-diacylglycerol (DAG) and ceramide are familiar examples of this concept. Along with their intracellular roles, however, lipid compounds may also serve important functions in the exchange of information between cells. Indeed, biochemical mechanisms analogous to those involved in the generation of DAG or ceramide give rise to biologically active lipids that leave their cell of origin to activate G protein-coupled receptors located on the surface of neighboring cells. Traditionally overshadowed by amino acid, amine, and peptide transmitters, biologically active lipids are now emerging as essential mediators of cell-to-cell communication within the CNS, where G protein-coupled receptors for multiple families of such compounds, including lysophosphatidic acid and eicosanoids, have been identified (67, 285).

In this section, we discuss the biochemical properties of endogenous lipids that activate brain cannabinoid receptors. These compounds share two common structural motifs: a polyunsaturated fatty acid moiety (e.g., arachidonic acid) and a polar head group consisting of ethanolamine or glycerol (Fig. 1). Because of these features, endocannabinoid substances seemingly resemble the eicosanoids, ubiquitous bioactive lipids generated through the enzymatic oxygenation of arachidonic acid. However, the endocannabinoids are clearly distinguished from the eicosanoids by their different biosynthetic routes, which do not involve oxidative metabolism. The two best characterized endocannabinoids, anandamide (arachidonoylethanolamide) (85) and 2-AG (242, 330), may be produced instead through cleavage of phospholipid precursors present in the membranes of neurons, glia, and other cells. In the following sections, we will first focus on the biochemical pathways that lead to the formation of endocannabinoids in neurons and then turn to the mechanism by which these compounds are deactivated.

B. Biosynthetic Pathways

1. Anandamide biosynthesis

Anandamide formation via energy-independent condensation of arachidonic acid and ethanolamine was described in brain tissue homogenates soon after the discovery of anandamide and was attributed to an enzymatic activity that was termed "anandamide synthase" (81, 83, 201). Subsequent work has demonstrated, however, that this reaction is in fact catalyzed by fatty acid amide hydrolase (FAAH), the primary enzyme of anandamide hydrolysis, acting in reverse (203). Since FAAH requires high concentrations of arachidonate and ethanolamine to synthesize anandamide, higher than those normally found in cells, this enzyme is unlikely to play a role in the physiological formation of anandamide (for further discussion, see sect. II*C6*).

Another model for anandamide biosynthesis is illustrated schematically in Figure 2. According to this model, anandamide may be produced via hydrolysis of the phospholipid precursor *N*-arachidonoyl phosphatidylethanolamine (PE), catalyzed by a phospholipase D (PLD)-type activity (89, 331, 332). The precursor consumed in this reaction may be resynthesized by a separate enzyme activity, *N*-acyltransferase (NAT), which may transfer an arachidonate group from the *sn*-1 glycerol ester position of phospholipids to the primary amino group of PE (89). The validity of this model was initially questioned, be-

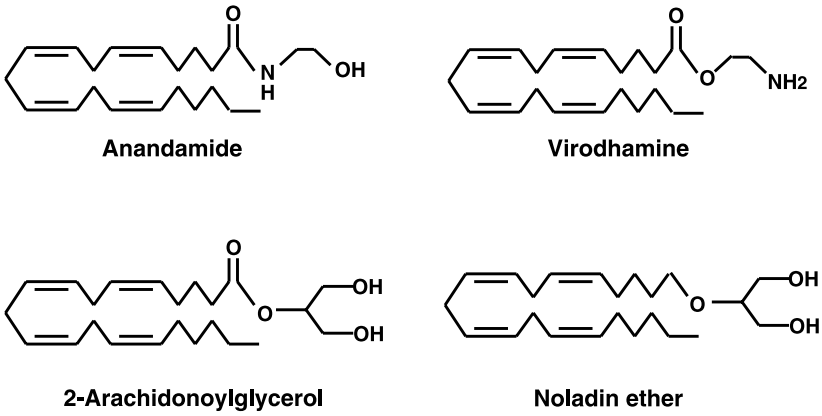


FIG. 1. Molecular structure of endogenous lipids that activate brain cannabinoid receptors. These endocannabinoid compounds share two common structural motifs: a polyunsaturated fatty acid moiety (e.g., arachidonic acid) and a polar head group consisting of ethanolamine or glycerol. For details, see section II, *A* and *B4*.

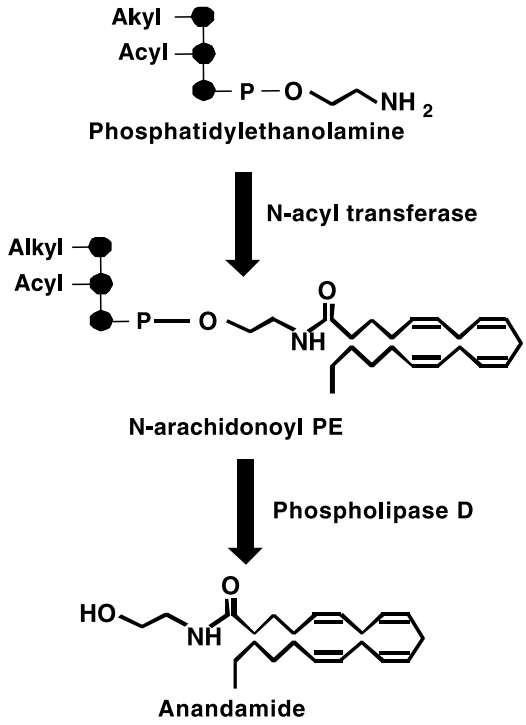


FIG. 2. Scheme illustrating the possible mechanism of anandamide formation. PE, phosphatidylethanolamine. For details, see section II*B1*.

cause previous studies had failed to detect *N*-arachidonoyl PE in mammalian tissues (266, 267, 318). More recent chromatographic and mass spectrometric analyses have unambiguously shown, however, that *N*-arachidonyl PE is present in brain and other tissues, where it may serve as a physiological precursor for anandamide (44, 46, 89, 332).

Although biochemically distinct, anandamide formation and *N*-arachidonoyl PE synthesis are thought to proceed in parallel. Both reactions may be initiated by intracellular Ca^{2+} rises (44, 45, 89, 315, 331, 332) and/or by activation of neurotransmitter receptors (125, 327). For example, administration of dopamine D_2-receptor agonists to rats in vivo causes a profound stimulation of anandamide release in the striatum (125), which is likely mediated by de novo anandamide synthesis (A. Giuffrida and D. Piomelli, unpublished observations). Unfortunately, the two key enzyme activities responsible for these reactions, PLD and NAT, have only been partially characterized, and their molecular properties are still unknown (44, 45, 282, 283).

2. 2-AG biosynthesis

There are two possible routes of 2-AG biosynthesis in neurons, which are illustrated in Figure 3. Phospholipase C (PLC)-mediated hydrolysis of membrane phospholipids may produce DAG, which may be subsequently converted to 2-AG by diacylglycerol lipase (DGL) activity. Alternatively, phospholipase A_1 (PLA_1) may generate a lysophos-

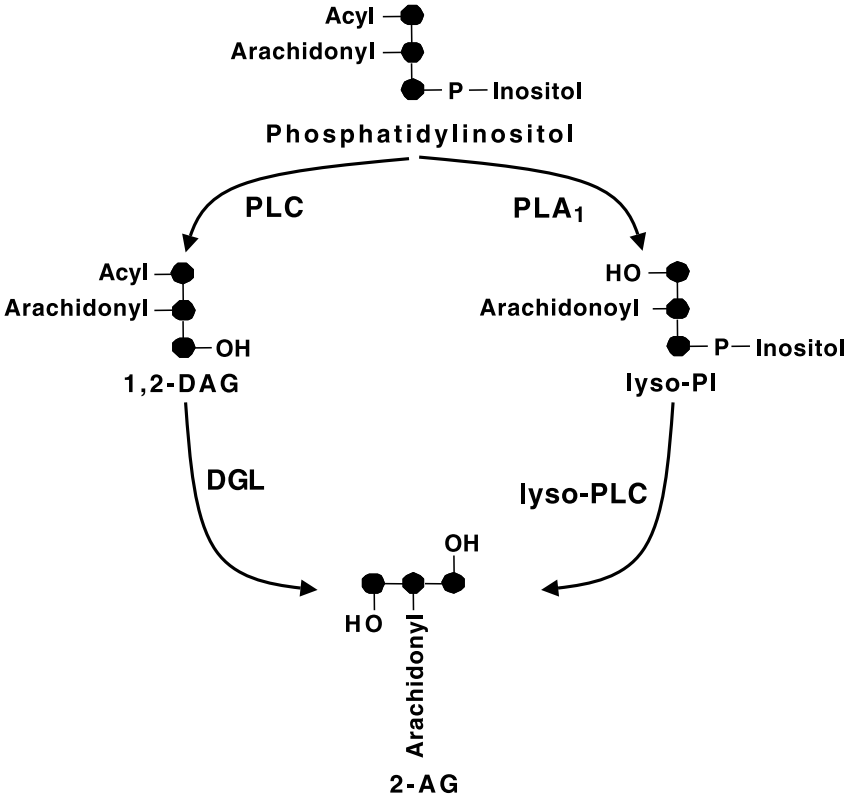


FIG. 3. Scheme illustrating the possible mechanism of 2-arachidonoylglycerol (2-AG) formation. DAG, 1,2-diacylglycerol; DGL, 1,2-diacylglycerol lipase; PI, phosphatidylinositol; PLC, phospholipase C; PLA_1, phospholipase A_1. For details, see section II*B2*.

pholipid, which may be hydrolyzed to 2-AG by a lyso-PLC activity. In the intestine, where 2-AG was originally identified (242), this compound accumulates during the digestion of dietary triglycerides and phospholipids, catalyzed by pancreatic lipases (39). The fact that various, structurally distinct inhibitors of PLC and DGL activities prevent 2-AG formation in cultures of cortical neurons indicates that the PLC/DGL pathway may play a primary role in this process (328). The molecular identity of the enzymes involved remains undefined, although the purification of rat brain DGL has been reported (100, 101).

As first suggested by experiments with acutely dissected hippocampal slices, neural activity may evoke 2-AG biosynthesis in neurons by elevating intracellular Ca^{2+} levels (327, 328). In the hippocampal slice preparation, electrical stimulation of the Schaffer collaterals (a glutamatergic fiber tract that projects from CA3 to CA1 neurons) produces a fourfold increase in 2-AG formation, which is prevented by the Na^+ channel blocker tetrodotoxin or by removing Ca^{2+} from the medium. Noteworthy, the local concentrations reached by 2-AG after stimulation are in the low micromolar range (328), which should be sufficient to activate the dense population of CB_1 receptors present on axon terminals of hippocampal GABAergic interneurons (187, 188). The possible significance of this process for hippocampal network activity is discussed in sections IV and V*C*.

In addition to neural activity, certain neurotransmitter receptors also may be linked to 2-AG formation. For example, in primary cultures of cortical neurons, glutamate stimulates 2-AG synthesis by allowing the entry of Ca^{2+} through activated *N*-methyl-D-aspartate (NMDA) receptor channels (327). Interestingly, this response is strongly enhanced by the cholinergic agonist carbachol, which has no effect on 2-AG formation when applied alone (327). The molecular basis of the synergistic interaction between NMDA and carbachol is unclear at present but deserves further investigation in light of the potential roles of 2-AG in hippocampal retrograde signaling (see sect. V*C*).

3. Fatty acid ethanolamides that do not interact with known cannabinoid receptors

The anandamide precursor *N*-arachidonoyl PE belongs to a family of *N*-acylated PE derivatives, which contain different saturated or unsaturated fatty acids linked to their ethanolamine moieties and give rise to the corresponding fatty acid ethanolamides (FAE). These compounds generally lack CB_1 receptor-binding activity but display a number of remarkable effects and possible biological functions. In this regard, two FAE have been studied in some detail, palmitoylethanolamide (PEA) and oleoylethanolamide (OEA).

PEA exerts profound analgesic and anti-inflammatory effects in vivo, which have been attributed to its ability to interact with a putative receptor site sensitive to the CB_2-preferring antagonist SR144528 (48, 99, 174, 238). The molecular identity of this site is unknown, although it is probably distinct from the CB_2 receptor whose gene has been cloned (260). PEA is present at high levels in skin and other tissues where, together with locally produced anandamide, may participate in the peripheral control of pain initiation (48).

Despite its chemical similarity with PEA, OEA shows weak analgesic properties (49) but exerts potent appetite-suppressing effects in the rat (303). Because these effects are prevented by sensory deafferentation, and intestinal OEA biosynthesis is linked to the feeding state (increasing in fed and decreasing in starved animals), it has been suggested that OEA may be involved in the peripheral regulation of feeding (303).

4. Other endogenous agonists at cannabinoid receptors

A series of close structural analogs of anandamide with activity at cannabinoid receptors have been isolated from brain tissue. These compounds, which include eicosatrienoylethanolamide and docosatetraenoylethanolamide (144), may be generated through the same enzymatic route as anandamide, albeit in smaller quantities.

Distinct from these polyunsaturated ethanolamides as well as from 2-AG are two recently discovered brain lipids: 2-arachidonoyl glyceryl ether (noladin ether) (143) and *O*-arachidonoyl ethanolamine (virodhamine) (291) (Fig. 1). Noladin ether was isolated from porcine brain and identified by using a combination of mass spectrometry, nuclear magnetic resonance, and chemical synthesis. The compound binds to CB_1 receptors with high affinity in vitro [dissociation constant (K_D) 21 nM] and produces cannabinoid-like effects in the mouse in vivo, including sedation, immobility, hypothermia, and antinociception (143). Virodhamine was identified in rat brain by mass spectrometry and chemical synthesis and shown to weakly activate CB_1 receptors in a ^{35}S-labeled guanosine 5′-*O*-(3-thiotriphosphate) (GTPγS) binding assay (half-maximal effective concentration, 1.9 μM) in which the compound also displayed partial agonist activity (291). Moreover, virodhamine decreases body temperature in the mouse, although less effectively than anandamide, and inhibits anandamide transport in RBL-2H3 cells (291). A possible confounding factor in these studies is due, however, to the chemical instability of virodhamine, which in an aqueous environment is rapidly converted to anandamide. The formation and inactivation of these molecules, as well as their physiological significance, is the subject of ongoing investigations (105).

5. Endocannabinoid release

Both anandamide and 2-AG may be generated by and released from neurons through a mechanism that does not require vesicular secretion. However, unlike classical or peptide neurotransmitters, which readily diffuse across the synaptic cleft, anandamide and 2-AG are hydrophobic molecules and, as such, are constrained in their movements through the aqueous environment surrounding cells. How may these compounds reach their receptors on neighboring neurons?

Experiments with bacterial PLD suggest that, in cortical neurons, ~40% of the anandamide precursor *N*-arachidonoyl PE is localized to the cell surface (45), which also contains 2-AG precursors such as phosphoinositol phosphate and bisphosphate (341). This suggests that both endocannabinoids may be generated in the plasmalemma, where they are ideally poised to access the external medium. As with other lipid compounds, the actual release step may be mediated by passive diffusion and/or facilitated by the presence of lipid-binding proteins such as the lipocalins (9).

The existence of different routes for the synthesis of anandamide and 2-AG suggests that these two endocannabinoids could in principle operate independently of each other. This idea is supported by three main findings. First, electrical stimulation of hippocampal slices increases the levels of 2-AG, but not those of anandamide (328). Second, activation of dopamine D_2 receptors in the striatum enhances the release of anandamide, but not that of 2-AG (125). Third, activation of NMDA receptors in cortical neurons in culture increases 2-AG levels but has no effect on anandamide formation, which requires instead the simultaneous activation of NMDA and α-7 nicotinic receptors (327). It is unclear at present whether these differences reflect regional segregation of the PLC/DGL and PLD/NAT pathways, the existence of receptor-activated mechanisms linked to the generation of specific endocannabinoids, or both.

C. Termination of Endocannabinoid Effects: Transport and Degradation

1. Anandamide transport

Carrier-mediated uptake into nerve endings and glia, probably the most frequent mechanism of neurotransmitter inactivation, is also involved in the clearance of lipid messengers. This idea may appear at first counterintuitive: why should a lipid molecule need a carrier protein to cross plasma membranes when it could do so by passive diffusion? A large body of evidence indicates, however, that even very simple lipids such as fatty acids are transported into cells by protein carriers, several families of which have now been molecularly characterized (2, 160, 316). Indeed, carrier-mediated transport may provide a rapid and selective means of delivering lipid molecules to specific cellular compartments (for example, enzyme complexes implicated in lipid metabolism). Thus it is not surprising that neural cells might adopt the same strategy to interrupt lipid-mediated signaling (Fig. 4).

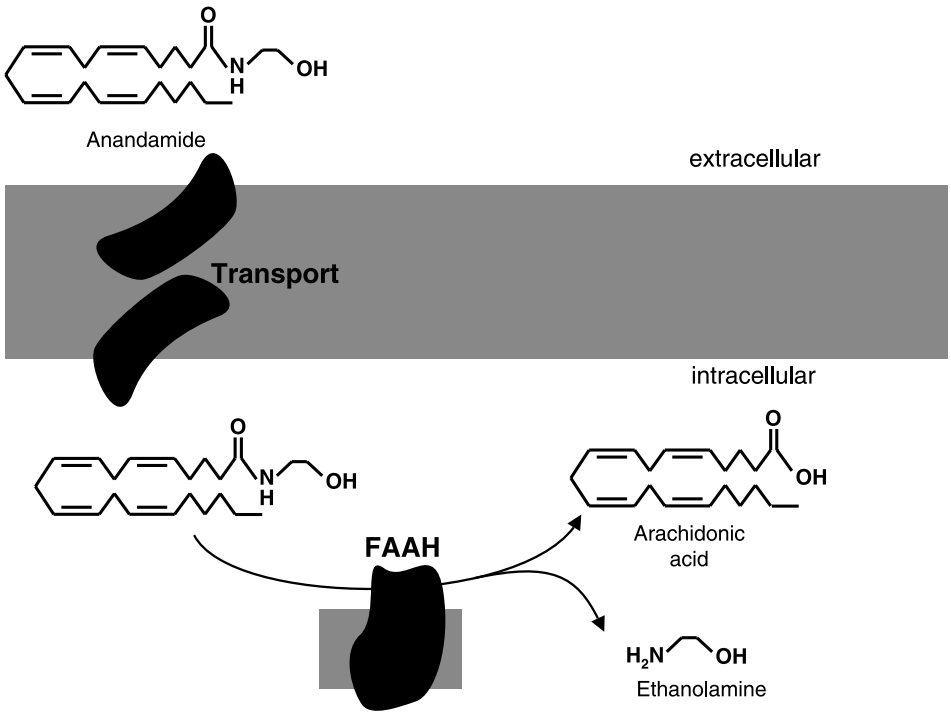


FIG. 4. Scheme illustrating the possible mechanism of anadamide uptake and degradation by an as yet unidentified transporter and a hydrolytic enzyme, fatty acid amide hydrolase (FAAH), respectively. For details, see section II, *C1* and *C6*.

Anandamide transport meets four key criteria of a carrier-mediated process: saturability, fast rate, temperature dependence, and substrate selectivity (25, 89, 156). In rat cortical neurons in primary culture, the uptake of exogenous [^{3}H]anandamide is saturable at 37°C, reaches 50% of its maximum within 4 min, and displays a Michaelis constant (K_m) of 1.2 μM and a maximum accumulation rate (V_{max}) of 90.9 pmol · min^{-1} · mg protein^{-1} (25). Comparable kinetic values are observed in rat cortical astrocytes (K_m = 0.32 μM; V_{max} = 171 pmol · min^{-1} · mg protein^{-1}) and human astrocytoma cells (K_m = 0.6 μM; V_{max} = 14.7 pmol · min^{-1} · mg protein^{-1}) (25, 286), as well as in a variety of nonneural cells (for a review, see Ref. 108). For example, RBL-2H3 basophilic leukemia cells accumulate [^{3}H]anandamide with a K_m of 11.4 μM and a V_{max} of 17.5 × 10^{-17} mol/cell (293).

Anandamide transport differs from that of amine and amino acid transmitters in that it does not require cellular energy or external Na^+, implying that it may be mediated through facilitated diffusion (25, 156, 286, 293). Because anandamide is rapidly hydrolyzed within cells (see sect. II*C6*), it is reasonable to hypothesize that intracellular breakdown contributes to the rate of anandamide transport. Accordingly, HeLa cells that overexpress the anandamide-hydrolyzing enzyme FAAH also display higher than normal rates of [^{3}H]anandamide accumulation (73). However, in primary cultures of rat neurons and astrocytes or in adult rat brain slices, FAAH inhibitors have no effect on [^{3}H]anandamide transport at concentrations that completely abrogate [^{3}H]anandamide hydrolysis (23, 25, 124). From these results it is reasonable to conclude that anandamide transport in the CNS is largely independent of intracellular hydrolysis. Whether persistent disruption of FAAH activity may eventually change the distribution of anandamide between intracellular and extracellular pools is an interesting question that warrants examination.

The substrate selectivity of anandamide transport has been investigated in rat cortical neurons and astrocytes (25, 89) and, more systematically, in human astrocytoma cells (286). In the latter model, [^{3}H]anandamide uptake is not affected by a variety of lipids that bear close structural resemblance to anandamide, including arachidonic acid, PEA, ceramide, prostaglandins, leukotrienes, hydroxyeicosatetraenoic acids, and epoxyeicosatetraenoic acids. Furthermore, [^{3}H]anandamide accumulation in these cells is insensitive to substrates or inhibitors of fatty acid transport (phloretin), organic anion transport (*p*-amino-hippurate and digoxin), and P-glycoproteins (verapamil, quinidine) (286). However, [^{3}H]anandamide uptake is competitively blocked by nonradioactive anandamide (IC_{50} = 15.1 μM) and by the anandamide analog *N*-(4-hydroxyphenyl)-arachidonamide (AM404) (IC_{50} = 2.2 μM) (24, 286). A similar sensitivity to AM404 has been reported for rat cortical and cerebellar neurons (25, 176), rat cortical astrocytes (25), and rat brain slices (24). Inhibitory effects of AM404 on anandamide accumulation also have been observed in a number of nonneural cells, although the concentrations of AM404 needed to produce such effects are generally higher than in neurons (for a review, see Ref. 108). Together, these data are consistent with the view that anandamide is internalized by neurons and astrocytes through a selective process of facilitated diffusion. The molecular identity of the protein(s) responsible for this process is, however, unknown.

2. 2-AG transport

Nonradioactive 2-AG prevents [^{3}H]anandamide uptake in various cell types, suggesting that the two endocannabinoids may compete for the same transport system. Three observations support this hypothesis. First, in astrocytoma and other cells, [^{3}H]anandamide and [^{3}H]2-AG are accumulated with similar kinetic properties (26, 286). For example, in C6 glioma cells, [^{3}H]2-AG uptake displays a K_m of 1.7 μM and a V_{max} of 240 pmol · min^{-1} · mg protein^{-1}, values that are very close to those obtained with [^{3}H]anandamide (26). Second, anandamide and 2-AG can prevent each other's transport (24, 26). Third, the accumulation of either endocannabinoid is blocked with similar potencies by the transport inhibitor AM404 (24, 26). Thus AM404 inhibits [^{14}C]anandamide and [^{3}H]2-AG accumulation in C6 glioma cells with IC_{50} values of 7.5 and 10.2 μM, respectively (26).

Despite these similarities, differences in the properties of anandamide and 2-AG uptake also have been documented. For example, incubation with arachidonic acid causes a marked reduction in [^{3}H]2-AG uptake by astrocytoma cells, but it has no effect on [^{3}H]anandamide accumulation (24). Two alternative explanations may be offered for this discrepancy. Arachidonic acid may directly interfere with a 2-AG carrier distinct from anandamide's, or the fatty acid may indirectly prevent the facilitated diffusion of [^{3}H]2-AG by inhibiting its conversion to arachidonic acid (possibly through product inhibition) in the intracellular compartment. If the latter explanation is correct, agents that interfere with the incorporation of arachidonic acid into phospholipids, such as triacsin C (an inhibitor of acyl-CoA synthesis), also should decrease [^{3}H]2-AG uptake. Accordingly, triacsin C selectively prevents the uptake of [^{3}H]2-AG by astrocytoma cells, but not that of [^{3}H]anandamide (24). Thus, although anandamide and 2-AG may utilize similar transport mechanisms, or even share a common one, they may differ in how their intracellular degradation affects the rate of transport.

3. Structure-activity relationship

Anandamide and 2-AG share three common structural features: *1*) a highly hydrophobic fatty acid chain, *2*) an amide (anandamide) or an ester (2-AG) moiety, and *3*)

a polar head group (Fig. 1). Systematic modifications in the hydrophobic carbon chain indicate that the structural requisites for substrate recognition by the putative anandamide transporter may be different from those of substrate translocation. Substrate recognition may require the presence of at least one *cis* double bond in the middle of the fatty acid chain, indicating a preference for substrates (or competitive inhibitors) with a fatty acid chain that can adopt an extended U-shaped conformation. In contrast, a minimum of four *cis* nonconjugated double bonds may be required for translocation, suggesting that a closed "hairpin" conformation is required in order for substrates to be moved across the membrane (286). Molecular modeling studies show that transport substrates (such as anandamide and 2-AG) have both extended and hairpin low-energy conformers (286). In contrast, extended but not hairpin conformations may be thermodynamically favored in pseudo-substrates such as oleoylethanolamide, which displace [^{3}H]anandamide from transport without being internalized (286, 295).

The effects of head group modifications on anandamide transport have also been investigated (176, 286). The results suggest that ligand recognition may be maintained when the head group is removed (as in arachidonamide), or replaced with substantially bulkier moieties (as in AM404), and when an ester bond substitutes the amide bond (as in 2-AG). Notably, ligand recognition appears to be favored by replacing the ethanolamine group with a substituted hydroxyphenyl group [as in AM404 and its derivative *N*-(2-methyl-4-hydroxy-phenyl)arachidonamide (25, 79) or a furane group (215)] (Fig. 1).

4. Distribution of anandamide transport in the CNS

Biochemical experiments have demonstrated the existence of anandamide transport in primary cultures of rat cortical neurons and astrocytes (25), as well as rat cerebellar granule cells (156). But what brain regions express the transporter is still unclear, primarily due to a lack of molecular understanding of the transporter(s) involved in this process. In one study, the CNS distribution of anandamide transport was investigated by exposing metabolically active rat brain slices to [^{14}C]anandamide and measuring the distribution of radioactivity by autoradiography. The CB_1 antagonist SR141716A (rimonabant) was included in the incubation medium to prevent binding of [^{14}C]anandamide to CB_1 receptors, and AM404 was used to differentiate transport-mediated [^{14}C]anandamide accumulation from nonspecific association with cell membranes and cell debris (124). These experiments suggest that the somatosensory, motor, and limbic areas of the cortex, as well as the striatum, contain substantial levels of AM404-sensitive [^{14}C]anandamide uptake. Other brain regions showing detectable transport include the hippocampus, the amygdala, the septum, the thalamus, the substantia nigra, and the hypothalamus (124).

5. Inhibitors of anandamide transport

Although a variety of compounds have been shown to inhibit anandamide transport, the anandamide analog AM404 remains a standard of reference, mainly because of its relatively high potency and its ability to block anandamide transport both in vitro and in vivo (24, 127, 156, 176, 286, 293).

AM404 inhibits [^{3}H]anandamide uptake in rat brain neurons and astrocytes (25), human astrocytoma cells (286), rat brain slices (24), and a variety of nonneural cell types (see, for review, Ref. 108). The inhibitor also enhances several CB_1 receptor-mediated effects of anandamide, without directly activating cannabinoid receptors (24, 25). For example, AM404 increases anandamide-evoked inhibition of adenylyl cyclase activity in cortical neurons (25), augments the presynaptic inhibition of GABA release produced by anandamide in the midbrain periaqueductal gray (PAG) (358), and mimics the effects of cannabinoid agonists on hippocampal depolarization-induced suppression of inhibition (375) (see sect. v*C*). The fact that the cannabinoid antagonist SR141716A prevents these effects suggests that AM404 may act by preventing anandamide inactivation and enhancing its interactions with cannabinoid receptors. Importantly, however, AM404 also can be transported inside cells (286), where it may reach levels that are sufficient to inhibit anandamide degradation by FAAH (176).

The target selectivity of AM404 has been investigated in some detail. Initial studies showed that AM404 has no affinity for a panel of 36 potential targets, including G protein-coupled receptors, ligand-gated channels, and voltage-dependent channels (22). Subsequent work suggested, however, that AM404 may activate the capsaicin-sensitive VR1 vanilloid receptor in vitro (27, 325, but see Ref. 215 for opposing results). It is unlikely that this effect occurs in vivo, since AM404 does not display any of the pharmacological properties of a vanilloid agonist (see below). Yet, these findings underscore the need to design novel inhibitors of anandamide transport endowed with greater target selectivity. Ongoing research efforts in this direction have led to the development of several arachidonic acid derivatives that are equivalent or slightly superior to AM404 in inhibiting anandamide transport in vitro (79, 215) and in vivo, with effects similar to those of AM404 (77).

Consistent with its low affinity for CB_1 receptors, AM404 does not act as a direct cannabinoid agonist when administered to live animals. The compound has no antinociceptive effects in the mouse hot-plate test (25) and does not reduce arterial blood pressure in the urethane-anesthetized guinea pig (47). In the same models, how-

ever, AM404 magnifies the responses elicited by exogenous anandamide, actions that are prevented by the CB_1 antagonist SR141716A (25, 47). Furthermore, when administered alone, AM404 reduces motor activity (22), attenuates apomorphine-induced yawning (22), decreases the levels of circulating prolactin (132), and alleviates the motor hyperactivity induced in the rat by striatal 3-nitropropionic acid lesions (206). These actions resemble those of anandamide and are blocked by SR141716A (22, 127), suggesting that endogenous anandamide may be involved. In keeping with this notion, systemic administration of AM404 in the rat causes a time-dependent increase in circulating anandamide levels (127).

The participation of anandamide in the effects of AM404 in vivo has been questioned (108) based on the ability of this compound to interact with vanilloid receptors in vitro (27, 325; but see Ref. 215). Yet, the fact that SR141716A blocks the motor inhibitory actions of AM404 at doses that are selective for CB_1 receptors strongly argues for a predominant, if not unique, role of the endocannabinoid system in the behavioral response to AM404 administration. Furthermore, the pharmacological properties of AM404 are very different, often opposite to those of capsaicin and other vanilloid agonists. For example, capsaicin produces pain and bronchial smooth muscle constriction (336), whereas AM404 has no such effect when administered alone, and in fact enhances anandamide's analgesic and bronchodilatory actions (22, 49). The ability of intraperitoneal capsaicin to inhibit movement, described by Di Marzo et al. (91), superficially mimics one property of AM404, but should be viewed with caution, as it most likely results from the strong visceral pain and subsequent "freezing response" elicited by capsaicin. In conclusion, current evidence suggests that AM404 may magnify the actions of anandamide primarily by inhibiting the clearance of this compound from its sites of action.

6. Anandamide hydrolysis: role of FAAH

Almost a decade before anandamide was discovered, Schmid and collaborators (265) identified a hydrolase activity in rat liver that catalyzes the hydrolysis of fatty acid ethanolamides to free fatty acid and ethanolamine (265). That anandamide may be a substrate for such an activity was first suggested by biochemical experiments (80, 81, 89, 159, 351) and then demonstrated by molecular cloning, heterologous expression, and genetic disruption of the enzyme involved (68, 69).

FAAH (previously called anandamide amidohydrolase and oleamide hydrolase) is an intracellular membrane-bound protein whose primary structure displays significant homology with the "amidase signature family" of enzymes (69, 119). It acts as a hydrolytic enzyme for fatty acid ethanolamides such as anandamide, but also for esters such as 2-AG (134, 204) and primary amides such as oleamide (70). Site-directed mutagenesis experiments indicate that this unusually wide substrate preference may be due to a novel catalytic mechanism involving the amino acid residue lysine-142. This residue may act as a general acid catalyst, favoring the protonation and consequent detachment of reaction products from the enzyme's active site (279). This mechanism was recently confirmed by the solution of the crystal structure of FAAH complexed with the active site-directed inhibitor methoxy arachidonyl fluorophosphonate (34).

In addition to FAAH, other enzymes may participate in the breakdown of anandamide and its fatty acid ethanolamide analogs. A PEA-hydrolyzing activity distinct from FAAH was described in rat brain membranes (80) and human megakaryoblastic cells (352). This activity was purified to homogeneity from rat lung and shown to possess a marked substrate preference for PEA over anandamide (353). PEA does not bind to any of the known cannabinoid receptors but produces profound analgesic and anti-inflammatory effects (48, 238), which are prevented by the CB_2-preferring antagonist SR144528 (48, 49). Future studies will undoubtedly address the relative roles of FAAH and this newly discovered enzyme in the biological disposition of PEA and anandamide.

The ability of FAAH to act in reverse (i.e., to synthesize anandamide from arachidonic acid and ethanolamine) has generated some confusion as to the mechanism of anandamide formation. Early reports of anandamide synthesis from free arachidonate and ethanolamine (81, 83) have now been unambiguously attributed to the reverse of the FAAH reaction (16, 181, 203). Because high concentrations of arachidonic acid and ethanolamine are needed to drive FAAH to work in reverse, it is unlikely that this reaction plays a physiological role in anandamide generation (see sect. II*B1*). One possible exception is represented by the rat uterus, where substrate concentrations in the micromolar range are required for the synthetic reaction to occur, implying that FAAH or a similar enzyme might contribute to anandamide biosynthesis in this tissue (319).

7. Structure-activity relationship

Systematic structure-activity relationship investigations have identified several general requisites for substrate recognition by FAAH. First, FAAH accommodates a wide range of fatty acid amide substrates, but reducing the number of double bonds in the fatty acid chain generally results in a decrease in hydrolysis rate (29, 30, 80, 351). Second, replacing the ethanolamine moiety with a primary amide yields excellent substrates. For example, the rate of hydrolysis of arachidonamide is two to three times greater than anandamide's (29, 204). Third, anandamide congeners containing a tertiary nitrogen in the ethanolamine moiety are poor substrates (204). Fourth,

introduction of a methyl group at the C2, C1′, or C2′ positions of anandamide yields analogs that are resistant to hydrolysis, probably due to increased steric hindrance around the carbonyl group (1, 204). Fifth, substrate recognition at the FAAH active site is stereoselective, at least with fatty acid ethanolamide congeners containing a methyl group in the C1′ or C2′ positions (1, 204). Finally, fatty acid esters such as 2-AG also are excellent substrates for FAAH activity in vitro (134, 279).

8. FAAH distribution in the CNS

Early biochemical experiments showed that FAAH activity is abundant throughout the CNS, with particularly high levels in the neocortex, the hippocampus, and the basal ganglia (80, 159). Subsequent investigations have confirmed this wide distribution. Thus, in situ hybridization studies in the rat have found that FAAH mRNA expression is higher in the neocortex and hippocampus; intermediate in the cerebellum, thalamus, olfactory bulb, and striatum; and lower in the hypothalamus, brain stem, and pituitary gland (340). Immunohistochemical experiments suggest that large principal neurons in the cerebral cortex, hippocampus, cerebellum, and olfactory bulb have the highest levels of FAAH immunoreactivity (95, 347). For example, large pyramidal neurons in the neocortex are prominently stained together with their apical and basal dendrites in layer V (347). Moderate immunostaining is observed also in the amygdala, the basal ganglia, the ventral and posterior thalamus, the deep cerebellar nuclei, the superior colliculus, the red nucleus, and motor neurons of the spinal cord (347). A more recent study reported staining of principal cells and astrocytes in various regions of the human brain (307). However, the protein recognized by the antibody utilized in these experiments has an apparent molecular mass of ~50 kDa (by SDS-polyacrylamide gel electrophoresis), which does not correspond to that of native FAAH (~60 kDa) (307).

Many FAAH-positive neurons throughout the brain are found in close proximity to axon terminals that contain CB_1 cannabinoid receptors (see sect. III), providing important evidence for a role of FAAH in anandamide deactivation. Yet, there are multiple other regions of the brain where there is no such correlation, a discrepancy that likely reflects the participation of FAAH in the catabolism of other bioactive fatty acid ethanolamides, such as OEA (302) and PEA (48, 49).

9. Inhibitors of FAAH activity

A number of inhibitors of anandamide hydrolysis have been described, including fatty acid trifluoromethylketones, fluorophosphonates, α-keto esters and α-keto amides (30, 82, 198), bromoenol lactones (23), and nonsteroidal anti-inflammatory drugs (109, 110). These compounds lack, in general, target selectivity and biological availability; thus attempts to use them in vivo (64) should be interpreted with caution.

An emerging second generation of FAAH inhibitors comprises three groups of molecules. The first are fatty acid sulfonyl fluorides, such as palmitylsulfonylfluoride (AM374). AM374 irreversibly inhibits FAAH activity with an IC_{50} of 10 nM and displays a 50-fold preference for FAAH inhibition versus CB_1 receptor binding (82). Systemic administration of AM374 enhances the operant lever-pressing response evoked by anandamide administration, but exerts no overt behavioral effect per se (310), raising the possibility that AM374 may protect anandamide from peripheral metabolism but may not have access to the brain. The second group of FAAH inhibitors is represented by a series of substituted α-keto-oxazolopyridines, which are both reversible and extremely potent (30), but whose pharmacological selectivity and in vivo properties are not yet known. The third group is constituted by a class of aryl-substituted carbamate derivatives (185). The most potent member of this class, the compound URB597, inhibits FAAH activity with an IC_{50} value of 4 nM in brain membranes and an ID_{50} value of 0.1 mg/kg in live rats. This compound has 25,000-fold greater selectivity for FAAH than cannabinoid receptors, which is matched by an apparent lack of cannabimimetic effects in vivo (185). The pharmacological profile of URB597, which is currently under investigation, includes profound antianxiety effects accompanied by modest analgesia (185).

10. Physiological roles of FAAH

The generation of mutant mice in which the *faah* gene was disrupted by homologous recombination has shed much light on the role of FAAH in anandamide inactivation (68). FAAH −/− mice cannot metabolize anandamide and are therefore extremely sensitive to the pharmacological effects of this compound: doses of anandamide that are inactive in wild-type mice exert profound cannabimimetic effects in these mutants. FAAH −/− mice also have markedly elevated brain anandamide levels and reduced nociception (68). This finding is consistent with the roles of anandamide in the modulation of pain sensation (see, for review, Refs. 49, 173) and is supported by the analgesic activity of FAAH inhibitors (185).

Recently, a single nucleotide polymorphism in the human gene encoding for FAAH, which produces a proteolysis-sensitive variant of the enzyme, was found to be strongly associated with street drug and alcohol abuse (324). This important observation reinforces the central role played by the endocannabinoid system in the control of motivation and reward (228).

11. 2-AG hydrolysis: the role of monoglyceride lipase

The fact that FAAH catalyzes the hydrolysis of 2-AG along with anandamide's has prompted the suggestion

that this enzyme may be responsible for eliminating both endocannabinoids. There is, however, strong evidence against this hypothesis. First, inhibitors of FAAH activity have no effect on [^{3}H]2-AG hydrolysis at concentrations that completely block anandamide degradation (24). Second, 2-AG hydrolysis is preserved in mutant FAAH −/− mice, which do not degrade either endogenous or exogenous anandamide (212).

In agreement with these results, a 2-AG hydrolase activity distinct from FAAH has been identified and partially purified from porcine brain (133). This activity likely corresponds to monoglyceride lipase (MGL), a cytosolic serine hydrolase that converts 2- and 1-monoglycerides to fatty acid and glycerol (180). Several findings support this conclusion (93). First, heterologous expression of rat brain MGL confers strong 2-AG-hydrolyzing activity and MGL immunoreactivity to HeLa cells. Second, adenovirus-mediated transfer of the MGL gene in intact neurons increases MGL expression and shortens the life span of endogenously produced 2-AG, without any effect on either 2-AG synthesis or anandamide degradation. Third, MGL mRNA and protein are discretely distributed in the rat brain, with highest levels in regions where CB_1 receptors are also present (93).

The distribution of MGL in the rat hippocampus is particularly noteworthy. The high density of MGL immunoreactivity in the termination zones of the glutamatergic Schaffer collaterals suggests a presynaptic localization of this enzyme at CA3-CA1 synapses. 2-AG may be produced by CA1 pyramidal cells during Schaffer collateral stimulation (328), and the newly generated endocannabinoid may mediate depolarization-induced suppression of inhibition (271, 374, 375; see sect. v*C*), if able to diffuse to the nearby GABAergic boutons, or a suppression of excitation (273, but see sect. v*B2*). Thus MGL is exquisitely poised to terminate the actions of 2-AG at hippocampal synapses.

III. REGIONAL AND CELLULAR DISTRIBUTION OF NEURONAL CB_1 CANNABINOID RECEPTORS

A. Characteristic Differences in CB_1 Receptor Distribution in the Brain

In a landmark study published in 1990, Herkenham and co-workers took advantage of the newly developed cannabinoid agonist [^{3}H]CP-55,940, the same highly selective ligand that had helped identify cannabinoid receptors two years earlier (84), to investigate for the first time the distribution of cannabinoid binding sites in the brain (152). Their results showed that these sites strikingly coincide with the neural substrates for cannabinoid actions predicted from behavioral experiments and started a season of intense research on the CNS distribution of cannabinoid receptors. In the following pages, we will summarize the current status of this research, highlighting the correspondence between cannabinoid receptor distribution and behavioral effects of cannabimimetic agents. In the next sections, we focus on the cellular and subcellular localization of cannabinoid receptors and on the consequences of their physiological or pharmacological activation.

Various radioactive ligands (both agonists and antagonists) have been used to identify the sites of action of cannabimimetic drugs at the regional and cellular level (129, 149–152, 225, 299, 370). One surprising observation stemming from these binding experiments, and confirmed later with other neuroanatomical techniques, is that cannabinoid receptors are much more densely expressed in the rat brain than are any other G protein-coupled receptors (Fig. 5, *A* and *C*) (152). Indeed, in several brain regions cannabinoid receptors are present in densities that are comparable to those of GABA or glutamate receptor channels, which, owing to their relatively low ligand affinities, are highly concentrated at synapses to allow fast neurotransmission to occur. This puzzling finding is still unexplained but can be conceptualized in the light of recent discoveries suggesting that the synaptic functions served by the endocannabinoid system may be much broader than previously suspected. These functions, which will be discussed in detail in section v*C*, appear to be primarily concerned with the short-range, activity-dependent regulation of synaptic strength and to extend to a diversity of CNS structures.

The broad regulatory roles of the endocannabinoids also may be surmised from the diverse effects of cannabimimetic drugs on physiology and behavior. In both animals and humans, these agents elicit a wide, but very distinctive spectrum of biological responses (166), which are epitomized by a tetrad comprising rigid immobility (catalepsy), decreased motor activity, analgesia, and hypothermia. This tetrad assay, developed by Billy R. Martin and his collaborators (see for example, Ref. 65), provides a convenient early screening to identify novel cannabimimetic drugs and highlights the role of the endocannabinoid system in motor behavior. Consistent with such a role, two brain regions that are intimately involved in movement regulation, the basal ganglia and the cerebellum, stand out among others for their very high densities of cannabinoid binding sites (Fig. 5, *A* and *C*). On the other hand, the marked binding capacities observed in limbic areas of the cerebral cortex, especially the cingulate and frontal cortices, as well as the amygdala, concord with the potent analgesic and antihyperalgesic properties of cannabinoid agonists and with their impact on emotional reactivity (Fig. 5, *A* and *C*) (115, 229). Although not as dense, significant cannabinoid binding is also found in other pain-processing areas of the CNS, including the

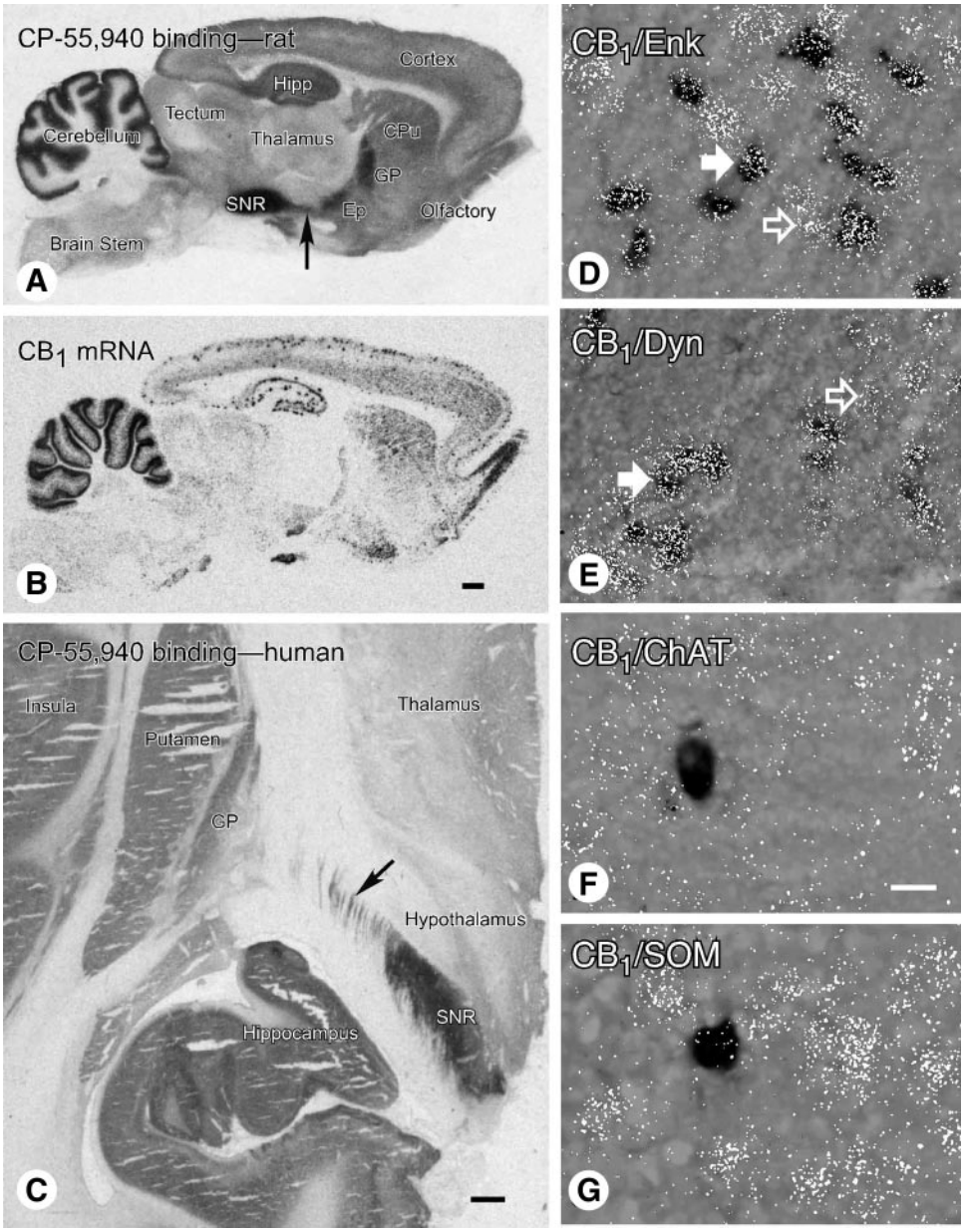


FIG. 5. Autoradiographic film images (*A–C*) show cannabinoid receptor localization in rat (*A*) and human brain (*C*) marked by the tritiated ligand CP-55,940 in an in vitro binding assay described by Herkenham et al. (152). Sagittal slide-mounted section of rat brain hybridized with a CB_1-specific oligonucleotide probe (*B*) shows locations of neurons that express the mRNA at this level. In both rat and human, high levels of receptor protein are visible in the basal ganglia structures globus pallidus (GP), entopeduncular nucleus (Ep), and substantia nigra pars reticulata (SNR). High binding is also seen in the cerebellum; moderate binding levels are found in the hippocampus (Hipp), cortex, and caudate putamen (CPu); and low binding is seen in the brain stem and thalamus. Note that the GP, Ep, and SNR do not contain CB_1 mRNA-expressing cells (*B*); this is because the receptors in these areas are on axons (large arrows in *A* and *C*) and terminals, and the mRNA-expressing cells of origin reside in the caudate and putamen. High-magnification photomicrographs (*D–G*) of rat CPu show that CB_1 mRNA-expressing neurons, marked by white dots (silver grains in the emulsion) are colocalized with enkephalin (Enk) and dynorphin (Dyn) mRNA-positive striatal projection neurons (*D* and *E*, respectively), but not with choline acetyltransferase (ChAT) or somatostatin (SOM) mRNA-positive striatal interneurons (*F* and *G*, respectively). Open arrows depict CB_1-positive but dynorphin- or enkephalin-negative somata, whereas solid arrows indicate double-labeled cells. Scale bars: *B*, 1 mm; *C*, 2 mm; *F*, 20 μm. [*D–G* from Hohmann and Herkenham (164); figure was kindly prepared by Miles Herkenham and Andrea Hohmann.]

PAG and the dorsal horn of the spinal cord. An important property of cannabimimetic agents, which is not modeled by the tetrad assay, relates to the ability of these compounds to influence cognitive functions, including short-term memory and attention (142). The high densities of cannabinoid binding sites in the hippocampus and other cortical structures provide a likely neural substrate for this property (Fig. 5, *A* and *C*).

Sheer density of CNS binding sites is not sufficient to precisely account for the spectrum of cannabinoid effects. Studies on the activation of G proteins by cannabinoid agonists in acutely dissected brain slices have revealed, indeed, the existence of an uneven coupling of cannabinoid receptors with G protein activation in different brain structures (37, 38, 304–306, 322). For example, receptors in structures such as the hypothalamus and the thalamus, although relatively low in number, display very tight G protein coupling, suggesting that they may be more efficacious than receptors found elsewhere in the brain. The molecular basis for these regional variations is unclear at present, but they may help reconcile the comparatively low density of cannabinoid receptors found in the hypothalamus with the profound neuroendocrine effects of cannabinoid drugs (261).

A quantitative summary of the distribution of cannabinoid binding sites in the rat brain has been provided (see Table 1 in Ref. 151). Similar distribution patterns have been found in other mammalian and nonmammalian species (see Fig. 5*C*), implying that the endocannabinoid system may play conserved roles in vertebrate phylogeny (57, 152).

B. Selective Expression of CB_1 Cannabinoid Receptors by Identified Cell Types of Complex Networks

The mapping of brain cannabinoid binding sites by Herkenham et al. (152) preceded by a few months the molecular identification of the first cannabinoid receptor, the G protein-coupled receptor that is now called CB_1 (236). A related gene encoding a second cannabinoid-sensitive G protein-coupled receptor, the CB_2, was identified soon afterward (260). The CB_1 receptor is distributed throughout the body but is predominantly found in neurons of the central and peripheral nervous systems. In contrast, the CB_2 receptor is highly concentrated in immune cells and appears to be absent from CNS neurons (41, 113). Genetic deletion studies have confirmed that CB_1 receptors contribute in a major way to the behavioral effects of cannabimimetic drugs. Thus mutant mice lacking functional CB_1 receptors do not exhibit the tetrad of behavioral responses evoked by cannabinoid agonists (208, 380). As mentioned above, the tetrad only partially illustrates the complexity of cannabinoid actions and ostensibly excludes those involving cognitive systems. It is conceivable therefore that certain responses to cannabimimetic agents may be preserved in mutant $CB_1^{-/-}$ mice (36, 88, 253). This possibility is strongly supported by electrophysiological experiments, which show that $CB_1^{-/-}$ mice, although impaired in their CB_1-mediated regulation of GABAergic transmission, retain an intact cannabinoid modulation of glutamate transmission (Fig. 12) (139). A parsimonious interpretation of these results, which is also consistent with current morphological data (Figs. 9*A* and 10, *A* and *B*) (138), is that glutamatergic axon terminals contain a cannabinoid-sensitive receptor that is molecularly distinct from CB_1 (see sect. IV*B1*B).

To understand the complex neurobiological effects of cannabinoid drugs and their endogenous counterparts, it is first necessary to precisely outline the neuronal cell types that express cannabinoid receptors. The molecular characterization of the CB_1 receptor opened the way to in situ hybridization studies on the CNS distribution of this receptor's mRNA (Fig. 5*B*) (236). The subsequent development of specific antibodies allowed the comparison of mRNA and protein expression, and investigators could now delve in greater detail into the cellular and subcellular localizations of this receptor (see Figs. 6–8) (138, 187, 188, 345). In this section, we synthesize the rapidly growing body of data from several laboratories about CB_1 cannabinoid receptor localization in particular cell types of given brain areas. Remarkably, these anatomical studies confirmed that, likewise to the patterned distribution of cannabinoid binding sites in certain brain regions, expression of the CB_1 receptor gene is restricted to specific cell types subserving distinct functional roles in certain neuronal networks, which may indeed account for the striking diversity of cannabinoid effects.

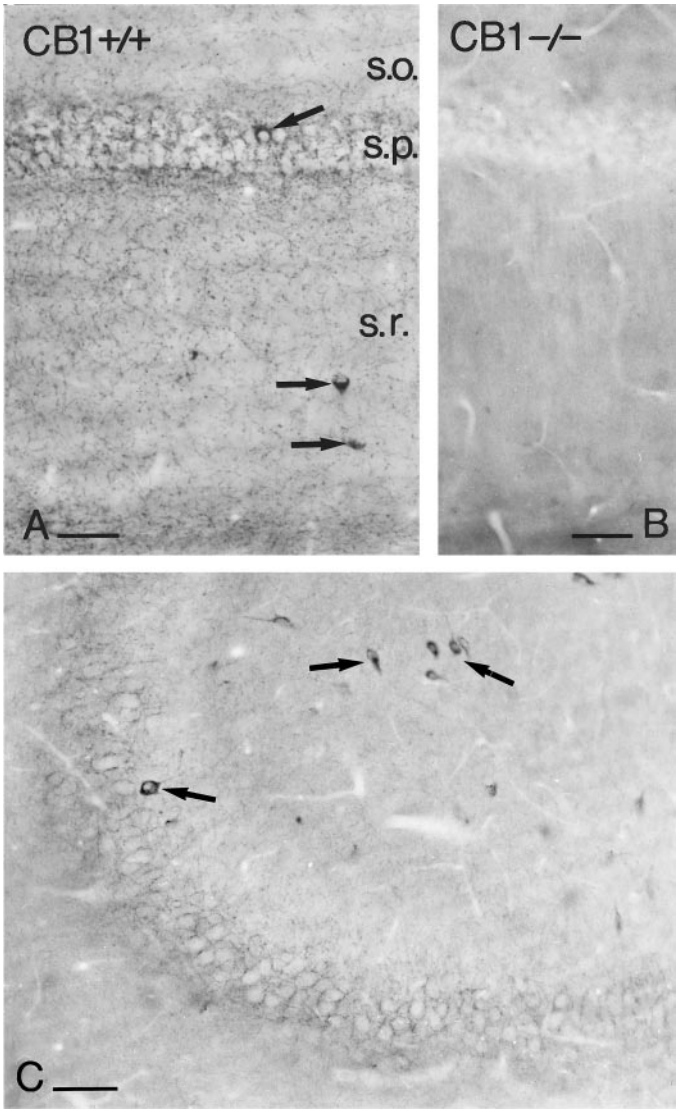


FIG. 6. Light micrographs of hippocampal sections (*A* and *B* from mouse, *C* from rat) immunostained for CB_1 receptor using an antibody raised against a COOH-terminal intracellular epitope (*A* and *B*, showing the CA1 region), and another recognizing the NH_2-terminal extracellular epitope (*C*, showing the CA3 region). The COOH-terminal antibody is more sensitive and provides somewhat stronger labeling, particularly in the dendritic layers, but the general staining pattern is similar. CB_1-positive axon terminals are seen in high density particularly in stratum pyramidale, where they surround the negative cell bodies of pyramidal cells in all subfields. Somatic staining appears only in interneurons mostly in strata radiatum and oriens, occasionally in stratum pyramidale (arrows). No immunostaining is visible in the CB_1 receptor knock-out mouse (*B*). s.o., Stratum oriens; s.p., stratum pyramidale; s.r., stratum radiatum; CB_1 +/+, wild-type mouse; CB_1 −/−, CB_1 receptor knock-out mouse. Scale bars, 50 μm. [Modified from Katona et al. (188) and Hájos et al. (138).]

1. Methodological considerations

Comprehensive in situ hybridization experiments have revealed three populations of brain cells that can be

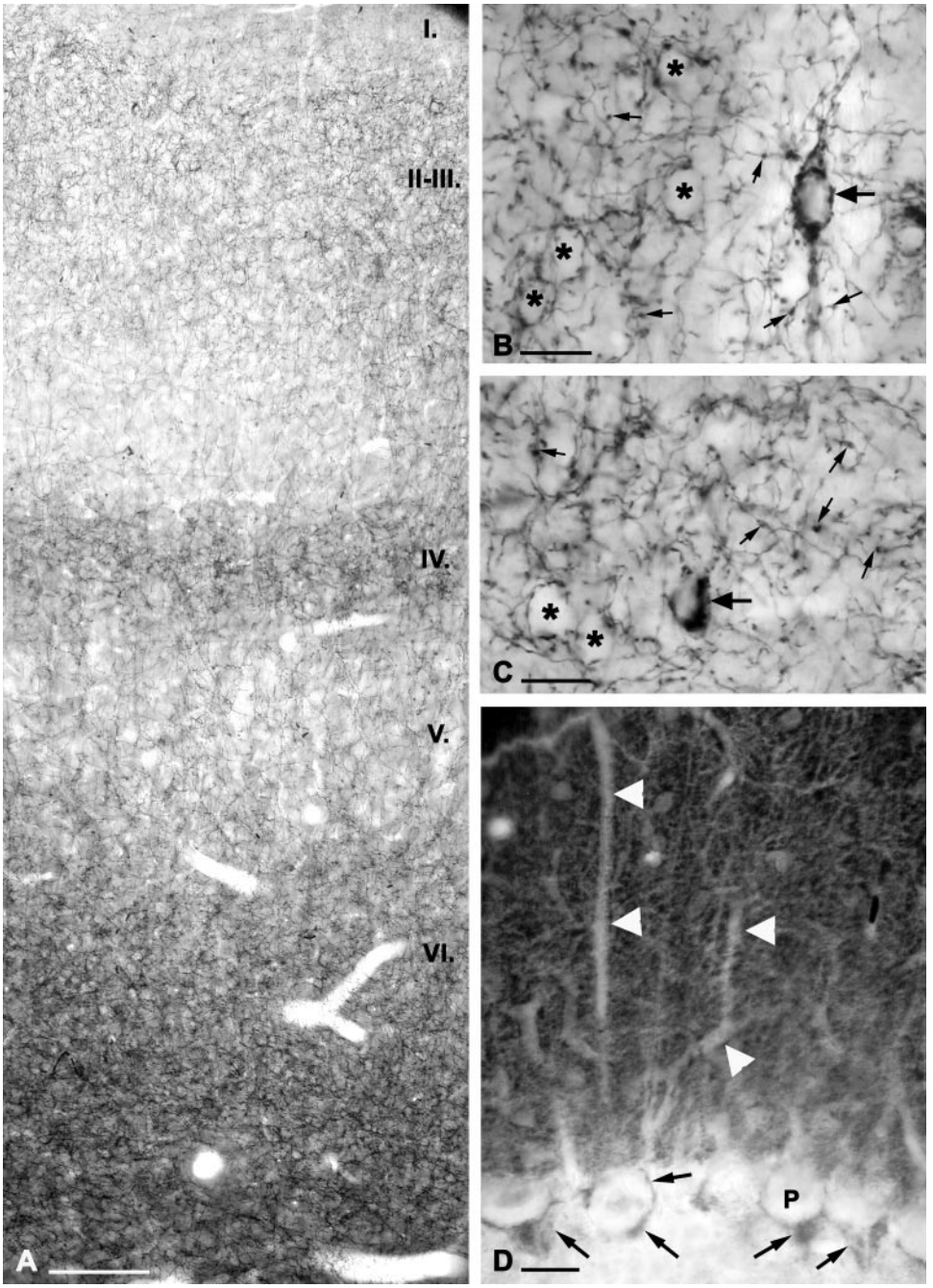


FIG. 7. *A–C*: immunostaining of the rat somatosensory cortex for CB_1 reveals a dense axon labeling in all layers with variable densities. The highest density of labeled fibers can be seen in layers II and upper III, as well as in layers IV and VI. A much smaller amount of stained axons is visible in layer V and deep layer III, whereas layer I has only negligible labeling. Both synaptic varicosities (small arrows) and preterminal, thin axon segments show strong staining, as seen at higher power in *B* (layer II-III) and *C* (layer VI). A large number of varicosities surround the CB_1-negative somata of pyramidal cells (asterisks). CB_1-immunoreactive cell bodies show the characteristics of interneurons (large arrows). *D*: in the cerebellar cortex, Purkinje cells (P) are negative for CB_1; their dendrites (arrowheads) appear as negative images in the otherwise strongly positive molecular layer. The dense terminal labeling in the molecular layer corresponds to both parallel and climbing fibers, and perhaps also includes stellate cell axon terminals. The somata and axon initial segments of Purkinje cells are surrounded by CB_1-positive axons of basket cells (arrows). Scale bars: *A*, 100 μm; *B–D*, 25 μm.

grouped according to their levels of CB_1 mRNA (225, 231, 235). Cells with very high CB_1 mRNA expression are found in many cortical regions, especially in the hippocampus, but also in the anterior olfactory nucleus, the neocortex, and the amygdala. Cells with moderate CB_1 mRNA levels are characteristically present in the striatum and the cerebellum, whereas cells with very weak CB_1 mRNA expression are widespread throughout the brain. Although this broad classification is generally accepted, more precise descriptions of CB_1 mRNA distribution are still controversial. Discrepancies have been reported not only in the intensity of labeling among brain regions, but even in the presence or absence of CB_1 mRNA in certain cell types (even though the authors used the same oligonucleotides). Subsequent immunocytochemical studies have helped clarify some of these issues but have left others unsolved and, indeed, generated their own share of unexplained results. For example, recent reports of strong CB_1 immunostaining in cerebellar Purkinje cells (252, 276) are in striking contrast to the lack of CB_1 mRNA noted in these cells by many investigators (225, 235). Surely, these problems will be appropriately ad-

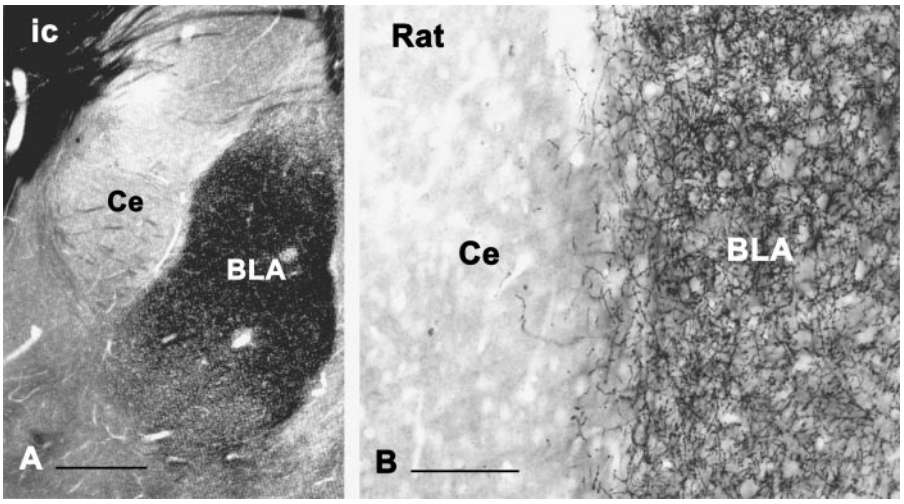


FIG. 8. *A*: remarkable subregional selectivity of CB_1 receptor expression was found in the amygdala. The basolateral nucleus (BLA) is heavily positive for CB_1, whereas the central nucleus (Ce) is devoid of any staining. The sharp border between them is visible at higher power in *B*. The CB_1-positive fibers form a dense network in BLA and surround the negative principal cell bodies and dendrites. The white matter (ic, internal capsule) is dark due to the osmium treatment, not to immunostaining. Scale bars: *A*, 500 μm; *B*, 100 μm. [From Katona et al. (186), copyright 2001 by the Society for Neuroscience.]

dressed and resolved in due time (e.g., by using $CB_1^{-/-}$ mice for probe and antibody testing, see Fig. 6, *A* and *B*). Meanwhile, here we will primarily discuss those results, which are unequivocally supported by a combination of multiple neuroanatomical and functional approaches.

2. Cortical areas

In situ hybridization and immunocytochemical studies consistently show that CB_1 receptors are highly abundant in many forebrain areas, including the anterior olfactory nucleus, the hippocampal formation (Fig. 6), the neocortex (Fig. 7, *A–C*), and the basolateral as well as the cortical amygdaloid nuclei (Fig. 8) (138, 186–188, 225, 231, 235, 345). CB_1-positive cells in these areas display a scattered distribution pattern, represent only a small percent of the total cell population, and belong to the heterogeneous population of GABAergic interneurons (188, 231, 345). In the forebrain, GABAergic interneurons can be divided into various classes based on the cell type-selective expression of neurochemical markers, two prominent examples of which are the neuropeptide cholecystokinin (CCK) and the calcium-binding protein parvalbumin (111, 190). Double-labeling studies have revealed that only one subset of GABAergic interneurons contains CB_1 receptors, those that also express and presumably release CCK. In contrast, other major interneuron types, such as those containing parvalbumin, lack CB_1 receptors. This pattern of expression is common to most forebrain areas, having been found in the anterior cortical nucleus (231), the basolateral amygdala (186, 231, 240), the cortical amygdaloid nuclei (186), the hippocampal formation (188, 231, 346), and the neocortex (28, 231). Moreover, an analogous pattern is also seen in the human hippocampal formation (187). This selective distribution implies that CB_1 receptor-dependent effects of cannabinoids on many of the physiological processes related to these forebrain areas (e.g., cognitive functions like learning and memory) might involve the modulation of a particular subpopulation of GABAergic interneurons and predicts that this interneuron population may be closely connected with the participation of the endocannabinoids in the short-range modulation of synaptic activity, which will be further discussed in section V*C*.

Although the strong expression of CB_1 receptors in GABAergic interneurons of the cortex is now well established, the presence of CB_1 receptors in principal cells of the forebrain is still debated. Initial in situ hybridization studies reported a modest CB_1 mRNA expression in principal neurons of the neocortex (225, 235). Subsequent double-labeling experiments showed, however, that all CB_1-expressing cells in this structure are also positive for the 65-kDa isoform of glutamic acid decarboxylase (GAD65), the GABA-synthesizing enzyme that marks GABAergic cells (231). Moreover, although several investigators have reported low CB_1 mRNA expression in principal neurons of the CA3 and CA1 subfields of the hippocampus (225, 231, 235), a more recent study suggested that CB_1 labeling may be restricted to GABAergic interneurons (254). Even looking at the original figures claiming CB_1 expression in pyramidal neurons (see, for example, Fig. 8*B* of Ref. 225), the density of labeling over the principal cells (5–10 silver grains) seems to be remarkably low compared with the interneuronal labeling (the cells are completely filled with a huge number of grains). This very low expression pattern within the principal neurons of cortical networks is similar in most other forebrain areas and was found in the human brain as well (231, 370).

This disagreement could not be settled by immunocytochemical localization of the CB_1 protein. Experiments with antibodies raised against the NH_2 terminus of the CB_1 receptor found labeling of principal neurons in many forebrain areas (252, 276, 284). However, these studies also report CB_1 immunoreactivity in cell populations from other brain areas, which were found to be

negative for CB_1 mRNA in all in situ hybridization studies (i.e., Purkinje cells in the cerebellum, cells in the laterodorsal nucleus in the thalamus, in the substantia nigra, and in glial cells). Other investigators utilized different antibodies directed either against the NH_2 or the COOH terminus of the CB_1 protein, and unequivocally established antibody specificity with control tests on brains of mutant $CB_1^{-/-}$ mice (Fig. 6, *A* and *B*). These carefully controlled studies found CB_1 immunostaining only in GABAergic interneurons of the cortex (138, 186–188, 345, 346). However, the fact that principal cells were not stained in these experiments does not rule out the possibility that a very low amount of CB_1 protein, undetectable by the antibodies, may be present in principal cells. Moreover, targeting of the receptor to axon terminals could further decrease antibody access to the antigen and account for the lack of cell body staining. Indeed, several laboratories have reported that glutamatergic synaptic currents in neurons of the prefrontal cortex and hippocampus are inhibited by cannabinoid agonists via a presynaptic mechanism (17, 139, 251, 323; see sect. IV*B1*B). Yet, the lack of CB_1 immunoreactivity on axon terminals forming asymmetrical synapses (which are typically excitatory) strongly argues against the presence of CB_1 receptors at these glutamatergic terminals (Figs. 9*A* and 10*B*) (138, 186–188). This clear morphological finding is also supported by work with $CB^{-/-}$ mice, which suggests that an additional receptor, pharmacologically related, but molecularly distinct from the CB_1, may mediate the cannabinoid modulation of glutamatergic transmission in the hippocampus (Fig. 12) (139). We will return to this hypothesis in section IV*B1*B.

3. Basal forebrain

CB_1 receptors are also present in several subcortical nuclei of the basal forebrain. Cells expressing moderate levels of this receptor are located mainly in the tenia

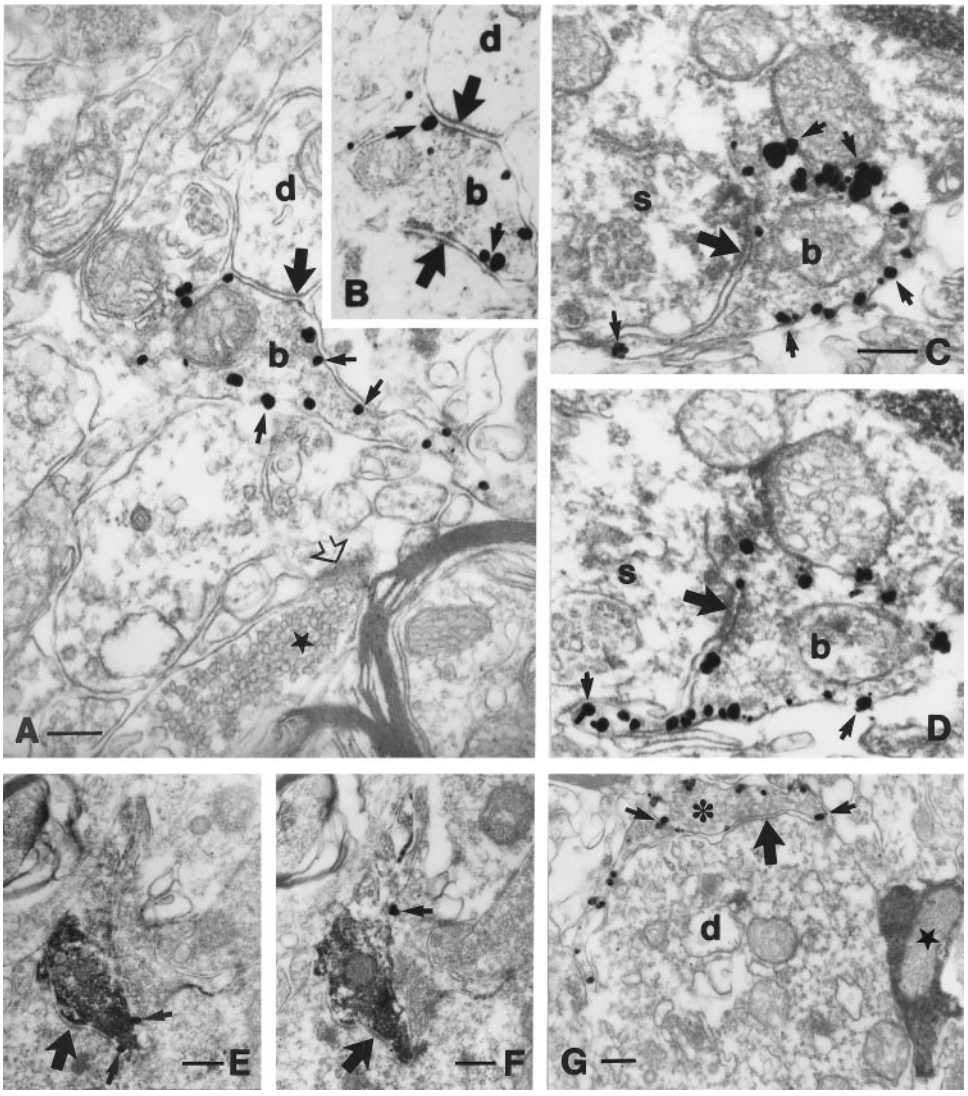


FIG. 9. *A–D*: subcellular localization of CB_1 receptors using an antibody raised against a COOH-terminal intracellular epitope, and the immunogold procedure in the hippocampal CA1 region of the rat. Silver-enhanced gold particles (small arrows) represent CB_1-immunoreactive sites. Labeling was found to outline the inner surface of the membrane of axon terminals (b) that established symmetrical synaptic contacts (large arrows in *A–D*) characteristic of GABAergic synapses. Boutons (asterisk in *A*) forming asymmetrical synaptic specializations (open arrow in *A*) were always negative. CB_1-receptor immunoreactivity was rarely seen on the plasma membrane of cell bodies or dendrites. Serial sections of the same boutons are shown in *A* and *B*, as well as in *C* and *D*, the former synapsing on a dendritic shaft (d) in stratum radiatum and the latter on a soma (s). *E* and *F*: colocalization of CB_1 and cholecystokinin (CCK) in the same axon terminals using the diffusible (homogeneous electron dense) DAB end product to label CCK, and silver-gold to label the NH_2-terminal extracellular epitope of CB_1 (small arrows). The outer surface of the CCK-positive bouton, which forms a symmetrical synapse on a cell body (large arrow), is decorated with silver grains. *G*: no colocalization was found between parvalbumin (star), a marker of another basket cell type, and CB_1 (small arrows) using the same technique. Both terminals form symmetrical synapses (large arrow) on the same proximal dendrite (d) of a pyramidal cell. Scale bars: *A–G*, 0.2 μm. [Modified from Katona et al. (188) and Hájos et al. (138).]

tecta, the lateral and medial septum, and the nuclei of the vertical and horizontal limbs of the diagonal band (225, 231, 235, 345). Colocalization experiments show that CB_1 receptors may be present in somatostatin-positive neurons of the lateral septum (164) and in cholinergic cells in the medial septum and the nucleus basalis of Meynert (216).

4. Basal ganglia

In keeping with the profound impact of cannabimimetic drugs on motor activity (for a review, see Ref. 313), in situ hybridization studies have invariably reported strong expression of CB_1 mRNA in the striatum (225, 235). Detailed analysis at the regional and cellular level uncovered a selective expression pattern in specific components of basal ganglia networks (164, 231). In rodents, the highest density of CB_1 mRNA is found in the dorsolateral portion of the striatum, where the transcript is primarily localized to GABAergic medium spiny cells, which constitute >90% of striatal neurons. In contrast, CB_1 mRNA expression is rather low in two key output structures of the basal ganglia in the globus pallidus and in the substantia nigra. This is also true for the human basal ganglia, which lack however the dorsoventral gradient of mRNA expression seen in rodents (225, 370).

Although the globus pallidus and the substantia nigra pars reticulata contain little CB_1 mRNA, cannabinoid binding is remarkably dense in these structures, implying that CB_1 receptors may be mainly localized to the axons of striatonigral and striatopallidal GABAergic neurons (150). Indeed, two colocalization studies have now established that CB_1 mRNA is expressed by neurons that also contain high levels of the enzyme GAD65 and low levels of its higher molecular mass isoform, GAD67 (164, 231). In a separate study, CB_1 mRNA was found to be coexpressed with both preproenkephalin (a marker of striatopallidal neurons) and prodynorphin (a marker of striatonigral neurons), indicating that striatal projection neurons express CB_1 receptors irrespectively of their specific target region (Fig. 5, *D–E*) (164).

Interestingly, a small fraction of CB_1-positive neurons contain neither preproenkephalin nor prodynorphin (164) and express high levels of GAD67, which is typical of striatal interneurons. Thus, in addition to medium spiny projection cells, other neurons (presumably local-circuit interneurons) also may express CB_1 mRNA. Because colocalization experiments revealed that CB_1 is found neither in somatostatin-positive nor in cholinergic interneurons (Fig. 5, *F* and *G*), the presumptive candidates are the remaining parvalbumin-containing cells (164). Indeed, Marsicano and Lutz (231) demonstrated that ~15% of the CB_1-expressing neurons are positive for parvalbumin, providing direct evidence that striatal local-circuit neurons express CB_1 receptors. It is important to reiterate that this expression pattern is opposite to the one found in cortical and amygdaloid structures, where parvalbumin-positive interneurons do not express CB_1 receptors (186, 188, 231, 346). While the above results are based on the presence of CB_1 mRNA in striatal projection neurons and local-circuit cells, the cellular expression pattern has not been confirmed yet at the protein level, although the presence of the CB_1 protein in striatal neurons has already been demonstrated by immunostaining (345).

5. Thalamus

In situ hybridization studies have reported very low levels of CB_1 mRNA expression in the thalamus (225, 235). Subsequent work confirmed this finding both at the mRNA and at the protein level and extended it to the human brain (231, 345, 370). Neurons expressing moderate amounts of CB_1 mRNA were observed in the habenula and the anterior dorsal part of thalamus, while CB_1-immunoreactive cells were found in the reticular nucleus and zona incerta (225, 231, 235, 284, 345). Further studies are needed, however, to unambiguously identify these cells and solve remaining inconsistencies in the literature regarding their exact location in different nuclei. This need is further underscored by the finding that anterior and dorsal nuclei of the thalamus may express high levels of monoacylglycerol lipase, an intracellular serine hydrolase implicated in terminating the biological effects of the endocannabinoid, 2-AG (93).

6. Hypothalamus

There is a coherent body of evidence indicating that the endocannabinoid system participates in the hypothalamic regulation of feeding (90) and neuroendocrine function (261). Likewise, anatomical investigations agree in finding moderate levels of CB_1 receptor expression in the ventromedial and anterior nuclei of the hypothalamus (225, 231, 235), while pharmacological experiments suggest that these receptors may be particularly well coupled to G proteins (37, 38). Importantly, a double-labeling study showed that CB_1 receptors are colocalized with calretinin, a marker for glutamatergic neurons in select hypothalamic nuclei (193), but not with GAD65 (231). This suggests that glutamatergic, but not GABAergic, cells may express CB_1 receptors in these nuclei. Other hypothalamic nuclei display very low levels of CB_1 expression in a population of uniformly distributed cells. These nuclei include the medial and lateral preoptic nucleus, the magnocellular preoptic and hypothalamic nucleus, the premammilary nucleus and the lateral nucleus of the mammilary body, and the lateral hypothalamus (225, 231, 235). However, as elsewhere in the brain, there is still disagreement as to the precise identity and localization of hypothalamic CB_1-expressing neurons, which will undoubtedly foster further scrutiny.

7. Midbrain

The finding that noxious stimuli trigger anandamide release in the PAG, as assessed by in vivo microdialysis (365), implies that this midbrain structure may serve as a relay in the pain-processing circuit modulated by the endocannabinoids. Yet, a coherent description of the regional and cellular expression of CB_1 receptors in the midbrain is still lacking. Although current data suggest that several midbrain nuclei may have very low to moderate expression of CB_1 mRNA, they are in conflict regarding the exact identity of these nuclei (225, 235). Immunostaining studies have shown that the superior colliculus contains CB_1-positive neuronal cell bodies, but the identity of these cells was not determined (345). To be able to interpret the growing body of work on the analgesic and antihyperalgesic effects of cannabinoid agents, these morphological gaps need to be filled.

8. Medulla and pons

Detailed morphological studies of the hindbrain are also rare. A notable exception is represented by the recent immunocytochemical demonstration of CB_1 receptors in the dorsal vagal complex of the ferret, which may be relevant to the autonomic and antiemetic effects of cannabinoid agonists (355). The exclusive presence of these receptors in local GABAergic interneurons, but not in preganglionic motor neurons (355), shows how this intriguing morphological leitmotif may recurrently be found at most levels of the neuraxis.

9. Cerebellum

CB_1 receptor mRNA is highly abundant in the cerebellum (Fig. 5*B*) (225, 235). Owing to the well-determined circuitry of the cerebellar cortex, along with its laminar structure, the identification of neuronal elements expressing CB_1 receptors in this region is relatively straightforward. Strong expression levels are found in glutamatergic granule cells, but not in the GABAergic Purkinje cells (Fig. 7*D*). In the molecular layer, several large cells were also reported to express CB_1, which might belong to the basket and stellate cells (345). However, it is not known whether all cerebellar interneurons express CB_1 or a subtype selectivity exists among them.

10. Spinal cord

One of the most important aspects of cannabinoids in terms of medicinal usefulness is their analgesic and antihyperalgesic effect at multiple stages of the pain-processing pathway, from high cognitive centers of the forebrain to the midbrain and down to peripheral tissues (48, 229, 234, 245, 297, 365). The spinal cord, where cells expressing CB_1 receptors have been extensively characterized, is obviously an integral component of this circuit. Most in situ hybridization and immunostaining studies agree that CB_1 receptors are present in select neuronal populations of the spinal dorsal horn (7, 102, 165, 311). In lamina II, GABAergic neurons expressing CB_1 also contain nitric oxide synthase (NOS), a marker for a subset of spinal interneurons called islet cells (311). In addition, CB_1-positive cells have also been found in lamina X, which surrounds the central canal of the spinal cord (311); however, by using a different antibody, these cells could only be visualized after spinal transection (102).

The presence of CB_1 receptors in the dorsal root ganglia is now well established (for review, see Ref. 258). Primary sensory neurons in these ganglia are classified based on the selective expression of various neuropeptides [calcitonin gene-related peptide (CGRP), substance P, somatostatin], or the responsiveness to neurotrophic factors [nerve growth factor (NGF), glial-derived growth factor (GDNF), present in nociceptive neurons]. These cell-specific markers are rather heterogeneously colocalized with CB_1 receptors. In a small population of dorsal root ganglion cells, CB_1 receptors are present in CGRP- and substance P-expressing neurons, but not in somatostatin-positive cells (165). This suggests that CB_1 receptors may be expressed only by a subset of peptidergic nociceptive neurons, which represent ~25% of all CB_1-positive cells, whereas the remaining CB_1-expressing cells may belong to other subpopulations of nociceptive or nonnociceptive neurons. Work in dorsal root ganglion cultures suggests that CB_1 receptors colocalize with another nociceptor marker, the acid- and heat-sensitive vanilloid receptor 1 (VR1) (8). Further triple immunolabeling experiments confirmed this observation and suggested that ~25% of CB_1-bearing neurons are nonnociceptive and that distinct types of nociceptive neurons express the receptor as well (7, 258). This highly heterogeneous distribution may contribute to explain the unprecedented analgesic effectiveness of cannabinoid agents, particularly in animal models of persistent pain of neuropathic origin (154, 173).

IV. ANATOMICAL, PHYSIOLOGICAL, AND PHARMACOLOGICAL EVIDENCE FOR THE PRESYNAPTIC LOCALIZATION OF CB_1 CANNABINOID RECEPTORS IN THE BRAIN

Based on the selective distribution of CB_1 receptors in the CNS and their pervasive association with GABAergic interneurons, one would predict that the endocannabinoid system may play important and, possibly, unique roles in the local control of neuronal network activity. A growing body of functional evidence supports this prediction. For example, microdialysis experiments have found that anandamide is released in the striatum by activation of dopamine D_2 receptor, where it may act as a short-

range mediator to counterbalance dopamine activity (22, 125). Furthermore, an endocannabinoid substance, which remains unfortunately uncharacterized, has been recently identified as a key component in two related forms of *trans*-synaptic communication, known as depolarization-induced suppression of inhibition (DSI) and depolarization-induced suppression of excitation (DSE) (200, 271, 375). In section v, we discuss how the endocannabinoid system may participate in these processes. But to do that, we first need to take a further step in the localization of CB_1 receptors, down to the subcellular level.

G protein-coupled receptors, such as the CB_1, are embedded within the lipid bilayer of the plasma membrane. The membrane surface of a nerve cell can be subdivided into two functionally distinct spatial domains. The dendritic tree and cell body are equipped to receive synaptic contacts at specialized structures called active zones, where receptors for fast-acting neurotransmitters such as glutamate or GABA are concentrated. G protein-coupled receptors are rarely associated with these structures; rather, a significant proportion of these receptors are found outside the synapse, within the so-called perisynaptic zone or even further away on the dendritic tree (see, for example, Ref. 19), where they can influence synaptic currents and neuronal excitability by triggering the formation of diffusible intracellular second messengers. Another group of G protein-coupled receptors is situated on axon terminals, where they are exquisitely poised to regulate the release of neurotransmitters, thereby controlling the final output of a neuron. Thus the question arises, in which neuronal surface domain are CB_1 receptors localized? The most direct way to approach this question consists, when a receptor-specific antibody is available, in analyzing the subcellular distribution of the receptor by using electron microscopy. This approach can also provide a wealth of information on the structure and function of the synapse, such as the complement of neurotransmitters and additional membrane receptors present. Evidence from anatomical studies such as these, as well as functional experiments, indicates that CB_1 receptors are predominantly found in axon terminal membranes, where they may be involved in the presynaptic regulation of neurotransmitter release.

A. Anatomical Evidence for Presynaptic Cannabinoid Receptors

Indirect anatomical evidence for the localization of CB_1 receptors on axon terminals was first provided by in situ hybridization (236) and receptor binding experiments (152). These studies showed that, in the basal ganglia, CB_1 receptor mRNA is almost exclusively localized to neurons within the striatum (236), whereas cannabinoid binding is strongest in the globus pallidus and the substantia nigra pars reticulata (Fig. 5, *A–C*) (152, 236). This mismatch implies that CB_1 receptors synthesized in the cell bodies of striatal projection neurons are transported to axon terminal fields in the pallidum and substantia nigra. In keeping with this hypothesis, ibotenic acid lesion of the rat striatum produces a marked loss of cannabinoid binding in these two regions (150). A similar presynaptic localization also has been suggested for CB_1 receptors in dorsal root ganglion neurons, because resection of the dorsal root significantly decreases cannabinoid binding in the dorsal horn of the spinal cord (163).

An important achievement in cannabinoid research was the development of specific antibodies recognizing CB_1 receptors, which have become indispensable research tools (94, 138, 284, 345). Antibodies raised against either the NH_2 terminus or the COOH terminus of the CB_1 protein provided crucial information about the precise localization of CB_1 receptors at the regional, cellular, and subcellular levels. However, immunohistochemical studies require careful investigation and well-designed controls, since it is rare that an antibody is absolutely specific for the desired target protein. Thus reports claiming immunoreactivity for CB_1 receptors in cells (e.g., cerebellar Purkinje neurons), which do not produce the mRNA of CB_1, or immunolabeling of glial cells due to the antibody recognizing the antigen carrier protein should be viewed with caution (240, 252, 276, 302). Essential in this regard was the generation of mutant $CB_1^{-/-}$ mice (208, 380), which were instrumental to demonstrate antibody specificity (Fig. 6, *A* and *B*) and limit the confusion resulting from staining artifacts (138, 186).

Initial light microscopy studies revealed the existence of numerous CB_1-immunoreactive fibers throughout the brain (Figs. 6, *A* and *C*, 7, *A–C*, and 8*B*) (94, 95, 138, 186–188, 345). Based on their distinctive morphological appearance, thin and rich in varicosities, these fibers were tentatively identified as axons. This identification received its first subcellular confirmation from work conducted in the rat hippocampus (188). The varicosities observed at the light microscopy level were found to correspond to axon terminals packed with synaptic vesicles and to be densely covered by CB_1 receptors (Figs. 9, *A–D*, and 10). Notably, when an antibody against the extracellular NH_2 terminus of the CB_1 receptor was used in combination with silver-impregnated gold particles, the particles were exclusively found at the outer surface of the axonal plasma membrane (Fig. 10, *C–E*) (188). On the other hand, when the staining was carried out with a different antibody, specific for the COOH terminus, the gold particles only labeled the intracellular surface of the boutons (Figs. 9, *A–D*, and 10, *A* and *B*) (138).

CB_1-positive axons have a scattered pattern of distribution, which largely parallels that obtained with radioli-

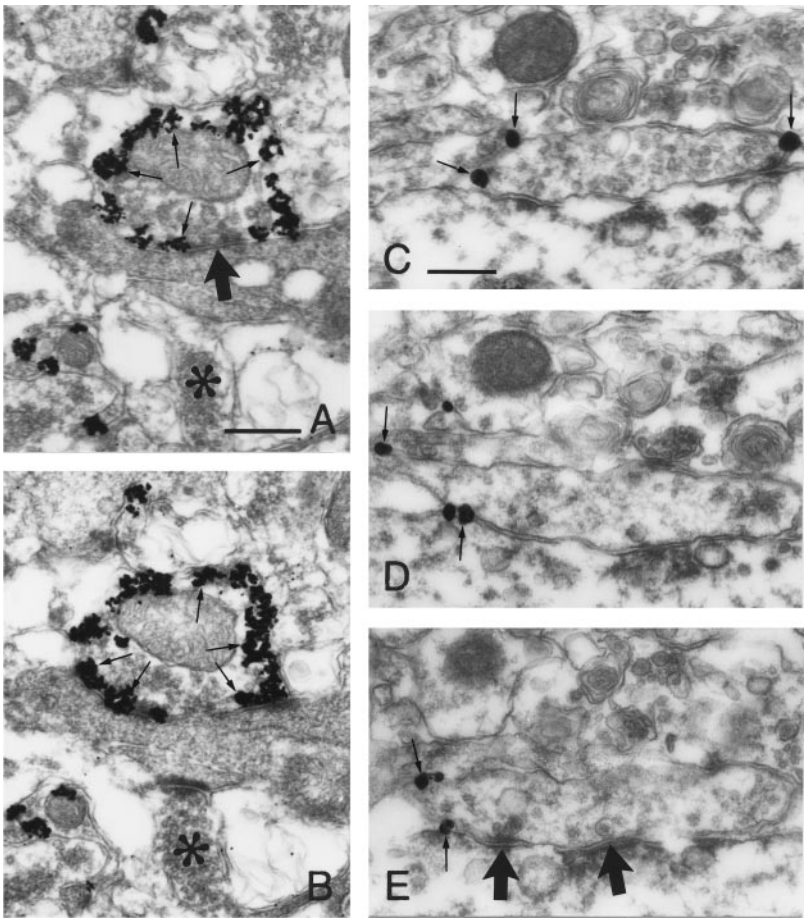
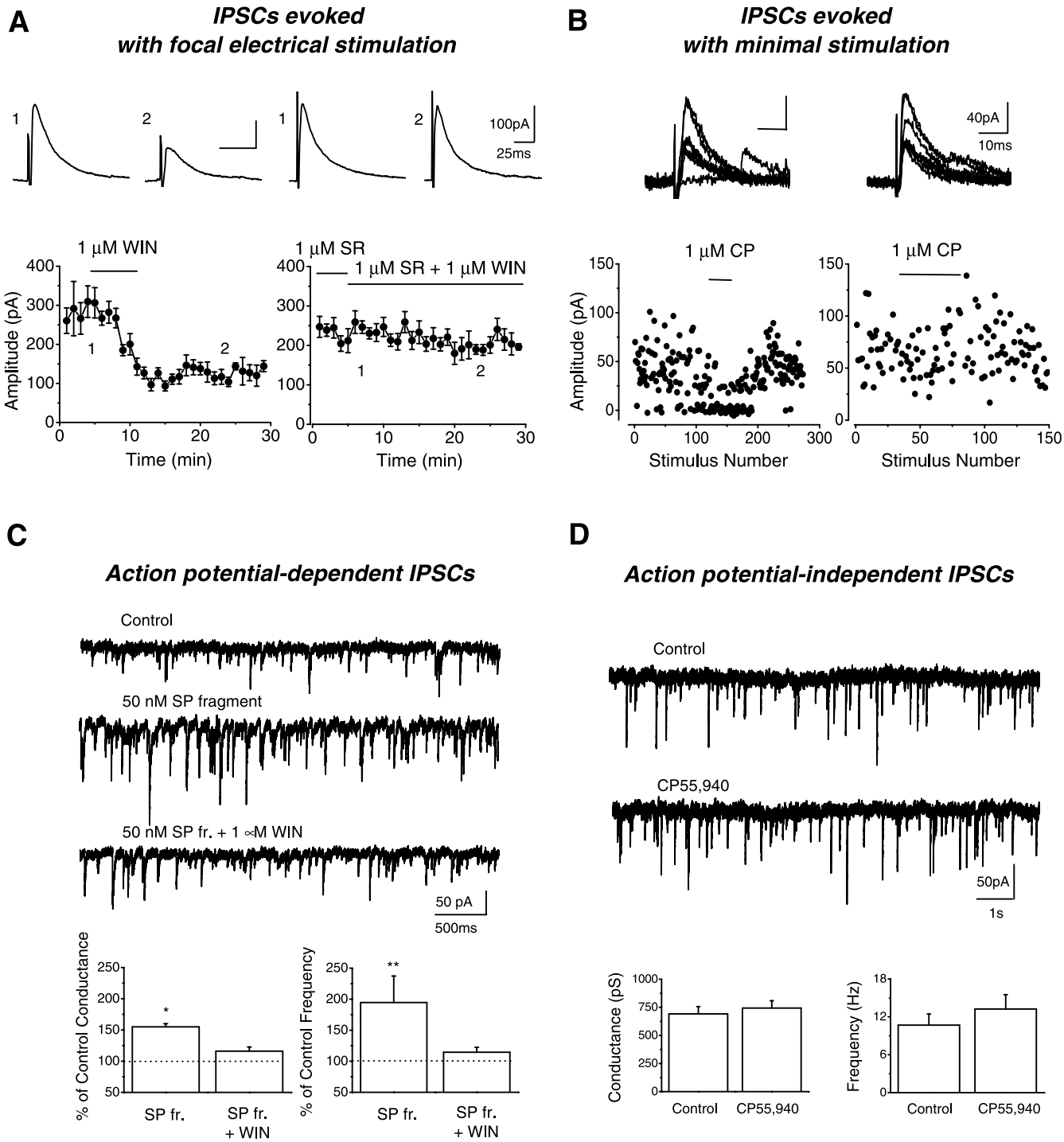


FIG. 10. Subcellular localization of CB_1 receptors in the human hippocampal CA1 region using an antibody raised against a COOH-terminal intracellular epitope (*A* and *B*) and another recognizing an NH_2-terminal extracellular epitope (*C–E*). Silver-enhanced gold particles (small arrows) represent CB_1-immunoreactive sites on the inner (*A* and *B*) and outer (*C–E*) surface of axon terminal membranes, corresponding to the subcellular localization of the respective epitopes. Only boutons forming symmetrical synapses (large arrows) were labeled, as in the rat, which is characteristic of GABAergic, but not of glutamatergic (asterisk) axons. Scale bars: *A–E*, 0.2 μm. [From Katona et al. (187), copyright 2000 with permission from Elsevier Science.]

gand binding (94). An especially dense fiber meshwork is observed in the globus pallidus, the substantia nigra pars reticulata, and the entopeduncular nucleus, probably on axons deriving from the striatum. In many cortical areas, as well as in olfactory systems, CB_1-immunoreactive axons are abundant and form pericellular baskets around CB_1-negative cell bodies. Likewise, CB_1-positive axons equipped with numerous boutons cover the somata of Purkinje cells in the cerebellum and shape the characteristic pinceaux (paint-brush) structures around the axon initial segments (Fig. 7*D*). In addition, the stratum moleculare of the cerebellum also exhibits strong CB_1 immunoreactivity, while leaving blank the dendritic tree of Purkinje cells (Fig. 7*D*).

The cell origin of these fibers can sometimes be inferred from the combination of cellular CB_1 expression pattern and the distribution of CB_1-positive axons. For example, in the cerebellum, the dense staining seen in the stratum moleculare likely results from axons of CB_1-expressing granule cells, which constitute the so-called parallel fibers. In most cases, however, the cell origin and phenotype of CB_1-carrying axons is still uncertain. Recent efforts have helped determine the precise subcellular distribution of CB_1 receptors in the rodent somatosensory cortex, the hippocampus, and the amygdala, as well as in the human hippocampus (28, 138, 186–188). In these areas, CB_1 receptors localize to specific types of axon terminals, and as a rule, boutons engaged in asymmetrical (excitatory) synapses do not carry CB_1 receptors, whereas boutons engaged in symmetrical (inhibitory) synapses do (see for example Fig. 10*B*). This indicates that GABAergic, but not glutamatergic, axon terminals contain the receptors. GABAergic interneurons are extremely heterogeneous, however, and not all of them express CB_1 receptors. Indeed, only a subpopulation of GABAergic interneurons, those that utilize CCK as a peptide cotransmitter, was found to be CB_1 positive (Fig. 9, *E* and *F*), whereas those marked by parvalbumin were not (Fig. 9*G*) (see sect. III*B2*). Because CCK- and parvalbumin-positive interneurons have distinct roles in the regulation of cortical activity, it is likely that endocannabinoid substances also have specific functions in the modulation of cortical network properties. This notion is strongly supported by the retrograde messenger role of endocannabinoids in DSI, which is clearly restricted to select inhibitory synapses within the hippocampus (233, 271, 374) (see sect. V).

Outside the cortex, detailed information on the subcellular distribution of CB_1 receptors is only available for the peripheral nervous system, where CB_1 receptors also appear to be concentrated at nerve endings. In the rat and guinea pig lung, sparse nerve fibers bearing CB_1 receptors are found among bronchial smooth muscle cells (46, 363). Although such fibers rarely form true synapses, immunogold labeling reveals that CB_1 receptors are located close to vesicle accumulations, where they may act to modulate neurotransmitter release. Importantly, neuropeptide Y, a neurochemical marker for noradrenergic sympathetic nerve fibers (18), was found to colocalize with CB_1 in these axon terminals (46, 363). Accordingly, cannabinoids potently inhibit norepinephrine release in peripheral tissues and organs through a presynaptic mechanism (131, 172, 344, 363).



B. Physiological and Pharmacological Evidence for Presynaptic Cannabinoid Receptors

Although anatomical studies may reveal the precise localization site of a particular receptor type, they may only provide predictions about its functional importance. In the last decade, two major approaches, electrophysiological recordings and neurochemical release studies, contributed fundamentally to our understanding of the physiological role of endocannabinoids and the consequences of cannabinoid receptor activation. Most of these studies point to the same conclusion as anatomical studies, i.e., CB_1 receptors presynaptically regulate the release of certain types of neurotransmitters from axon terminals. The major goal of these studies is to establish which of the numerous types of neurotransmitters are influenced by cannabinoids at certain brain areas. Not surprisingly, the release of nearly all major neurotransmitter types was shown to be affected by cannabinoid agents.

Similarly to CB_1-specific antibodies in anatomical experiments, the development of pharmacological probes, such as selective CB_1 receptor agonists and antagonists, was indispensable to advance the field (71, 158, 213, 298). However, as is the case with immunohistochemical experiments, the establishment of the role of CB_1 receptors in many of the described processes requires careful evaluation. Recent studies using $CB_1^{-/-}$ mice provided evidence that conventional cannabinoid receptor ligands, as well as the endocannabinoids, are not exclusively selective for CB_1 receptors (36, 60, 88, 139, 175, 227, 253, 381).

In the following sections, we survey the various lines of pharmacological evidence for the existence of presynaptic cannabinoid receptors on many different types of axons in several brain areas and aim to evaluate in the light of anatomical data whether CB_1 or another molecular target may underlie certain effects of cannabinoids.

1. Cortical areas

A) CANNABINOID EFFECTS ON GABA RELEASE IN CORTICAL AREAS. In the hippocampus, electrophysiological and neurotransmitter release experiments concord in indicating that cannabimimetic agents modulate GABA release via a presynaptic mechanism. Whole cell patch-clamp experiments show that cannabinoid agonists decrease amplitude and frequency of $GABA_A$ receptor-mediated inhibitory postsynaptic currents (IPSCs) elicited by action potentials (Figs. 11, *A–C*, and 12*B*) (138, 161, 171). These effects are mediated by CB_1 receptors, because they are blocked by the CB_1 antagonist SR141716A (Fig. 11*A*) and are completely absent in $CB^{-/-}$ mice (Fig. 12*B*) (138, 139). The presynaptic action of cannabinoids was suggested by the lack of effect on the amplitude of miniature IPSCs (Fig. 11*D*), as well as by a reduction in vesicle release probability (measured using the paired-pulse ratio). These data are in striking agreement with the anatomical studies showing the presynaptic localization of CB_1 receptors on GABAergic axon terminals. In the basolateral amygdala, which has a morphological architecture in many respects similar to the hippocampus, cannabinoid agonists produce comparable responses. The compounds inhibit synaptic $GABA_A$-mediated currents in principal neurons of this region, but cause no such effect in the central nucleus, which does not contain CB_1 receptors (186). The significance of these findings was also recently confirmed in vivo in the prefrontal cortex (104). In accordance with the exclusive expression of CB_1 by GABAergic neurons in the neocortex (231), the cannabinoid receptor agonist WIN 55,212–2 reduced cortical GABA levels, which was prevented by the cannabinoid receptor antagonist SR 141716A (104). Moreover, neurochemical release experiments extended the validity of this finding from the rat (188) to the human hippocampus (187). Taken together, these results indicate that GABAergic axon terminals are one of the major targets of cannabinoids in cortical networks, where they reduce the release of GABA in a CB_1 receptor-mediated manner.

B) CANNABINOID EFFECTS ON GLUTAMATE RELEASE IN CORTICAL AREAS: INVOLVEMENT OF A NEW RECEPTOR? Results from a variety of cortical tissue preparations are consistent in indicating that cannabinoid agonists can reduce excitatory

FIG. 11. Synthetic cannabinoids suppress inhibitory postsynaptic currents (IPSC) in hippocampal pyramidal cells as revealed by whole cell patch-clamp recordings. *A*: plot of the IPSC amplitude shows an ~50% reduction of monosynaptic responses evoked by focal electrical stimulation after bath application of the cannabinoid receptor agonist WIN55,212–2 (WIN). Pretreatment with a cannabinoid receptor antagonist, SR141716A, and its coapplication with WIN55,212–2 prevents the suppression of the evoked IPSC amplitude. *B*: consistent with the anatomical results, only a subset of inhibitory axons is responsive to CB_1 receptor activation. Representative traces evoked by minimal stimulation from two different stimulus sites are shown. As the amplitude plot shows, one of the evoked IPSCs (*left panel*) was sensitive to another cannabinoid ligand, CP55,940, as indicated by the increased number of transmission failures. After washout, the synaptic responses returned to control levels. The IPSCs evoked by stimulation of a different site (*right panel*) were insensitive to the agonist application, since there was no obvious change in their failure rate during the CP55,940 treatment. *C*: activation of substance P receptors enhances the firing rate of predominantly those hippocampal interneurons (5), which express CB_1 receptors (188). As a consequence of increased interneuron firing, both the conductance and frequency of IPSCs increased significantly, the increment of which could be reduced by 1 μM WIN55,212–2 (bar graphs, $n = 7$). *D*: raw traces depicting mIPSCs in the presence of 0.5 μM tetrodotoxin are shown before (*top panel*) and after (*bottom panel*) bath application of the synthetic cannabinoid CP55,940. The averaged mIPSC conductance or the averaged frequency did not differ significantly before or after applying CP55,940 (bar graphs, $n = 8$). [Modified from Hájos et al. (138); figure kindly prepared by Dr. Norbert Hájos.]

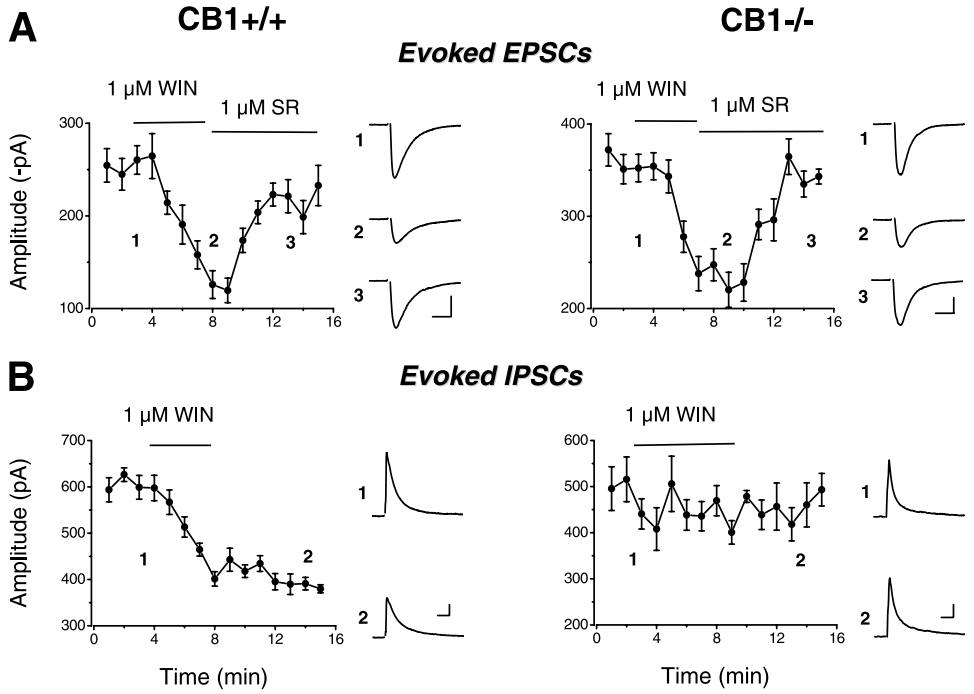


FIG. 12. The cannabinoid receptor agonist WIN55,212–2 (WIN) inhibits glutamatergic but not GABAergic synaptic transmission in CB_1 receptor knock-out mice. *A*: in CA1 pyramidal neurons of both CB_1 +/+ and CB_1 −/− mice, the amplitudes of monosynaptically evoked excitatory postsynaptic currents (EPSCs) were reduced in a similar manner by bath application of 1 μM WIN, the effects of which could be readily reversed by 1 μM SR141716A (SR), a cannabinoid receptor antagonist. *B*: 1 μM WIN decreased the amplitudes of evoked IPSCs in CB_1 +/+ mice but had no effect in CB_1 −/− animals. [Modified from Hájos et al. (139); figure kindly prepared by Dr. Nobert Hájos.]

synaptic neurotransmission (Fig. 12*A*) (15, 17, 251, 323, 333). These actions are probably exerted at a presynaptic locus, for three reasons: *1*) cannabinoid agonists increase paired-pulse facilitation, *2*) they do not change postsynaptic responses to glutamate or kainate applications, and *3*) they cause a characteristic increase in response failures and coefficient of variation of excitatory postsynaptic currents (EPSCs). The ability of the CB_1 antagonist SR141716A to prevent these inhibitory responses suggested early on that CB_1 receptors might be involved. Nevertheless, the fact that careful anatomical analyses negated this hypothesis sent the field up an apparent cul-de-sac: how could cannabinoid agonists inhibit glutamate release if CB_1 receptors are only weakly, if at all, expressed by glutamatergic neurons and are absent from glutamatergic terminals (138, 186–188, 231)? The use of $CB_1^{-/-}$ mice offered a solution to this conundrum. Cannabimimetic agents reduce glutamatergic EPSCs in $CB_1^{-/-}$ mice to the same degree as they do in wild-type ones, although they no longer affect GABAergic IPSCs (Fig. 12) (139). The most economical hypothesis compatible with this result is that glutamatergic axon terminals contain a novel cannabinoid-sensitive site, which is blocked by SR141716A, but is molecularly distinct from the cloned CB_1 receptor.

Further pharmacological characterization revealed that the new cannabinoid-sensitive receptor has an order of magnitude lower affinity for WIN55,212–2 compared with CB_1 (137), as the EC_{50} for the suppression of EPSCs was 2.01 μM, whereas for IPSCs 0.24 μM (161). In addition, cannabinoid effects on EPSCs could be antagonized by the vanilloid antagonist capsazepine, and mimicked by the agonist capsaicin, whereas vanilloid compounds were without effect on GABAergic IPSCs (Fig. 13) (137). These data clearly indicate that cannabinoid receptors controlling IPSCs versus EPSCs are pharmacologically distinct. The latter type is unlikely to be the vanilloid receptor VR1, since WIN55,212–2 does not bind to VR1 on sensory nerves (381). Moreover, VR1 forms a nonselective cation channel (55), whereas cannabinoid effects on glutamatergic EPSCs are mediated via a pertussis toxin-sensitive G protein-coupled process (251, 329), which is in accordance with the ability of WIN 55,212–2 to stimulate [^{35}S]GTPγS binding in several brain regions of CB_1 knockout mice (36). It is reasonable therefore to conclude that a cannabinoid-sensitive receptor other than CB_1 or VR1 is located on glutamatergic, but not on GABAergic, axons in the hippocampus and possibly other brain areas (though we do not know whether this site corresponds with the one identified by Breivogel and collaborators, Ref. 36).

C) CANNABINOID EFFECTS ON ACETYLCHOLINE RELEASE IN CORTICAL AREAS. The cannabinoid receptor agonist WIN 55,212–2 decreases acetylcholine release from electrically stimulated rat hippocampal slices (120). This effect is mimicked by other synthetic cannabinoid agonists, as well as by the endocannabinoid anandamide, and is prevented by the CB_1 antagonists SR141716A and AM281 (121–123, 182–184). Comparable inhibitory actions also have been demonstrated in the rodent neocortex (121, 183). The role of CB_1 receptors in these responses, suggested by the effects of CB_1 antagonists, is further supported by anatomical and genetic data. CB_1 receptors are expressed by neurons in the medial septum and ventral diagonal band, where cholinergic innervation of the hip-

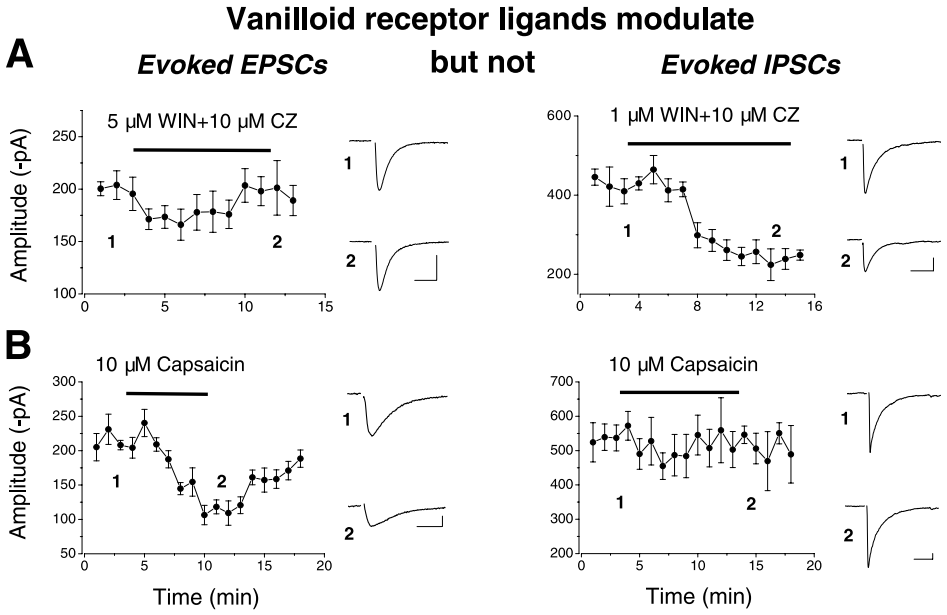


FIG. 13. Vanilloid receptor ligands regulate glutamatergic, but not GABAergic, neurotransmission in the rat hippocampus. *A*: bath coapplication of the synthetic cannabinoid agonist WIN55,212–2 (1–5 μM, WIN), with the vanilloid receptor antagonist capsazepine (10 μM; CZ), prevented the suppression of monosynaptically evoked EPSC amplitude, but not the amplitude of evoked IPSCs in CA1 pyramidal cells. *B*: 10 μM capsaicin, a vanilloid receptor agonist, suppressed the amplitude of evoked EPSCs, but not of evoked IPSCs. [Modified from Hájos and Freund (137); figure kindly prepared by Dr. Nobert Hájos.]

pocampus originates (225, 235). In the monkey forebrain, septal CB_1-immunoreactive cells, along with other CB_1-positive neurons in the nucleus basalis of Meynert (where the cortical cholinergic pathway originates), express choline acetyltransferase (ChAT), the synthetic enzyme for acetylcholine (216). Furthermore, the cannabinoid modulation of acetylcholine release was reduced in "knockdown" experiments with antisense oligonucleotides (182) and abolished in the hippocampus and the neocortex of CB_1 knock-out mice (184). Although unequivocal anatomical demonstration of CB_1 receptors on cholinergic axon terminals is still needed, physiological evidence also supports their existence. In hippocampal slices perfused with a Ca^{2+}-free, K^+-rich medium containing the Na^+ channel blocker tetrodotoxin, cannabinoid agonists attenuate Ca^{2+}-evoked acetylcholine release, probably by inhibition of voltage-gated Ca^{2+} channels (183). Importantly, a parallel result was obtained in cortical and hippocampal synaptosomes, again implying a presynaptic site of action (121).

What is the functional significance of these in vitro findings? Cholinergic innervation of cortical brain regions is thought to play an important role in cognitive processes, many of which are strongly impaired by cannabinoid treatment (35). An appealing causal link between these observations is strengthened by the finding that cannabinoid agonists reduce acetylcholine levels in rat cortical and hippocampal microdialysates, when administered at relatively high doses (mg/kg) (54, 118). However, recent experiments uncovered that lower doses of these drugs (μg/kg) cause an opposite effect, elevating acetylcholine level in the prefrontal cortex and the hippocampus (3, 4). Such an "inverted U" dose-response relationship warrants further investigation but may be explained by the activation of different cannabinoid receptor types, likely possessing distinct agonist sensitivity (see Refs. 36, 137), or by the dose-dependent engagement of excitatory or inhibitory afferent pathways of the basal forebrain, which may enhance or reduce the intrinsic activity of cholinergic neurons. In any case, the predominant effect of presynaptic CB_1 receptors present on cholinergic axon terminals within the cortex is likely to be the inhibition of acetylcholine release, although such an effect alone may not entirely explain cannabinoid actions on cognition (268).

D) CANNABINOID EFFECTS ON NOREPINEPHRINE RELEASE IN CORTICAL AREAS. Along with cholinergic fibers, ascending noradrenergic pathways are also sensitive to cannabinoid modulation (182, 317). Norepinephrine release is inhibited by cannabinoid agonists, albeit in a species-specific manner, being reduced in human and guinea pig hippocampus and cortex, but not in rat hippocampus or mouse hippocampus, neocortex, and amygdala (122, 123, 153, 184, 317, 344). These species differences are intriguing, especially in light of the highly conserved distribution of CB_1 receptors on the axon terminals of hippocampal GABAergic (138, 139, 187, 188) and septohippocampal cholinergic neurons (184, 216). In the rat locus coeruleus, where ascending noradrenergic pathways originate, CB_1 mRNA expression is very low (225, 235). Thus it would be interesting to determine whether a diverging pattern of CB_1 receptor expression might explain the greater sensitivity of human and guinea pig noradrenergic transmission to cannabinoid regulation.

E) CANNABINOID EFFECTS ON SEROTONIN RELEASE IN CORTICAL AREAS. The finding that cannabinoid agonists reduce both electrically and Ca^{2+}-evoked serotonin release in mouse brain cortical slices (264) accords with the prevailing

presynaptic localization of CB_1 receptors. But whether serotonergic terminals do in fact contain such receptors is still unknown. Notably, the observed maximal reduction (~20%) in serotonin release is quite low compared with other transmitters like acetylcholine or GABA (50–80%) (120, 188, 264). Furthermore, in situ hybridization studies in the raphe nuclei have yielded inconsistent results (225, 235), and immunohistochemical investigations have not yet been reported.

F) CANNABINOID EFFECTS ON CCK RELEASE IN CORTICAL AREAS. As we have already pointed out, CB_1 receptors are located on the axon terminals of a specific GABAergic cell population in several cortical networks characterized by the expression of CCK (Fig. 9, *E* and *F*) (28, 186–188). Therefore, it is not unexpected that cannabinoid agonists inhibit potassium-evoked CCK release in rat hippocampal slices (21). More surprising, however, and still unexplained, is the observation that CCK release is unchanged in the frontal cortex (21). This discrepancy is surprising in light of the coexpression of CB_1 and CCK in the entire neocortex and in the hippocampus (231). In addition, the observed maximal reduction of cholecystokinin was only ~40% in the hippocampus, which seems to be quite low considering the fact that nearly all CCK-containing axon terminals carry CB_1 receptors in this brain region (188). Clarifying this point is particularly important in view of the possible interactions of CCK and anandamide in regulating anxiety and other emotional states (185).

2. Basal ganglia

A) CANNABINOID EFFECTS ON GABA RELEASE IN THE BASAL GANGLIA. CB_1 cannabinoid receptors are expressed by at least three different GABAergic cell populations in the striatum (164, 231). Hence, cannabinoid effects on GABA release in different regions of the basal ganglia are well documented, and solid evidence for presynaptic cannabinoid receptors on GABAergic axon terminals derives from several different pharmacological approaches. Application of cannabinoid agonists to parasagittal slices of the rat midbrain causes a significant reduction of $GABA_A$ receptor-mediated currents recorded in substantia nigra pars reticulata neurons after stimulation of the internal capsule (367). Comparable results were obtained in coronal midbrain sections, although in this preparation GABAergic currents are more likely to derive from local GABAergic interneurons (58). The presynaptic nature of these responses is supported by the increased paired-pulse ratio of evoked IPSCs and by the lack of cannabinoid modulation on $GABA_A$ receptor-mediated currents elicited by bath application of GABA (59, 367).

In vivo experiments have provided additional insight on the roles of CB_1 receptors on striatonigral GABAergic terminals (for review, see Ref. 313). Both systemically and locally applied cannabinoid agonists increase spontaneous activity of substantia nigra pars reticulata neurons, probably by removing an ongoing GABAergic inhibition (247, 339). Moreover, striatal stimulation inhibits the firing of nigral neurons, which is also alleviated by cannabinoids. Blocking of $GABA_A$ receptors by bicuculline reverses this effect, indicating that cannabinoid treatment suppresses GABA release (247).

Striatal stimulation also results in reduced firing of pallidal neurons, and this effect is antagonized by systemic administration of a cannabinoid agonist (248). Surprisingly, local administration of the compound into the globus pallidus does not reverse this effect, raising doubts as to the role of striatopallidal GABAergic projections (249).

Although cannabinoid binding is lower in the striatum compared with its output structures, it is still quite abundant (152). In addition, endocannabinoid release and local cannabinoid receptors may participate in the modulation of striatal neuronal activity (125). Szabó et al. (334) provided electrophysiological evidence that cannabinoids inhibit the amplitude of IPSCs recorded from medium spiny neurons. One presumptive site for this action is the axon terminals of intrinsic inhibitory interneurons (parvalbumin positive; Ref. 231), which provide the major inhibitory control over the activity of striatal projection neurons (197). The contribution of recurrent axon collaterals of medium spiny neurons cannot be excluded at present.

In the shell of the nucleus accumbens, cannabimimetic agents decrease the amplitude of evoked IPSCs and increase the paired-pulse ratio, but do not alter the amplitude of miniature IPSCs, indicating a presynaptic inhibitory effect on GABA release (162, 230). In situ hybridization and immunostaining studies of this region report low CB_1 receptor levels (94, 225, 226, 235, 345), but this low signal may simply reflect a restricted distribution of the receptor to select interneuronal subtypes, as is the case elsewhere in the CNS. The important functions served by the nucleus accumbens in motivational and reward processes and the impact that cannabinoid drugs exert on such processes should encourage further studies aimed at establishing the precise localization of CB_1 receptors in this structure.

B) CANNABINOID EFFECTS ON GLUTAMATE RELEASE IN THE BASAL GANGLIA. The glutamatergic innervation of the basal ganglia derives from three main sources. Neurons in the striatum receive glutamatergic axon terminals from cortical and thalamic projection neurons, whereas neurons in the substantia nigra pars reticulata and globus pallidus receive glutamatergic input from the subthalamic nucleus. Both pathways can be modulated by cannabimimetic agents, which inhibit excitatory postsynaptic currents in the striatum as well as the substantia nigra pars reticulata (116, 168, 169, 335). The increased paired-pulse ratio and coefficient of variation, together with the lack of

effect of cannabinoids on response to bath-applied glutamate, support a presynaptic site of action. In addition, currents evoked by direct glutamate application are not modulated by cannabinoids, demonstrating the lack of a postsynaptic component in these effects. A recent study by Gerdeman et al. (117) provided definitive evidence that the reduction of glutamate release in the striatum is mediated by CB_1 receptors, by showing that this effect is absent in $CB_1^{-/-}$ mice. Furthermore, these authors also demonstrated that the ability of cannabinoid agonists to acutely inhibit glutamate release is a crucial factor in the initiation of striatal long-term depression (117), a form of synaptic plasticity characterized by a persistent diminution in excitatory transmission.

The cannabinoid modulation of glutamatergic neurotransmission in the globus pallidus and the substantia nigra pars reticulata may be of considerable functional importance (313). Indeed, in contrast to striatal GABAergic projections to the output nuclei, which are usually quiescent, the subthalamic glutamatergic innervation to these two structures is tonically active. The administration of cannabinoid agonists produces changes in the firing of pallidal and nigral neurons, which are consistent with a decrease in this intrinsic activity (249, 314). It will be interesting to determine whether the endocannabinoid system plays a similar role and, if so, under which physiological circumstances.

In the nucleus accumbens, cannabinoid agonists reduce the amplitude of field excitatory postsynaptic potentials (EPSPs) as well as EPSCs recorded from medium spiny neurons in the core, but not the shell region of this nucleus (162, 300). The relatively high cannabinoid concentrations required to produce these effects, and the low expression of CB_1 receptors in the projection neurons of the prefrontal cortex, basolateral amygdala, and thalamus that innervate the nucleus accumbens, suggest that in this region, as in the hippocampus (139), cannabimimetic agents target a CB_1-like receptor distinct from CB_1. However, the possibility that the reduced cannabinoid sensitivity may reflect the very low expression level of CB_1 receptors in glutamatergic neurons cannot be excluded, and recent experiments demonstrating that evoked EPCS are not modulated by cannabinoids in CB_1 knock-out mice may also favor this explanation (301).

3. Cerebellum

A) CANNABINOID EFFECTS ON GABA RELEASE IN THE CEREBELLUM. The cerebellum contains one of the highest densities of CB_1 receptors in the brain. Expression of these receptors in local GABAergic interneurons (both basket and stellate cells) has been suggested by many studies, whereas Purkinje cells do not contain CB_1 mRNA (225, 235). Immunostainings revealed CB_1-positive putative GABAergic axon terminals forming a pericellular matrix around the axon initial segment and cell body of Purkinje cells or impinging upon their dendritic tree (Fig. 7*D*) (87, 345).

In accordance, GABAergic synaptic currents recorded from Purkinje cells are strongly modulated by cannabinoids. Takahashi and Linden (337) provided the first evidence that spontaneous IPSCs are suppressed by cannabinoid agonists. They estimated that the amplitude of action potential-dependent IPSCs is reduced by ~75%, whereas the amplitude of miniature IPSCs is not affected, suggesting a presynaptic mechanism of action. Subsequent experiments using paired recording and imaging of calcium transients in inhibitory axon terminals confirmed this observation (87) and extended it, by showing that endocannabinoids may also regulate afferent inhibitory inputs to Purkinje cells in a retrograde manner (see details in sect. V) (87, 199, 377). Verifying the role of CB_1 receptors, this response is absent from $CB_1^{-/-}$ mice (377).

B) CANNABINOID EFFECTS ON GLUTAMATE RELEASE IN THE CEREBELLUM. In the cerebellum, as in many other brain regions, cannabinoids can effectively modulate neurotransmission not only at inhibitory but also at excitatory synapses. Two pathways provide excitatory input to the cerebellum, the climbing fibers originating from the inferior olive and the parallel fibers deriving from local glutamatergic granule cells. Early anatomical studies reported a high density of cannabinoid binding sites in the molecular layer of the cerebellar cortex along with the expression of CB_1 mRNA in granule cells (152, 225, 235), indicating, but not proving, the presence of CB_1 receptors on parallel fibers. Subsequent immunohistochemical experiments supported this notion by revealing a dense CB_1-positive axonal meshwork in the molecular layer (94, 345). Hence, CB_1 receptors are situated in a central position to modulate the excitatory input of Purkinje cells. Indeed, whole cell patch-clamp studies revealed that cannabinoids effectively decrease parallel fiber EPSCs (211, 337). Experimental evidence supports a presynaptic effect, in which activation of cannabinoids results in a reduced probability of glutamate release. Neither the excitability of parallel fibers nor the response to locally applied glutamate was modified by cannabinoid receptor agonists (211, 337). Moreover, although the frequency of miniature EPSCs was decreased, the amplitude was also unchanged, indicating the presynaptic localization of cannabinoid receptors. An important consequence of this phenomenon is that cannabinoids may impair cerebellar long-term depression (211). In addition, recent experiments uncovered that endocannabinoids also serve as retrograde signaling molecules in DSE, a phenomenon discussed in detail in section V (200).

In contrast to the cannabinoid effects on parallel fibers, the localization of cannabinoid receptors on climbing fibers seems to be more modest. While a cannabinoid

agonist strongly reduced the amplitude of parallel fiber EPSCs (~12% of baseline level), EPSCs deriving from putative climbing fibers were only slightly modulated (~74% of baseline level) (337). However, experimental evidence shows that modulation of glutamate release at climbing fibers is also under the control of endocannabinoids released by the postsynaptic Purkinje cells (200, 223). In this case, the role of CB_1 receptors is not clear yet, because only low levels of CB_1 mRNA were found in the inferior olive, where climbing fibers originate (235). Recent data also suggest the existence of additional cannabinoid binding sites in the cerebellum distinct from CB_1, although the molecular identity and precise localization of these putative sites is still unknown (253).

4. Areas and pathways involved in pain perception

A) CANNABINOID EFFECTS ON GABA AND GLYCINE RELEASE IN SPINAL AND SUPRASPINAL NOCICEPTIVE AREAS. Cannabinoid agonists regulate pain sensation by acting at the supraspinal, spinal, and peripheral level (48, 154, 234, 245, 281, 297, 365). One common feature of the regulatory actions of these compounds is their ability to reduce inhibitory neurotransmission in the rostral ventromedial medulla, the PAG, and the trigeminal nucleus caudatus (177, 358, 359). Patch-clamp experiments revealed that in these three structures cannabinoid agonists reduce GABA release through a presynaptic mechanism. In the trigeminal nucleus, the release of another inhibitory transmitter, glycine, is also reduced (177). Although the presynaptic localization of cannabinoid receptors is confirmed by several experiments, the role of CB_1 receptors remains equivocal. In addition, detailed studies clarifying the precise localization of CB_1 receptors at the subcellular level in these brain areas have not yet been conducted.

B) CANNABINOID EFFECTS ON GLUTAMATE RELEASE IN SPINAL AND SUPRASPINAL NOCICEPTIVE AREAS. The regulation of glutamatergic neurotransmission may also contribute to the antinociceptive activity of cannabinoid agonists. In the dorsal horn of the spinal cord, these agents suppress glutamate release from primary sensory afferents. Whole cell patch-clamp recordings in substantia gelatinosa neurons have indeed demonstrated that cannabinoid agonists reduce both the amplitude and frequency of spontaneous EPSCs (259). The frequency, but not the amplitude, of miniature EPSCs was diminished, indicating a presynaptic effect. The presumptive target sites of these effects are the axon terminals of afferent sensory fibers, since evoked EPSCs are also significantly decreased by cannabinoid agonists upon stimulation of the neighboring dorsal root ganglion. The stimulation protocol used in this study indicated that mainly Aδ- and C-fibers were affected, which was also confirmed by using the vanilloid agonist capsaicin (219, 259). These results parallel anatomical evidence that dorsal root ganglion neurons express CB_1 receptors (165), and cannabinoid binding sites are reduced after dorsal rhizotomy or neonatal capsaicin treatment, although only 16% of the total CB_1 receptor population was estimated to be located on C-fibers (163, 165).

Along with the spinal cord, glutamatergic neurotransmission is also affected in neurons of the PAG (358), which may also contribute to the role of cannabinoids in alleviating pain sensation. Interestingly, however, it seems that the inhibitory effect of cannabinoids on glutamate release cannot be extended to all regions involved in antinociceptive activity of cannabinoids. Remarkably, while GABAergic neurotransmission was massively inhibited by cannabinoids in the trigeminal nucleus caudalis, the evoked EPSCs upon stimulation of the trigeminal tract remained unaffected (177). However, the trigeminal tract contains a mixed population of glutamatergic axons with different conduction velocities and activation thresholds. The C-fibers exhibit a higher activation threshold, and thus the effect of cannabinoids on a selected small population of fibers (see above) may be masked by the use of different stimulation protocols (0.07–0.1 Hz in Ref. 177 and 10 Hz in Ref. 259).

C) CANNABINOID EFFECTS ON NEUROPEPTIDE RELEASE IN SPINAL AND SUPRASPINAL NOCICEPTIVE AREAS. As observed throughout the brain, CB_1-bearing terminals in the spinal cord contain modulatory neuropeptides in addition to fast-acting amino acid neurotransmitters. Two neuropeptides, substance P (SP) and CGRP, are coexpressed with CB_1 receptors in dorsal root ganglia (165). Accordingly, low doses of the endocannabinoid anandamide inhibit capsaicin-evoked CGRP release from both central and peripheral axon terminals of primary sensory neurons in the dorsal horn of the spinal cord, as well as in hindpaw skin (296, 297). In addition, at low concentrations, anandamide also inhibits SP and CGRP release elicited by electrical field stimulation (342), an effect that probably results from the activation of CB_1 receptors and may contribute to the analgesic and anti-inflammatory properties of this lipid mediator (48, 297, 342). At high concentrations, which are unlikely to be attained in vivo, anandamide activates capsaicin-sensitive VR1 receptors, thereby stimulating SP and CGRP release (342). Similar concentrations of the compound also increase the frequency of miniature EPSCs in the spinal cord (258). The physiological significance of these findings, if any, is unknown at present.

C. Are There Postsynaptic CB_1 Receptors?

Cannabinoids can evoke physiological responses, which may not be mediated by presynaptic cannabinoid receptors. Recent reports indicate that both endocannabinoids and synthetic cannabinoid agonists modify the ex-

citability of neurons via regulation of distinct potassium conductances present on the extrasynaptic dendritic surface of neurons (74, 75, 148, 222, 227, 320). Within the synapse, the modulation of excitatory postsynaptic responses mediated by NMDA receptors was also reported (103, 141). In addition, cannabinoids are also able to induce or suppress gene expression patterns by activating signal transduction pathways likely to occur in the postsynaptic domain of neurons (32, 33, 128, 290, 354).

However, in most cases, the molecular substrates of these effects have not been unequivocally identified. Certain cannabinoid compounds were shown to activate ion channels and receptors other than CB_1 receptors (36, 60, 139, 227, 381). In addition, although many of the cannabinoid effects mentioned above were blocked by the CB_1 antagonist SR141716A, experiments with $CB_1^{-/-}$ mice demonstrate that this antagonist also recognizes other CB_1-like receptors (Fig. 12) (139, 175). Further studies on genetically modified animals and novel, more selective pharmacological tools are thus needed to dissect all the molecular components of cannabinoid neuromodulation.

In contrast to the well-established evidence of presynaptic CB_1 receptors in various brain regions and on the axon terminals of a number of distinct cell types, the presence of functional CB_1 receptors on the plasma membrane of the dendritic tree or somata of neurons requires more solid evidence than available at present. Although postsynaptic CB_1 receptors have been suggested to exist (276, 302, 311, 312), published data show a clear mismatch between the subcellular localization of the protein epitope used to generate the antibody and the distribution pattern of immunolabeling. The antibody used in these studies was generated against the NH_2 terminus of the CB_1 receptor protein (345), which is expected to be situated on the outer surface of the plasma membrane. In contrast, in these studies dendritic CB_1 labeling is invariably found inside cells and is often distant from the plasma membrane. This pattern might represent a labeling artifact, common in immunogold and immunoperoxidase staining, or may be biologically relevant. In some cases, dendritic CB_1 immunolabeling is clearly associated with intracellular organelles participating in the processing or degradation of proteins, such as the rough endoplasmic reticulum, the Golgi apparatus, or multivesicular bodies (302, 311, 312). Since these intracellular organelles usually intrude into the cytoplasm of proximal dendrites, in single electron microscopic sections they appear to be located within dendritic segments. Nevertheless, these segments belong functionally to the somatic (perinuclear) region, because they compose a continuous network within these structures. In our view, the most likely explanation for this labeling pattern is that the antibody also recognizes the freshly synthesized or degraded CB_1 protein. In support of this idea, correlated light and electron microscopy using high-resolution immunogold technique provide clear-cut evidence that the CB_1 immunostaining visualizing cell bodies and proximal dendrites of interneurons at the light microscopical level is always associated with intracellular organelles, but never with the somatic or dendritic plasma membrane (186, 188). Moreover, the antibody recognizing the NH_2 terminus of the CB_1 receptor selectively labels the axon terminals of these interneurons, and the gold particles are found exclusively on the outer surface of the plasma membrane, demonstrating the availability of the NH_2-terminal epitope for this antibody in conventional electron microscopic preparations (Fig. 10, *C–E*) (188). Thus, in contrast to presynaptic CB_1 receptors, establishing the presence of such receptors on the plasma membrane of the dendritic tree or somata of neurons will require further experimentation.

V. PHYSIOLOGICAL ROLES OF ENDOCANNABINOIDS

In the year 2001, we witnessed the merger of two independent lines of research, namely, decades of investigations into the cellular and network effects of exogenous cannabinoids, and studies on the characteristics of DSI, a form of retrograde synaptic signaling. Wilson and Nicoll (375) and Ohno-Shosaku et al. (271) provided the missing link between these two lines by demonstrating that an as-yet-unidentified endocannabinoid substance mediates DSI. If we want to evaluate the studies that led to the present understanding of endocannabinoid functions, we should follow the milestones of research not only in the field of cannabinoid pharmacology, but also the sequence of discoveries that led to the establishment of the phenomenon, as well as the pharmacology and physiology of DSI, namely, the work that was initiated by the groups of Alger and Marty in the early 1990s (214, 288). Thus this section will synthesize the findings deriving from these two roots of research with the aim to better understand the functional roles of endocannabinoids at the synaptic and network levels.

A. The Cannabinoid Root

It has been known for decades that cannabinoids have a profound influence on learning and memory (76, 155, 250). This may be related *1*) to the impairment of long-term potentiation (LTP) that is generally believed to be linked to learning-associated synaptic plasticity of glutamatergic connections, *2*) to a disturbance of fast and slow oscillations maintained by GABAergic interneurons that secure the necessary synchrony in the discharges of connected neurons, or *3*) to alterations in the activity or release properties of monoaminergic and cholinergic subcortical pathways known to influence cortical plasticity and activity states. Thus the major questions here concern

the brain region(s) and transmitter system(s) involved, as well as the network mechanisms underlying the cannabinoid effects.

In addition to showing an intense CB_1 receptor binding, the hippocampus is known to be a crucial area involved in learning and memory. LTP, as well as fast and slow oscillations, has been investigated most extensively and reproducibly in this brain region, and the underlying synaptic connectivity is relatively well understood. These features together provided sufficient reason to focus the majority of cannabinoid electrophysiology, and a large part of this section of the present review, on the hippocampus. On the other hand, the cerebellum and the basal ganglia are also extensively studied in cannabinoid physiology due to the well-known behavioral effects associated with these regions (for review, see Ref. 313), as well as to the very high density of cannabinoid binding sites (152). In addition, the cerebellum was one of the areas where DSI (and later DSE) was discovered (200, 214). Cannabinoid effects in these two brain regions have been discussed at the cellular level in sections III and IV; here we only focus on implications for DSI/DSE and, whenever data are available, possible network mechanisms.

Glutamate is the major mediator of intracellularly recorded as well as field EPSPs in the hippocampus, and it is the transmitter at synapses that are best known to show long-term plastic changes in strength. In addition, the laminar distribution of CB_1 receptor binding in the hippocampus overlaps with glutamatergic pathways, which together explains why this transmitter has been investigated most extensively. On the other hand, both fast and slow oscillations rely on local GABAergic interneurons in the hippocampus (and neocortex), and in addition, GABA is by far the most dominant neurotransmitter in the cerebellum and basal ganglia as well, providing ample reason for focusing studies also on this transmitter. In addition to influencing learning and memory, cannabinoids have a profound effect on mood, emotions, and motivation, which are known to involve subcortical monoaminergic pathways. Therefore, effects of cannabinoids on dopaminergic, cholinergic, serotonergic, and noradrenergic transmission have also been extensively studied, mostly in the basal ganglia, and to some extent also in the hippocampus, amygdala, and neocortex (see sect. IV).

1. *Effects on evoked potentials and long-term synaptic plasticity*

In general, any drug actions on field EPSPs, population spikes, and paired-pulse (short term) synaptic plasticity are difficult to interpret, since several mechanisms may underlie any observed changes. These mechanisms should be studied by intracellular recordings from single cells or connected cell pairs, and parallel population data should be provided. Such combined studies are rather rare in the cannabinoid field; therefore, we chose to present the data from the literature without necessarily attempting to provide an explanation for the mechanism of cannabinoid actions, and for the conflicting data. Many of the conflicting results may be due to the dual cannabinoid actions on CB_1 and on the new cannabinoid-sensitive receptor that is present on glutamatergic axons in the hippocampus (and possibly also in other areas), which can be influenced by several of the agonists and antagonists that are extensively used today as "selective" CB_1 ligands (36, 139; see sect. IV*B1*B). Some controversial interpretations of earlier studies may result from the shortage of data on the pre- or postsynaptic localization of the cannabinoid receptor(s). Our interpretation of these earlier results rests on the recent knowledge that these receptors are mostly, if not exclusively, presynaptic, as reviewed in section IV.

One of the earliest electrophyiological studies using cannabinoid agoinsts found that cannabinoids suppressed sensory-evoked or spontaneous firing of dentate granule cells and elicited characteristic changes in evoked potential waveform (50, 51). In the hippocampus, Wilkison and Pontzer (372) showed that CB_1 agonists and antagonists had negligible effects on field EPSPs and population spikes, whereas in some other studies cannabinoids were shown to have a dose-dependent biphasic effect on evoked population spikes. At low doses, delta-9-THC augments evoked field EPSPs as well as orthodromically or antidromically evoked population spikes, whereas at higher doses the responses are depressed (202, 269, 356, 369). In recent studies, the endogenous ligand anandamide was shown to decrease the slope of Schaffer collateral-evoked field EPSPs, as well as the amplitude of population spikes in the CA1 region at relatively low (1 μM) and high (10 μM) concentrations as well (15). The antagonist SR141716 prevented the effect of anandamide and when applied on its own induced a small increase in population spike amplitude. This suggests that endogenously released cannabinoids may be capable of inhibiting glutamate release. In contrast, another endocannabinoid, 2-AG, had no effect on the slope of evoked field EPSPs in CA1 (328), implying that the endogenous cannabinoid action observed by Ameri et al. (15) using SR141716A is likely exerted by anandamide alone. This effect is probably due to presynaptic inhibition of glutamate release, since population spike amplitudes evoked by antidromic stimulation (a reflection of excitability) did not change upon cannabinoid receptor activation (15). Higher doses of WIN55,212–2 also reduced paired pulse faciliation in the dentate gyrus, where the effect is again likely to be the inhibition of glutamate release from perforant path terminals (192).

Cannabinoid agonists were also found to decrease

paired-pulse depression of population spikes in the CA1 region (12, 13, 278). Paired-pulse depression of population spikes is thought to be due to recruitment of GABAergic inhibition, which normally decreases pyramidal cell excitability on the second stimulus; thus the interpretation of this result was that cannabinoids may be decreasing this paired-pulse effect by reducing feedback inhibition. Interestingly, a recent study from the same laboratory demonstrated that while 2-AG largely replicates the effect of WIN55,212–2 on paired-pulse inhibition, the other endocannabinoid, anandamide, had an opposite effect. It increased paired-pulse depression (PPD; Ref. 13), which was mimicked by the vanilloid agonist capsaicin, and antagonized by capsazepine. This may be interpreted as signifying an anadamide-induced reduction of glutamate release from axon terminals (including those that activate GABAergic feedback inhibition), which may result in a better activation of these interneurons by the second stimulus, and a concomittant increase in paired-pulse inhibition. These data, together with recent evidence that cannabinoid actions on GABAergic (CB_1) and glutamatergic transmission (new CBR), are mediated by distinct receptors (139, see also sect. IV*B1*B and Fig. 12), and that their effect on glutamatergic EPSCs, but not those on GABAergic IPSCs, can be antagonized by capsazepine (Fig. 13*A*) (137), suggest that the two endocannabinoids may differentially act on the two receptors. Anandamide may selectively inhibit glutamate release, whereas 2-AG may preferentially act on GABAergic terminals, as also suggested by the data of Ameri et al. (15) and Stella et al. (328) discussed above.

One of the first experiments with delta-9-THC was to test its effects, and later those of anandamide, 2-AG, and synthetic cannabinoid ligands, on LTP. The first report suggesting a reduction of LTP in the hippocampus by delta-9-THC came from Nowicky et al. (269). Reduction of LTP was also observed by other laboratories using endogenous ligands, or various agonists and antagonists, which at that time were thought to act on CB_1 receptors alone (62, 63, 251, 278, 328, 338). The mechanism of these cannabinoid actions is difficult to interpret knowing that the employed agonists (e.g., WIN55,212–2) and antagonists may act on both GABAergic and glutamatergic transmission (139). According to Terranova et al. (338), WIN55,212–2 had its maximal inhibitory effect on LTP at a concentration of 3 μM, which is 50% over the EC_{50} of this agonists on glutamatergic EPSCs, and it is more than 10 times the EC_{50} for GABAergic IPSC suppression (137, 161). Similarly, Paton et al. (278) observed a blockade of LTP by 5 μM WIN55,212–2, whereas low doses (250 nM) decreased but did not block LTP. Both doses were effective in reducing paired-pulse inhibition in the same slices (278). The order of magnitude difference between the affinity of CB_1 (located on GABAergic terminals) and the new CB receptor (located on glutamatergic terminals) for WIN55,212–2 suggests that the blockade of LTP is due to a direct inhibitory action of the higher agonist dose on glutamate release. GABA release should be decreased by both of these concentrations of the agonist, which likely accounts for the reduced inhibition of the population spike evoked by the second pulse in the PPD paradigm. Similar cannabinoid effects on LTP were observed also in the presence of picrotoxin (251), which further confirms that the site of action is the glutamatergic axon terminal. Anandamide was also shown to have a concentration-dependent effect on LTP, although it did not block it completely (338). Bath application of 2-AG had a similar effect (328), which is difficult to explain knowing that in the same study 2-AG did not reduce field EPSPs and therefore is unlikely to inhibit glutamate release. It does inhibit GABA release, but that should rather enhance LTP. One possibility is that 2-AG might have a direct (non-CB receptor mediated) action on NMDA receptors, but in the opposite direction than anandamide (141), i.e., reducing Ca^{2+} influx via NMDA receptors, or the inhibitory effect of 2-AG on glutamate release, if there is any, may become detectable only during high-frequency activation.

Another finding difficult to reconcile with the conclusions drawn so far is the enhanced LTP observed in CB_1 knock-out animals (31). It has been long known that suppression of inhibition (e.g., by pharmacological blockade of GABAergic neurotransmission or by induction of the DSI paradigm) facilitates the induction of LTP (53, 371). Thus a loss of endocannabinoid control of GABA release should increase inhibition, which likely counteracts LTP. An alternative explanation might involve a loss of CB_1 receptors from cholinergic or noradrenergic afferents to the hippocampus (see sect. IV) resulting in an enhanced release of these transmitters, which may contribute to the facilitation of LTP (40, 189, 195, 326).

2. Effects on population discharge patterns

The recent increase in cannabinoid research in the last 2–3 years brought about by the characterization of CB_1 receptor-mediated actions on identified neurons and circuits has not as yet resulted in a similar boosting of in vivo research into the effects on network activity patterns. Most of the data that can be reviewed here are over 20 years old and were obtained without the currently available more selective and reliable drugs.

The septal driving of hippocampal theta rhythm was shown to be decreased by delta-9-THC, and the effect was attributed to a reduction of noradrenergic transmission (135), which normally acts in the medial septum as well as in the neocortex and hippocampus. This interpretation is consistent with recent evidence for a cannabinergic reduction of norepinephrine release (see sect. IV*B1*D), but does not take into consideration direct cannabinoid effects on the GABAergic and glutamatergic components of

the cortical/hippocampal circuitry. The peak-to-peak voltage of cortical electroencephalogram (EEG) recorded with chronic electrodes was found to decrease after acute delta-9-THC application. This reduction in spectral power subsided within 8 h, coincident with behavioral recovery (42). High-voltage EEG bursts have been reported to accompany the reduced low-voltage fast-frequency (desynchronized) activity both in rats (42) and monkey (237). EEG spike-bursts predominated over the temporal and frontal cortices in monkey. Interestingly, in the rat, the single theta peak (~8 Hz) in the power spectrum recorded during rapid-eye-movement sleep was broken up by cannabinoids into two peaks at 7 and 11 Hz, suggesting that different theta oscillator mechanisms may have been decoupled (309). The rat and monkey data are partly contradicted by EEG studies in the rabbit, where hippocampal theta and cortical EEG spike-bursts were found to be disrupted, and cortical voltage output was generally increased by delta-9-THC in a dose-dependent manner (66). Solid implants of delta-9-THC into the ventral hippocampus, however, induced epileptic activity that produced afterdischarges in the contralateral hippocampus and other distant brain areas (321). Systemic administration of delta-9-THC increased afterdischarge duration in the rat and facilitated transcallosal cortical evoked potentials (348). Taken together with our present knowledge of CB_1 receptor distribution (see sects. III and IV), both studies suggest that disinhibition via CB_1 receptors on basket cells may be the dominant effect in these cases.

Tests of cannabinoid effects using in vitro models of network oscillations are essentially limited to a single study, where Hájos et al. (138) demonstrated that the cannabinoid agonist, CP 55,940, reversibly reduces kainate-induced gamma oscillations in hippocampal slices. These data are consistent with the well-known interference of cannabinoid actions with basket cell function, which includes synchronization of pyramidal cell activity at both high and slow frequencies (61, 111).

B. The DSI (DSE) Root: Control of GABAergic and Glutamatergic Synaptic Transmission via Retrograde Synaptic Signaling

Although modulation of synaptic transmission by retrograde messengers has been well established in the peripheral nervous system of vertebrates or invertebrates (72, 178), the potential importance and physiological role of this phenomenon in the vertebral CNS is still a debated question. Several classical and unconventional transmitters have been shown to be released from the postsynaptic neuron, and to influence synapse formation (107), as well as to modulate transmitter release from afferent boutons terminating on the same or an adjacent cell (10, 196, 373, 378, 379). Thus these signal molecules that include, e.g., amino acids, dopamine, neuropeptides, endocannabinoids, arachidonic acid, nitric oxide, or carbon monoxide, act in a retrograde fashion, whereby neurons may be able to regulate their own inputs and excitability in an activity-dependent manner.

1. DSI

A unique, slow, Ca^{2+}-dependent type of retrograde signaling was independently discovered a decade ago by two laboratories, one working in the cerebellum (214) and the other in the hippocampus (288). A train of postsynaptic action potentials, or a prolonged postsynaptic depolarization (0.1–2 s), was shown to induce a transient suppression of spontaneous or evoked GABAergic IPSP(C)s recorded in the postsynaptic neuron. This phenomenon was termed depolarization-induced suppression of inhibition, or DSI (10). Both in hippocampal pyramidal cells and cerebellar Purkinje cells evidence has been provided that DSI requires a large increase in intracellular Ca^{2+} concentration on the postsynaptic side, which results in the release of a retrograde messenger that acts on the presynaptic terminals, reducing the probability of GABA release. DSI can be blocked by postsynaptic Ca^{2+} buffers or initiated by activity restricted to the postsynaptic side, and likely involves the opening of voltage-gated Ca^{2+} channels (210, 214, 288, 289), or release from intracellular stores. Changes in postsynaptic $GABA_A$ receptor sensitivity have been excluded, since the response to iontophoretically applied GABA did not change, and DSI had no effect on the amplitude of miniature IPSCs. Despite the clearly postsynaptic site of initiation, numerous experiments demonstrated that DSI is expressed presynaptically, i.e., as a reduction in GABA release. With the use of minimal stimulation, DSI was found to increase failure rate, multiquantal components were also eliminated, and components of IPSCs were differentially influenced (11). In the cerebellum, axonal branch point conduction failure was shown to play a role (361). Furthermore, DSI was reduced by 4-aminopyridine and veratridine, both acting on the presynaptic terminal (11). Direct evidence for an inhibitory G protein-mediated presynaptic action has been provided by Pitler and Alger (289), as they showed that DSI was pertussis toxin sensitive.

Both laboratories hypothesized from the very beginning that they were dealing with a phenomenon that involves retrograde messengers. Llano et al. (214) stated that "Ca^{2+} rise in the Purkinje cell leads to the production of a lipid-soluble second messenger." This was a remarkable prediction 10 years before the discovery that, indeed, the lipid-soluble endocannabinoids are these messengers (271, 375, for details, see below), although the earlier claim of a retrograde action of arachidonic acid in the presynaptic control of LTP (373) made this assumption rather plausible at that time.

The quest for identifying the chemical nature of this retrograde messenger began with the discovery of DSI. The slow onset (~2–3 s to a maximal effect), the requirement of a lasting Ca^{2+} rise, and the Ca^{2+} buffer effects (see below) were all consistent with a hormone or peptide rather than classical vesicular neurotransmitter. Yet the first substance suggested by direct experimental evidence was glutamate. In the cerebellum, metabotropic glutamate receptor (mGluR) agonists, acting on presynaptic group II mGluRs, were shown to mimic and occlude DSI, whereas antagonists reduced it (130). Activation of adenylate cyclase by forskolin reduced DSI, which is consistent with the proposed reduction of cAMP levels by mGluR2/3 activation that is known to lead to a reduction of GABA release (52). In contrast, in the hippocampus, forskolin and group II or III mGluR ligands were without effect on DSI; however, group I agonists occluded, and antagonists reduced it (257). Pharmacology and the anatomical distribution of the receptors suggested that mGluR5 is likely to be involved in the reduction of GABA release (257), but it appeared to be confined to the somadendritic compartment of the neurons perisynaptically around glutamatergic contacts (217, 218), which was difficult to reconcile with the hypothesis of glutamate being the retrograde signal molecule (but see sect. IV*C* for the contribution of glutamate). The long duration of DSI is not due to the dynamics of the Ca^{2+} transient, as it was the same in EGTA and BAPTA (209), but probably to the slow disappearance of the retrograde messenger molecule from the site of action around the presynaptic terminal. This again is inconsistent with glutamate being the messenger (provided that it has no presynaptic mGluR-mediated effect, see above), since this transmitter is known to be rapidly taken up.

The fast buffer BAPTA and the slow buffer EGTA reduced DSI to a similar degree, suggesting that the site of Ca^{2+} entry (for example, the voltage-dependent Ca^{2+} channels) and the site of calcium's action in DSI induction are relatively far from each other (209). One possibility is that the target of incoming Ca^{2+} may be an intracellular Ca^{2+} store that is able to produce large Ca^{2+} transients required for the release of the signal molecule. On the other hand, the selective N-type Ca^{2+} channel blocker ω-conotoxin was able to block DSI (209), which, according to recent evidence (374), turned out to be an action on the presynaptic terminals that are sensitive to DSI and selectively express the N-type Ca^{2+} channel. These data suggest that Ca^{2+} plays a dual role: it is involved in the initiation (priming) phase via Ca^{2+}-induced Ca^{2+} release from intracellular stores in the postsynaptic side as well as in the effector phase via N-type Ca^{2+} channels on presynaptic terminals (for details, see sect. V*C*).

Obviously, DSI-like phenomena can have a functional role in neuronal signaling only if they can be induced by physiologically occurring activity patterns. In cerebellar Purkinje cells, 100-ms depolarization (from −60 to +20 mV) was required for a detectable reduction in IPSCs (214), which, under physiological conditions, may correspond to a few climbing fiber-induced complex spikes (30 ms each). Thus a short train of climbing fiber-induced spikes is expected to lead to an increased excitability of the innervated Purkinje cell for tens of seconds. Initiation by very few spikes, occasionally even two if closely spaced, has been reported in the hippocampus (288). With 100 μM BAPTA in the pipette, detectable DSI could be evoked already by depolarization as short as 25 ms, and half-maximal effect was produced by 187 ms, or by 109-ms depolarization in the absence of BAPTA (209). This suggests a lower threshold, but also a smaller magnitude and shorter time course of DSI compared with the cerebellum. The behavior-dependent electrical activity patterns in the hippocampus that may lead to DSI (induced by endocannabinoid release, see below) are discussed in section V*D*.

2. DSE

Recent studies by Kreitzer and Regehr (200) provided evidence that, at least in the cerebellum, excitatory synaptic transmission is also under the control of retrogradely acting signal molecules. Both parallel fiber and climbing fiber-evoked EPSCs were suppressed for tens of seconds by a 50- to 1,000-ms depolarization of the postsynaptic Purkinje cells from −60 to 0 mV. Due to the obvious similarity to DSI, this phenomenon has been termed depolarization-induced suppression of excitation (DSE). Paired-pulse experiments, showing that short-term plasticity is affected by the depolarization paradigm for both parallel and climbing fiber responses, demonstrated that the site of expression of DSE is presynaptic and involves a reduction in the probability of transmitter release. BAPTA in the recording pipette completely abolishes DSE, providing evidence for the requirement of postsynaptic Ca^{2+} rise to trigger the event (see further details in sect. V*C*). Earlier reports are consistent with the lack of DSE in the hippocampus (364), but a recent study using excessive depolarization for 5–10 s (i.e., for much longer than required for DSI) argues for its existence also in this brain region (273). Whether the mechanisms of DSE are similar in the hippocampus and cerebellum is dicussed in the following section.

C. Marriage of the Two Lines of Research Explains the Mechanism of DSI (and DSE) While Endowing Endocannabinoids With Function

The discovery by Wilson and Nicoll (375), Ohno-Shosaku et al. (271), and Kreitzer and Regehr (199, 200) that DSI/DSE are mediated by endocannabinoids revealed that investigations in both the cannabinoid and DSI/DSE

fields have been dealing accidentally with the same subject, i.e., the mechanism of retrograde synaptic signaling via endocannabinoids. Both receptor localization data and identification of the physiological actions of cannabinoids on synaptic transmission confirmed that cannabinoids act on presynaptic axons, reducing transmitter release (see sect. IV), whereas endocannabinoids are most likely released from the postsynaptic neuron upon strong stimuli that give rise to large Ca^{2+} transients. Thus the signal molecules, which turned out to be endocannabinoids, travel from the post- to the presynaptic site and thus enable neurons to influence the strength of their own synaptic inputs in an activity-dependent manner. This may be considered as a short definition of retrograde synaptic signaling and perhaps, at the same time, summarizes the function of the endocannabinoid system. However, before trying to correlate the findings of cannabinoid and DSI (DSE) studies, one should be aware of the major limitations. There are numerous examples of mismatch in receptor/transmitter distribution in the brain; receptors can be found in locations where they hardly ever see their endogenous ligand. Nevertheless, these receptors readily participate in mediating the effects of its exogenous ligands, e.g., during pharmacotherapy. We are facing the same problems with the relative distribution of cannabinoid receptors versus endocannabinoid release sites both at the cellular and subcellular levels. In addition, the distance to which anandamide and 2-AG are able to diffuse (in the presence or absence of transporter blockers) is also an important question from the point of identifying the degree of mismatch. Thus correlation of the sites of action of cannabinoid drugs and the sites of expression of DSI (and DSE) should reveal the regional, cellular, and subcellular domains where receptor and endogenous ligand distributions match, i.e., where endocannabinoids are likely to have a functional role in synaptic signaling.

Several lines of evidence have been provided that endocannabinoids represent the retrograde signal molecules that mediate DSI both in the hippocampus and cerebellum, as well as DSE in the cerebellum. Antagonists of CB_1 receptors fully block (Fig. 14, *A* and *B*) and agonists occlude DSI and DSE, whereas DSI is absent in CB_1 receptor knock-out animals (Fig. 14, *C* and *D*) (87, 199, 200, 271, 374, 375, 377). In these experiments either single-cell or paired recording has been used, and retrograde synaptic signaling has been evoked by the same procedures as described in the original work of Alger's and Marty's groups (214, 288). In addition, Wilson and Nicoll (375) demonstrated that uncaging of Ca^{2+} from a photo-

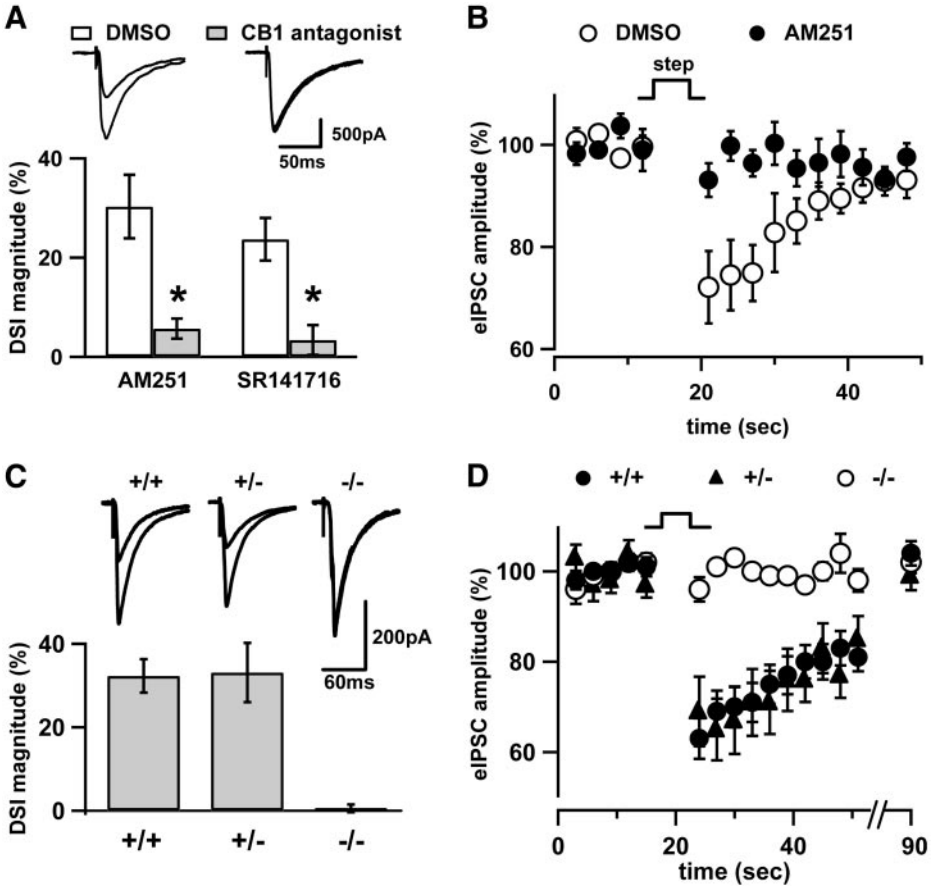


FIG. 14. *A*: in CA1 pyramidal neurons, a 5-s depolarizing step from −60 to 0 mV causes a transient suppression of GABAergic IPSCs. Depolarization-induced suppression of inhibition (DSI) is measured as a percentage depression of IPSC amplitude. Slices preincubated in the CB_1 antagonists AM251 (2 μM) or SR141716 (2 μM) show little or no DSI. *Insets* show average IPSCs in the 10 s before and 10 s just after the depolarizing step (overlaid). Glutamate receptor antagonists in the bath permit pharmacological isolation of IPSCs. *B*: average time course of eIPSC amplitudes after depolarization for control and AM251-treated slices. *C*: DSI is normal in CB_1 +/+ and CB_1 +/− mice, but completely absent in CB_1 −/− mice. *Insets* show eIPSCs for each genotype, with basal and depressed eIPSCs overlaid. *D*: average time course of eIPSC amplitudes after depolarization in CB_1 +/+, CB_1 +/−, and CB_1 −/− mice. (Figure was kindly prepared by Rachel Wilson and Roger Nicoll.)

labile chelator induces DSI that was indistinguishable from that evoked by depolarization. Thus a large intracellular Ca^{2+} rise is a necessary and sufficient element in the induction of the release of endocannabinoids. As expected from the membrane-permeant endocannabinoids, their release does not require vesicle fusion, since botulinum toxin delivered via the intracellular recording pipette did not affect DSI. A further crucial question concerns the range to which the released endocannabinoids are able to diffuse. Recordings at room temperature from pyramidal cells at various distances from the depolarized neuron releasing the signal molecules revealed that it is only the adjacent cell, at a maximum distance of 20 μm, to which endocannabinoids are able to diffuse in a sufficient concentration to evoke detectable DSI (360, 375). However, a considerably greater endocannabinoid uptake and metabolism should be expected at physiological temperatures, which likely results in a decreased spread and a more focused action.

Earlier data indicating the involvement of glutamate and mGluR receptors in DSI also needed clarification (256, 257). Varma et al. (357) demonstrated that enhancement of DSI by mGluR agonists could be blocked by antagonists of both group I mGluR and CB_1 receptors, whereas the same mGluR agonists were without effect in CB_1 receptor knock-out animals. This provides direct evidence that any mGluR effects on DSI published earlier were mediated by endocannabinoid signaling, and glutamate served here as a trigger for the release of endocannabinoids rather than as a retrograde signal molecule as thought earlier. These data were subsequently confirmed by paired recordings from cultured hippocampal neurons (272). In a recent paper, Maejima et al. (223) demonstrated that mGluR1 activation induces DSE in Purkinje cells even without changing the intracellular Ca^{2+} concentration. This suggests that, at least in the case of cerebellar Purkinje cells, two independent mechanisms may trigger endocannabinoid synthesis (and release); one involves a transient elevation of intracellular [Ca^{2+}], and the other is independent of intracellular [Ca^{2+}] and involves mGluR1 signaling. This may imply that, under normal physiological conditions, different induction mechanisms may evoke the release of different endocannabinoids. With the growing number of potential endocannabinoids (see sect. II*B4*), the question arises whether they are involved in distinct functions, i.e., by acting at different receptors and/or at specific types of synapses. This question represents one of the hot spots of current endocannabinoid research, and direct measurements of the different endocannabinoid compounds during retrograde signaling should provide an answer.

There are several mechanisms by which endocannabinoids may suppress transmitter release. They may induce branch-point failure, decrease action potential invasion of axon terminals, reduce Ca^{2+} influx into the synaptic varicosities via N- or P/Q-type channels, or block the release machinery somewhere downstream from the Ca^{2+} signal. Using Ca^{2+} imaging of single climbing fibers (200) provided evidence that DSE involves a reduction of presynaptic Ca^{2+} influx, which has the same time course as the reduction of the EPSC. Branch-point failure was shown not to contribute to DSE, at least in the case of climbing fibers, as stimulation of the examined single axon evoked a uniform rise of Ca^{2+} throughout its entire arbor. These findings are supported by the fact that cannabinoids are known to block N-type Ca^{2+} channels in neuroblastoma cells (221) and reduce synaptic transmission by inhibiting both N- and P/Q-type channels in neurons (349). Inhibition of the release machinery is unlikely to play a role, particularly in GABAergic transmission, since CB_1 receptor activation has little if any effect on mIPSC frequency in the presence of tetrodotoxin and cadmium (138, 161, 186). Furthermore, CB_1 receptors tend to be localized away from the release sites, having a high density even on preterminal axon segments, which also argues against this possibility (138, 187, 188).

In the hippocampus, evidence has been provided that DSI likely involves a direct action of G proteins on voltage-dependent calcium channels. These included the demonstration that modulation of kinase and phosphatase activities or cAMP levels (257, 374) has no effect on DSI, while the relatively rapid onset (on average 1.2 s) of IPSC suppression makes a phosphorylation-mediated change in channel activity less likely, since that would typically require several seconds. They confirmed the findings of Lenz et al. (210) that ω-conotoxin, but not ω-aga-toxin is able to block DSI, which means that the G protein-mediated endocannabinoid actions target only the N-type but not the P/Q-type Ca^{2+} channels in the hippocampus (Fig. 15). DSI or the selective Ca^{2+} channel inhibitors never block IPSCs completely, which may be due to a partial reduction of release from all terminals, or to the selective expression of CB_1 receptors together with the N-type channels only on a particular subset of interneurons. Wilson et al. (374) provided an elegant resolution to this dilemma using paired recordings, which revealed that interneurons producing IPSCs with distinct kinetics express different presynaptic Ca^{2+} channels, and those that show DSI possess only N-type channels (see Fig. 15). This finding correlates well with the anatomical observations that CCK-containing basket cells selectively express CB_1 receptors, whereas another basket cell type (that contains parvalbumin) lacks CB_1 receptors (Fig. 9, *E–G*) (188). The differences in IPSC kinetics observed by Wilson et al. (374) may be due to CCK cells forming synapses that are enriched in α_2-subunit-containing $GABA_A$ receptors (270), whereas parvalbumin-containing basket cells synapse onto $GABA_A$ receptors with five times less α_2-subunits (likely having α_1 instead). Taken together, these data suggest that CCK-containing basket

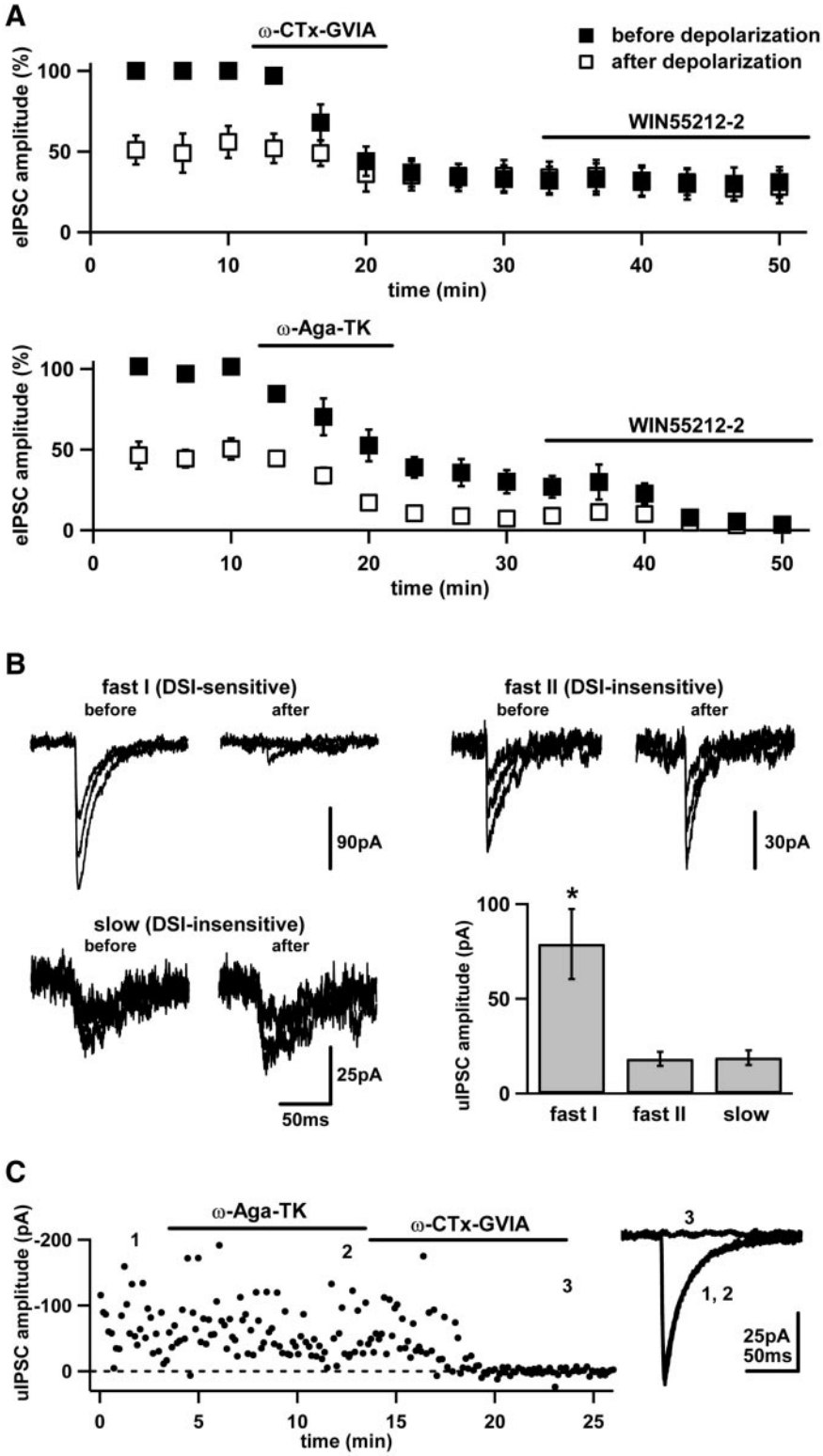


FIG. 15. *A*: DSI was monitored by comparing eIPSC amplitudes just before (solid symbols) and just after (open symbols) depolarizing steps. After a stable baseline period, the N-type VDCC antagonist ω-conotoxin GVIA (ω-CTx-GVIA) was washed onto the slice, causing a depression of basal IPSC amplitude and a complete block of DSI. Subsequent wash-in of WIN55212–2 had no effect, indicating that N-type VDCCs are required for presynaptic inhibition by cannabinoids. Conversely, the P/Q-type VDCC antagonist ω-agatoxin TK (ω-Aga-TK) depressed basal IPSC amplitude but increased DSI magnitude. Subsequent wash-in of WIN55212–2 blocked most of the remaining IPSCs, indicating that the component of release mediated by N-type VDCCs is highly sensitive to cannabinoids. *B*: raw traces from unitary GABAergic connections, classified accordings to kinetics and DSI sensitivity. Three overlaid sweeps acquired just before depolarization are displayed next to three overlaid sweeps acquired just after depolarization. "Fast I" connections show both failures and small-amplitude successes after depolarization, whereas connections from the other two groups ("fast II", "slow") are not affected by depolarization. Average unitary IPSC amplitude is significantly larger for fast I connections compared with either of the other two groups. *C*: a representative experiment showing that ω-CTx-GVIA completely blocks synaptic transmission at a fast I synapse, whereas ω-Aga-TK has no effect. *Inset* shows averaged traces corresponding to baseline (*1*), ω-Aga-TK (*2*), and ω-CTx-GVIA (*3*). (Figure was kindly prepared by Rachel Wilson and Roger Nicoll.)

cell terminals selectively express N-type Ca^{2+} channels together with CB_1 receptors predisposing them to DSI, whereas parvalbumin-containing interneurons may express only the P/Q-type Ca^{2+} channels, lack CB_1 receptors, and are therefore unaffected by DSI.

This conclusion also suggests that the success of DSI induction in any hippocampal slice preparation depends on the relative contribution of the two basket cell types to the examined spontaneous or evoked IPSCs samples. Carbachol is known to enhance DSI, but the mechanism has

not been revealed to date (232). One possibility is that carbachol activates the inositol 1,4,5-trisphosphate (IP_3) system via muscarinic receptors, thereby contributing to the large Ca^{2+} transient required for endocannabinoid release (263, 292). This sounds unlikely as in most experimental paradigms massive depolarizations or uncaging of calcium has been used; thus it would be difficult to further enhance calcium levels by activation of IP_3 receptors on intracellular stores. Furthermore, a recent study showed in sympathetic neuronal cultures that muscarinic receptor-mediated activation of PLC-β results in limited if any IP_3-mediated intracellular Ca^{2+} release (78); thus the major signaling pathway there is the production of DAG. However, under physiological conditions in the hippocampus, a cholinergic activation of PLC may well contribute to endocannabinoid release via both the IP_3 cascade and the DAG limb (see below). Another likely explanation for the experimental results with carbachol is that it may suppress IPSCs produced by parvalbumin-containing basket cells via presynaptic m2 receptors, which are selectively expressed by this interneuron type (140), whereas the spontaneous activity of CCK-containing interneurons may be increased via m_1 muscarinic, or perhaps even nicotinic actions of carbachol. The mutually exclusive distribution of CB_1 and m_2 receptors on two subsets of basket cell terminals is shown in Figure 16 (Katona and Freund, unpublished data). If this reasoning is correct, DSI could be facilitated via other receptors as well that are selectively (or preferentially) present on CCK cells but not on parvalbumin cells, e.g., substance P receptors (5) or 5-HT_3 receptors (255). Indeed, Hájos et al. (138) demonstrated that the increase in the amplitude and frequency of spontaneous IPSCs after bath application of substance P fragment was brought back to near control levels by the coapplication of the CB_1 receptor agonist WIN55212–2.

Although endocannabinoid-mediated DSE has been convincingly demonstrated in the cerebellum, the existence of this phenomenon in the hippocampus could not be established with the same paradigm used for DSI (364) or DSE in the cerebellum (see sect. IV*B*). Cannabinoids do reduce glutamatergic EPSCs in the hippocampus (139, 251, 323), but the receptor involved is unlikely to be CB_1 (Fig. 12), since the effect was found to be the same in CB_1 knock-out and wild-type animals (139; for details see sect. IV). However, in a recent study, prolonged (5–10 s) depolarization was found to readily induce DSE in hippocampal slices, which was absent in CB_1 knock-out mice (273). This is in conflict with the data of Hájos et al. (139) and may be due to age or strain differences. Retrograde endocannabinoid signaling was shown to be responsible for another type of synaptic plasticity of glutamatergic transmission in the striatum. Long-term depression (LTD) of EPSCs induced by high-frequency stimulation of afferent fibers disappeared in CB_1 receptor knock-out animals

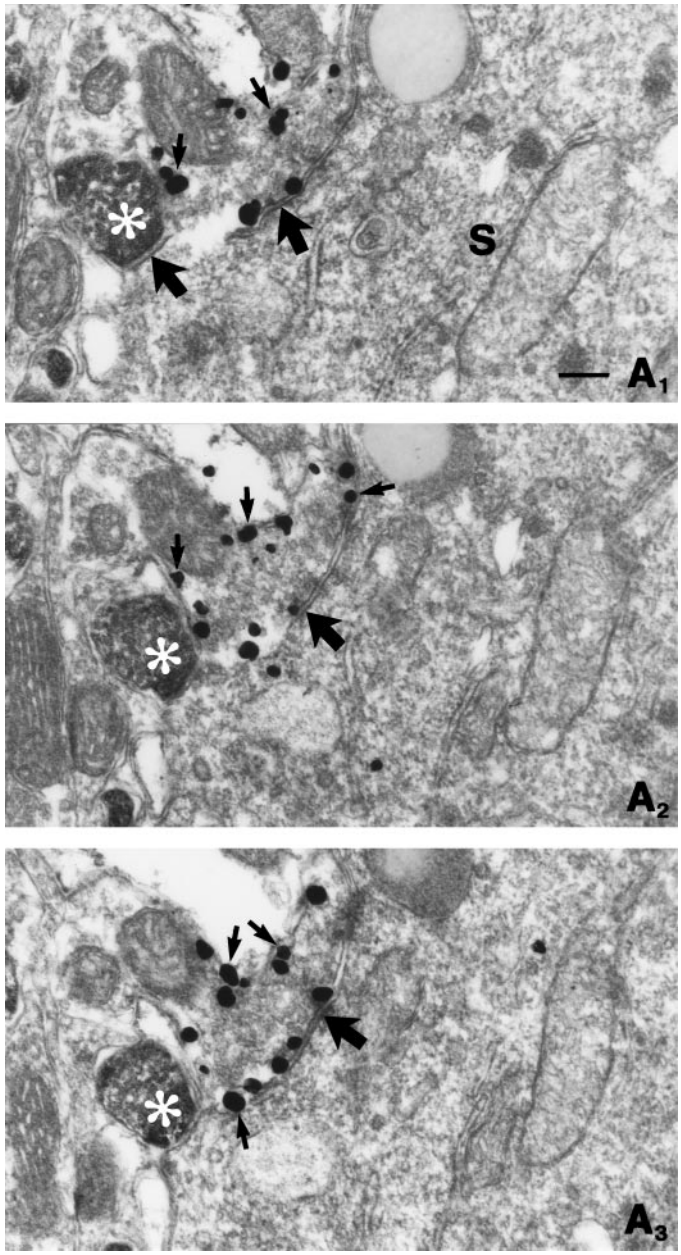


FIG. 16. Two nonoverlapping subsets of perisomatic axon terminals express CB_1 receptors (silver-gold particles labeled with small arrows) and muscarinic m_2 receptors (diffuse DAB labeling, asterisks) in the rat hippocampus. *A1–A3* show three adjacent ultrathin sections of the same boutons. The two axon terminals, one likely belonging to parvalbumin-containing (m_2-positive) and the other to CCK-containing (CB1-positive) basket cells, form symmetrical synapses (large arrows) on the same pyramidal cell body. Scale bars: 0.2 μm.

(117, 301). Anatomical data to support or explain this phenomenon are still lacking (see sect. III). Interestingly, recent experiments uncovered that activation of postsynaptic type I mGluR receptors induce LTD in the hippocampus by decreasing glutamate release presynaptically (368). The striking similarity of induction parameters, as well as the potential role of type I mGluRs in

endocannabinoid synthesis (223, 357), suggests that retrograde signaling via postsynaptic release of endocannabinoids is likely to account for this phenomenon. Thus an important question for future research is to determine how DSE and mGluR-dependent LTD are related, along with the identification of how postsynaptic release of endocannabinoids may contribute to these phenomena.

The paragraphs above dealt with cannabinoid signaling phenomena that are, or could be, brought about by endogenously released cannabinoids. Some thought should be given also to those cannabinoid actions that are unlikely to be reproduced by endogenously released cannabinoids but may still be important for the interpretation of the mechanisms of action of delta-9-THC or synthetic ligands. For example, endogenously released cannabinoids are unlikely to act on LTP in the hippocampus, since *1*) DSE could be evoked in this region only by prolonged (5–10 s) depolarization (273), *2*) cannabinoids had no effect on LTP or LTD when Mg^{2+}-free solution or pairing with strong postsynaptic depolarization was used (251), and *3*) LTP induction under quasi-physiological conditions may be insufficient stimulation for a detectable endocannabinoid release (328). Single postsynaptic spikes are able to induce LTP if paired with presynaptic spikes or bursts (224, 280), and excess endocannabinoid release that would be capable of inhibiting glutamate release is unlikely to occur under these conditions. Thus whether endogenously released cannabinoids are able to influence the efficacy or plasticity of glutamatergic transmission in the hippocampus via a direct action on glutamate release is still to be shown. However, Carlson et al. (53) showed that a weak train of stimuli that normally does not induce LTP will induce NMDA-dependent LTP if given during the DSI period. The simultaneously recorded field EPSPs do not undergo LTP, showing that the weak stimulus train was indeed subthreshold for LTP induction except in disinhibited cells. The single-cell LTP was prevented by pretreatment with AM251, suggesting that locally released endocannabinoids can enhance LTP by causing disinhibition of a pyramidal cell.

D. Electrical Activity Patterns Required for the Release of Endocannabinoids

As dicussed above, several lines of experimental evidence suggest that rather large increases in intracellular [Ca^{2+}] are required for the induction of DSI and DSE via the release of endocannabinoids (199, 200, 209, 288, 357, 375), and this elevation of Ca^{2+} is essential for the synthesis rather than the release of endocannabinoids (92, 223, 287, 375). Such profound Ca^{2+} transients may occur only under special physiological conditions, e.g., upon the release of Ca^{2+} from IP_3- or ryanodine-sensitive intracellular stores via simultaneous activation of metabotropic receptors and voltage-gated Ca^{2+} channels (Fig. 17) (146, 147, 262, 263, 292, but see Ref. 210). Back-propagating action potentials are most likely responsible for the voltage-gated Ca^{2+} influx both in the proximal dendritic (perisomatic) and distal dendritic regions (spines), although in small cellular compartments like a spinehead, a single NMDA-mediated synaptic event may be sufficient to release Ca^{2+} from the local intracellular stores (96). In the perisomatic region (including the proximal main dendrites), type I mGluRs appear to supply IP_3 both in pyramidal and Purkinje cells (106, 262, 263), which may partly explain the apparent involvement of this receptor type in DSI (257). Indeed, recent papers (223, 272, 357) provide evidence that metabotropic glutamate effects on DSI are mediated by endocannabinoids, as described above. Pairing back-propagating action potentials with mGluR activation increases Ca^{2+} release severalfold compared with spiking alone (262, 263). The largest amplitude Ca^{2+} transient was observed in the most proximal segment of the apical dendrite, an ideal location for endocannabinergic modulation of GABAergic axon terminals that innervate this region. Electron microscopic studies demonstrate the lack of glutamatergic synapses on the cell bodies and proximal apical shafts of pyramidal cells (244, 277), which suggests that intracellular Ca^{2+} release in this region has to have a role other than conveying plasticity to glutamatergic synapses. One possibility is that this Ca^{2+} rise is sufficiently close to the nucleus to trigger transcriptional changes. Alternatively, it may be critically involved in the induction of endocannabinoid release, which results in the downregulation of perisomatic inhibition. Thereby action potentials could better back-propagate into the distal dendrites allowing associative LTP of distal glutamatergic synapses, or would enable the neuron to dissociate itself from the population oscillation maintained by basket cell-mediated inhibition (61, 246, 343, 376; for review, see Ref. 111). One problem with this hypothesis, and with the interpretation of the mGluR studies (223, 262, 263, 272, 357), is the source of glutamate required to activate mGluRs in the somatic/proximal dendritic region, since these parts of pyramidal cells do not receive glutamatergic synapses (244, 277). Thus, if mGluRs get activated at all in this region under physiological conditions, it either has to involve extrasynaptic mGluRs reached by diffusion of glutamate from distant synaptic sites, or mGluRs may be activated further away from the proximal apical dendrite (mostly on spines), and IP_3 would have to be able to diffuse very fast to its receptors located on the perisomatic or proximal dendritic endoplasmic reticulum. The latter alternative is possible, since IP_3 was calculated to be able to diffuse 50 μm in 0.5 s, which is faster than Ca^{2+} diffusion in the cytosol containing Ca^{2+} buffers (14). Diffusion of synaptically released glutamate, however, is unlikely, since it is limited by the efficient glial and neuronal uptake machinery; a spillover even to the adjacent

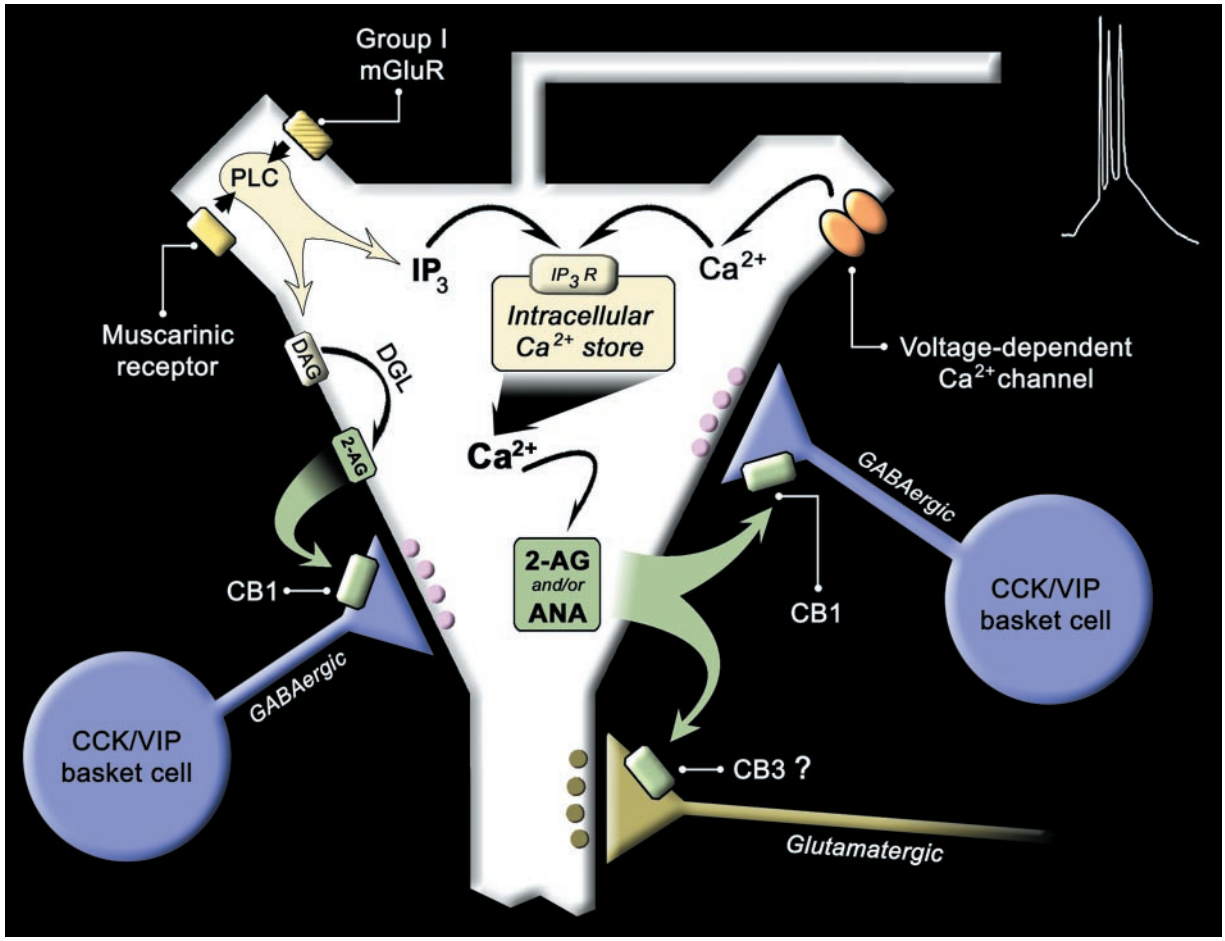


FIG. 17. Schematic diagram of endocannabinoid-mediated retrograde synaptic signaling. The possible physiological mechanisms that may trigger endocannabinoid synthesis and release from hippocampal pyramidal neurons are outlined (similar mechanisms are likely to operate in most brain areas where endocannabinoid signaling takes place). The large Ca^{2+} transient required for endocannabinoid synthesis likely involves Ca^{2+} mobilization from intracellular stores upon activation of the inositol 1,4,5-trisphosphate (IP_3) system (metabotropic receptors) and voltage-dependent Ca^{2+} channels (burst firing). Another root independent of intracellular Ca^{2+} transients is illustrated on the *left* of the schematized pyramidal cell body. Activation of phospholipase C (PLC) via group I metabotropic glutamate (mGluR) or muscarinic cholinergic receptors will produce, in addition to IP_3, 1,2-diacylglycerol (DAG), which likely remains in the plasma membrane. This could then be converted to 2-arachidonoylglycerol (2-AG) by the enzyme 1,2-diacylglycerol lipase (DGL) still within the membrane, which may ensure a rapid diffusion into the extracellular space. The released endocannabinoids act on CB_1 receptors located on axon terminals of GABAergic interneurons that contain CCK, or on a new cannabinoid receptor subtype (CB3?) expressed by glutamatergic axons. Activation of CB_1 reduces GABA release via G_i-mediated blocking of N-type Ca^{2+} channels, whereas the new receptor likely reduces glutamate release via a similar mechanism.

synapse is limited (86). An alternative trigger for IP_3 synthesis is muscarinic activation. Indeed, Martin and Alger (232) demonstrated that DSI is enhanced by muscarinic m_1 or m_3 receptor stimulation. Varicose cholinergic fibers are abundant in all layers of the hippocampus and particularly enriched in stratum pyramidale and near the granule cell layer (220). Furthermore, principal cells are known to express muscarinic receptors on their perisomatic membrane (308). Activation of muscarinic receptors induces a profound Ca^{2+} rise in the soma, or Ca^{2+} waves that propagate into the soma, and increases the Ca^{2+} transients evoked by trains of action potentials (263, 292). Thus it is important to emphasize that, in addition to group I mGluRs, cholinergic transmission may also contribute to the generation of sufficient IP_3 levels to trigger large Ca^{2+} transients followed by endocannabinoid release when coinciding with trains of action potentials. However, muscarinic receptor-mediated activation of PLC-β in sympathetic neuronal cultures results in limited if any IP_3-mediated intracellular Ca^{2+} release; thus the major signaling pathway there is the production of DAG (78), which, on the other hand, is the precursor of 2-AG synthesis (328). Whether muscarinic activation uses primarily the DAG limb in hippocampal endocannabinoid signaling remains to be established, although the lack of an antagonist (atropine) effect on DSI suggests that rest-

ing levels of acetylcholine are not involved in the generation of the required DAG pool (232). The same question arises also for the mechanism of mGluR-mediated endocannabinoid release, since in a recent study in hippocampal cultures, group I mGluR activation was shown to enhance DSI without increasing intracellular calcium signals (272). This raises the possibility that under some conditions, group I mGluR activation uses the alternative root; it may increase 2-AG synthesis via the DAG limb (328), and thereby could cooperate with depolarization-induced Ca^{2+} transients to enhance endocannabinoid release.

The physiologically most relevant question here is which are the behavior-dependent activity patterns that could ensure the coincidence of metabotropic receptor activation (IP_3 and DAG synthesis) and bursts of action potentials that are able to induce sufficiently large Ca^{2+} transients to release endocannabinoids in the hippocampus (Fig. 17). Spontaneous or low magnesium-evoked burst potentials that resemble physiological bursts were shown to induce DSI (20). Hippocampal pyramidal cells typically produce bursts of two to six action potentials at <6-ms intraburst intervals (294). These bursts were shown to invade parts of the dendritic tree quite efficiently, and therefore their pairing with presynaptic activity readily induces LTP (224, 280). Even much slower trains of action potentials (10–30 Hz) result in a buildup of Ca^{2+} in pyramidal cell dendrites. This Ca^{2+} level perfectly correlates with spike frequency (146); therefore, it may induce endocannabinoid release in an activity-dependent manner, if coupled to a coincident activation of the IP_3 cascade and releases Ca^{2+} from intracellular stores. The probability of bursts was found to be highest at firing rates around theta frequency (145), and bursts at this frequency are particularly suitable for inducing LTP in hippocampal pyramidal cells (170, 205). Acetylcholine release in the hippocampus is large during theta activity, and it correlates with theta power (191), while muscarinic receptor activation induces Ca^{2+} transients (and DAG synthesis). Therefore, theta is likely to be the behavior-dependent EEG pattern that best couples burst-induced Ca^{2+} influx with metabotropic activation of IP_3/DAG synthesis. Endocannabinoid release that follows the resulting high Ca^{2+} transients may reduce perisomatic inhibition of the burst-firing cells, which could facilitate LTP of distal dendritic synapses by allowing a more efficient back-propagation of action potentials. Interestingly, acetylcholine appears to use at least three different mechanisms to enhance communication between the soma and distal dendrites: *1*) it is able to close transient K^+ channels (I_A) in the apical dendrites (179), *2*) it reduces GABA release from parvalbumin-containing basket cell terminals via presynaptic m_2 receptors (140), and *3*) it may induce endocannabinoid release to reduce inhibition deriving from the other subset of perisomatic inhibitory cells, i.e., from those that contain CCK and express presynaptic CB_1 receptors (188).

Another result of endocannabinoid-mediated downregulation of perisomatic inhibition may be that individual cells could dissociate the timing of their action potential firing from network oscillations during theta activity. During exploratory behavior and theta activity pyramidal cells tend to fire in specific areas of their environment, which are called place fields (274). When the animal enters the place field of the recorded neuron, it starts to fire at earlier phases of the theta waves relative to the population, which was called phase precession (275). Burst firing starts to occur preferentially at the periphery of the place field (145), creating, together with muscarinic receptor acivation, ideal conditions for endocannabinoid release. This would result in a gradual downregulation of basket cell-mediated inhibition, allowing the cell to fire at earlier and earlier phases of the theta cycle. The cell could still fire phase-locked to gamma oscillation, if the other basket cell population (the parvalbumin cells lacking CB_1 receptors) is able to convey this effect. The timing of the presumed endocannabinoid effect also seems optimal for this function, since the onset of DSI is ~1.2 s after the somatic Ca^{2+} rise, and lasts for a few seconds, or occasionally for >10 s (288, 374).

Another EEG pattern accompanied by synchronous pyramidal cell firing at relatively high frequencies are the sharp-wave bursts, which occur during non-theta behaviors (43). These events, however, are rather short (40–120 ms), and whether this is sufficient for endocannabinoid synthesis/release remains to be established. Nevertheless, the synchronous burst discharge of a large proportion of pyramidal cells may result in extensive Ca^{2+} influx, activation of $mGluR_5$ receptors, and the synthesis of IP_3/DAG (Fig. 17). The induced endocannabinoid action may start suppressing inhibition within a few hundred milliseconds, leading to downregulation of inhibition and the generation of the next sharp wave burst. The variable second messenger delay may account for the irregular occurrence of sharp waves and may explain why the same pyramidal cells initiate the subsequent bursts.

VI. CONCLUSIONS

The aim of this review was to synthesize the currently available data about the life cycle of endocannabinoids; the conditions that result in their release in the brain; the precise sites of their action at the regional, cellular, and subcellular levels; and their physiological effects on neuronal networks. In addition, a major focus of this review was to generate testable hypotheses about the possible functional roles of endocannabinoids in complex integrative centers of the brain, such as the cerebral and cerebellar cortex and basal ganglia. A general view

emerging from the synthesis of the available data is that endocannabinoids serve as mediators in neuronal communication that is distinct from synaptic and nonsynaptic (volume) transmission in its range and function. Endocannabinoids mediate retrograde synaptic signaling, which has an intermediate range between synaptic and volume transmission. Synapses represent point-to-point connections where each of the contacts can be selectively activated and modified, whereas volume transmission employs mediators that can diffuse considerable distances (362) and are likely to be involved in the fine tuning of activity and plasticity in entire brain regions, subfields, or layers. In contrast, endocannabinoid diffusion is limited by uptake and metabolism basically to the axon terminals that form synapses on particular neurons that release them as retrograde signal molecules. Thus the generation of endocannabinoids by burst-firing and/or PLC activation via metabotropic receptors in a neuron will decrease the efficacy of the incoming inhibitory and/or excitatory synaptic signals primarily onto that neuron (Fig. 17). The functional importance of this mechanism is still under investigation. However, the available evidence suggests that endocannabinoids influence *1*) transmitter release dynamics that play crucial roles in synaptic plasticity, *2*) action potential back-propagation and timing relative to a phase-locked population activity in neuronal signaling, and *3*) oscillations that are involved in higher cognitive functions such as feature binding during learning and memory processes. Remarkably, these processes may also represent the neurobiological substrate of the various behavioral effects of cannabis smoking.

The recent findings presented in this review on the function of endocannabinoids suggest that we are just at the beginning of a revolution in endocannabinoid research that may shed light not only on normal brain operations, but also on disease mechanisms that are so far poorly understood, like schizophrenia, anxiety, and other brain disorders. Future research should focus on *1*) the molecular, physiological, and pharmacological characterization of missing key elements of the endocannabinoid system, such as new endocannabinoids and new cannabinoid receptors in the brain; *2*) their precise cellular and subcellular localization; *3*) the biochemical machinery involved in endocannabinoid synthesis, uptake, and degradation; *4*) the physiological conditions necessary and sufficient for endocannabinoid release; and, last but not least, *5*) the roles played by the endocannabinoid system in various neurological and psychiatric disorders.

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REFERENCES

1. Abadji V, Lin S, Taha G, Griffin G, Stevenson LA, Pertwee RG, and Makriyannis A. (*R*)-methanandamide: a chiral novel anandamide possessing higher potency and metabolic stability. *J Med Chem* 37: 1889–1893, 1994.
2. Abumrad N, Coburn C, and Ibrahimi A. Membrane proteins implicated in long-chain fatty acid uptake by mammalian cells: CD36, FATP and FABPm. *Biochim Biophys Acta* 1441: 4–13, 1999.
3. Acquas E, Pisanu A, Marrocu P, and Di Chiara G. Cannabinoid CB(1) receptor agonists increase rat cortical and hippocampal acetylcholine release in vivo. *Eur J Pharmacol* 401: 179–185, 2000.
4. Acquas E, Pisanu A, Marrocu P, Goldberg SR, and Di Chiara G. Delta9-tetrahydrocannabinol enhances cortical and hippocampal acetylcholine release in vivo: a microdialysis study. *Eur J Pharmacol* 419: 155–161, 2001.
5. Acsady L, Katona I, Gulyas AI, Shigemoto R, and Freund TF. Immunostaining for substance P receptor labels GABAergic cells with distinct termination patterns in the hippocampus. *J Comp Neurol* 378: 320–336, 1997.
6. Adams AR. Marihuana. *Harvey Lect* 37: 168–168, 1941.
7. Ahluwalia J, Urban L, Bevan S, Capogna M, and Nagy I. Cannabinoid 1 receptors are expressed by nerve growth factor- and glial cell-derived neurotrophic factor-responsive primary sensory neurones. *Neuroscience* 110: 747–753, 2002.
8. Ahluwalia J, Urban L, Capogna M, Bevan S, and Nagy I. Cannabinoid 1 receptors are expressed in nociceptive primary sensory neurons. *Neuroscience* 100: 685–688, 2000.
9. Akerstrom B, Flower DR, and Salier JP. Lipocalins: unity in diversity. *Biochim Biophys Acta* 1482: 1–8, 2000.
10. Alger BE and Pitler TA. Retrograde signaling at $GABA_A$-receptor synapses in the mammalian CNS. *Trends Neurosci* 18: 333–340, 1995.
11. Alger BE, Pitler TA, Wagner JJ, Martin LA, Morishita W, Kirov SA, and Lenz RA. Retrograde signalling in depolarization-induced suppression of inhibition in rat hippocampal CA1 cells. *J Physiol* 496: 197–209, 1996.
12. Al-Hayani A and Davies SN. Cannabinoid receptor mediated inhibition of excitatory synaptic transmission in the rat hippocampal slice is developmentally regulated. *Br J Pharmacol* 131: 663–665, 2000.
13. Al-Hayani A, Wease KN, Ross RA, Pertwee RG, and Davies SN. The endogenous cannabinoid anandamide activates vanilloid receptors in the rat hippocampal slice. *Neuropharmacology* 41: 1000–1005, 2001.
14. Allbritton NL, Meyer T, and Stryer L. Range of messenger action of calcium ion and inositol 1,4,5-trisphosphate. *Science* 258: 1812–1815, 1992.
15. Ameri A, Wilhelm A, and Simmet T. Effects of the endogeneous cannabinoid, anandamide, on neuronal activity in rat hippocampal slices. *Br J Pharmacol* 126: 1831–1839, 1999.
16. Arreaza G, Devane WA, Omeir RL, Sajnani G, Kunz J, Cravatt BF, and Deutsch DG. The cloned rat hydrolytic enzyme responsible for the breakdown of anandamide also catalyzes its formation via the condensation of arachidonic acid and ethanolamine. *Neurosci Lett* 234: 59–62, 1997.
17. Auclair N, Otani S, Soubrie P, and Crepel F. Cannabinoids modulate synaptic strength and plasticity at glutamatergic synapses of rat prefrontal cortex pyramidal neurons. *J Neurophysiol* 83: 3287–3293, 2000.

18. Barnes PJ. Modulation of neurotransmission in airways. *Physiol Rev* 72: 699–729, 1992.
19. Baude A, Nusser Z, Roberts JD, Mulvihill E, McIlhinney RA, and Somogyi P. The metabotropic glutamate receptor (mGluR1 alpha) is concentrated at perisynaptic membrane of neuronal subpopulations as detected by immunogold reaction. *Neuron* 11: 771–787, 1993.
20. Beau FE and Alger BE. Transient suppression of $GABA_A$-receptor-mediated IPSPs after epileptiform burst discharges in CA1 pyramidal cells. *J Neurophysiol* 79: 659–669, 1998.
21. Beinfeld MC and Connolly K. Activation of CB1 cannabinoid receptors in rat hippocampal slices inhibits potassium-evoked cholecystokinin release, a possible mechanism contributing to the spatial memory defects produced by cannabinoids. *Neurosci Lett* 301: 69–71, 2001.
22. Beltramo M, de Fonseca FR, Navarro M, Calignano A, Gorriti MA, Grammatikopoulos G, Sadile AG, Giuffrida A, and Piomelli D. Reversal of dopamine D(2) receptor responses by an anandamide transport inhibitor. *J Neurosci* 20: 3401–3407, 2000.
23. Beltramo M, di Tomaso E, and Piomelli D. Inhibition of anandamide hydrolysis in rat brain tissue by (*E*)-6-(bromomethylene) tetrahydro-3-(1-naphthalenyl)-2H-pyran-2-one. *FEBS Lett* 403: 263–267, 1997.
24. Beltramo M and Piomelli D. Carrier-mediated transport and enzymatic hydrolysis of the endogenous cannabinoid 2-arachidonylglycerol. *Neuroreport* 11: 1231–1235, 2000.
25. Beltramo M, Stella N, Calignano A, Lin SY, Makriyannis A, and Piomelli D. Functional role of high-affinity anandamide transport, as revealed by selective inhibition. *Science* 277: 1094–1097, 1997.
26. Bisogno T, MacCarrone M, De Petrocellis L, Jarrahian A, Finazzi-Agro A, Hillard C, and Di Marzo V. The uptake by cells of 2-arachidonoylglycerol, an endogenous agonist of cannabinoid receptors. *Eur J Biochem* 268: 1982–1989, 2001.
27. Bisogno T, Melck D, Bobrov M, Gretskaya NM, Bezuglov VV, De Petrocellis L, and Di Marzo V. *N*-acyl-dopamines: novel synthetic CB(1) cannabinoid-receptor ligands and inhibitors of anandamide inactivation with cannabimimetic activity in vitro and in vivo. *Biochem J* 351: 817–824, 2000.
28. Bodor A, Katona I, Mackie K, and Freund TF. Cellular and subcellular localization of CB1 cannabinoid receptor in rat somatosensory cortex. *FENS Abstr* 1: 284, 2002.
29. Boger DL, Fecik RA, Patterson JE, Miyauchi H, Patricelli MP, and Cravatt BF. Fatty acid amide hydrolase substrate specificity. *Bioorg Med Chem Lett* 10: 2613–2616, 2000.
30. Boger DL, Sato H, Lerner AE, Hedrick MP, Fecik RA, Miyauchi H, Wilkie GD, Austin BJ, Patricelli MP, and Cravatt BF. Exceptionally potent inhibitors of fatty acid amide hydrolase: the enzyme responsible for degradation of endogenous oleamide and anandamide. *Proc Natl Acad Sci USA* 97: 5044–5049, 2000.
31. Bohme GA, Laville M, Ledent C, Parmentier M, and Imperato A. Enhanced long-term potentiation in mice lacking cannabinoid CB1 receptors. *Neuroscience* 95: 5–7, 2000.
32. Bouaboula M, Bourrie B, Rinaldi-Carmona M, Shire D, Le Fur G, and Casellas P. Stimulation of cannabinoid receptor CB1 induces krox-24 expression in human astrocytoma cells. *J Biol Chem* 270: 13973–13980, 1995.
33. Bouaboula M, Poinot-Chazel C, Bourrie B, Canat X, Calandra B, Rinaldi-Carmona M, Le Fur G, and Casellas P. Activation of mitogen-activated protein kinases by stimulation of the central cannabinoid receptor CB1. *Biochem J* 312: 637–641, 1995.
34. Bracey MH, Hanson MA, Masuda KR, Stevens RC, and Cravatt BF. Structural adaptations in a membrane enzyme that terminates endocannabinoid signaling. *Science* 298: 1793–1796, 2002.
35. Braida D and Sala M. Cannabinoid-induced working memory impairment is reversed by a second generation cholinesterase inhibitor in rats. *Neuroreport* 11: 2025–2029, 2000.
36. Breivogel CS, Griffin G, Di Marzo V, and Martin BR. Evidence for a new G protein-coupled cannabinoid receptor in mouse brain. *Mol Pharmacol* 60: 155–163, 2001.
37. Breivogel CS, Selley DE, and Childers SR. Cannabinoid receptor agonist efficacy for stimulating [^{35}S]GTPγS binding to rat cerebellar membranes correlates with agonist-induced decreases in GDP affinity. *J Biol Chem* 273: 16865–16873, 1998.
38. Breivogel CS, Sim LJ, and Childers SR. Regional differences in cannabinoid receptor/G-protein coupling in rat brain. *J Pharmacol Exp Ther* 282: 1632–1642, 1997.
39. Brindley DN, Saxton J, Shahidullah H, and Armstrong M. Possible relationships between changes in body weight set-point and stress metabolism after treating rats chronically with D-fenfluramine. Effects of feeding rats acutely with fructose on the metabolism of corticosterone, glucose, fatty acids, glycerol and triacylglycerol. *Biochem Pharmacol* 34: 1265–1271, 1985.
40. Brocher S, Artola A, and Singer W. Agonists of cholinergic and noradrenergic receptors facilitate synergistically the induction of long-term potentiation in slices of rat visual cortex. *Brain Res* 573: 27–36, 1992.
41. Buckley NE, Hansson S, Harta G, and Mezey E. Expression of the CB1 and CB2 receptor messenger RNAs during embryonic development in the rat. *Neuroscience* 82: 1131–1149, 1998.
42. Buonamici M, Young GA, and Khazan N. Effects of acute delta 9-THC administration on EEG and EEG power spectra in the rat. *Neuropharmacology* 21: 825–829, 1982.
43. Buzsaki G. Hippocampal sharp waves: their origin and significance. *Brain Res* 398: 242–252, 1986.
44. Cadas H, di Tomaso E, and Piomelli D. Occurrence and biosynthesis of endogenous cannabinoid precursor, *N*-arachidonoyl phosphatidylethanolamine, in rat brain. *J Neurosci* 17: 1226–1242, 1997.
45. Cadas H, Gaillet S, Beltramo M, Venance L, and Piomelli D. Biosynthesis of an endogenous cannabinoid precursor in neurons and its control by calcium and cAMP. *J Neurosci* 16: 3934–3942, 1996.
46. Calignano A, Katona I, Desarnaud F, Giuffrida A, La Rana G, Mackie K, Freund TF, and Piomelli D. Bidirectional control of airway responsiveness by endogenous cannabinoids. *Nature* 408: 96–101, 2000.
47. Calignano A, La Rana G, Beltramo M, Makriyannis A, and Piomelli D. Potentiation of anandamide hypotension by the transport inhibitor, AM404. *Eur J Pharmacol* 337: R1–R2, 1997.
48. Calignano A, La Rana G, Giuffrida A, and Piomelli D. Control of pain initiation by endogenous cannabinoids. *Nature* 394: 277–281, 1998.
49. Calignano A, La Rana G, and Piomelli D. Antinociceptive activity of the endogenous fatty acid amide, palmitylethanolamide. *Eur J Pharmacol* 419: 191–198, 2001.
50. Campbell KA, Foster TC, Hampson RE, and Deadwyler SA. delta 9-Tetrahydrocannabinol differentially affects sensory-evoked potentials in the rat dentate gyrus. *J Pharmacol Exp Ther* 239: 936–940, 1986.
51. Campbell KA, Foster TC, Hampson RE, and Deadwyler SA. Effects of delta 9-tetrahydrocannabinol on sensory-evoked discharges of granule cells in the dentate gyrus of behaving rats. *J Pharmacol Exp Ther* 239: 941–945, 1986.
52. Capogna M, Gahwiler BH, and Thompson SM. Presynaptic enhancement of inhibitory synaptic transmission by protein kinases A and C in the rat hippocampus in vitro. *J Neurosci* 15: 1249–1260, 1995.
53. Carlson G, Wang Y, and Alger BE. Endocannabinoids facilitate the induction of LTP in the hippocampus. *Nat Neurosci* 5: 723–724, 2002.
54. Carta G, Nava F, and Gessa GL. Inhibition of hippocampal acetylcholine release after acute and repeated Delta9-tetrahydrocannabinol in rats. *Brain Res* 809: 1–4, 1998.
55. Caterina MJ, Schumacher MA, Tominaga M, Rosen TA, Levine JD, and Julius D. The capsaicin receptor: a heat-activated ion channel in the pain pathway. *Nature* 389: 816–824, 1997.
56. Caulfield MP and Brown DA. Cannabinoid receptor agonists inhibit Ca current in NG108–15 neuroblastoma cells via a pertussis toxin-sensitive mechanism. *Br J Pharmacol* 106: 231–232, 1992.
57. Cesa R, Mackie K, Beltramo M, and Franzoni MF. Cannabinoid receptor CB1-like and glutamic acid decarboxylase-like immunoreactivities in the brain of *Xenopus laevis*. *Cell Tissue Res* 306: 391–398, 2001.
58. Chan PK, Chan SC, and Yung WH. Presynaptic inhibition of GABAergic inputs to rat substantia nigra pars reticulata neurones by a cannabinoid agonist. *Neuroreport* 9: 671–675, 1998.
59. Chan PK and Yung WH. Occlusion of the presynaptic action of

cannabinoids in rat substantia nigra pars reticulata by cadmium. *Neurosci Lett* 249: 57–60, 1998.
60. CHEMIN J, MONTEIL A, PEREZ-REYES E, NARGEOT J, AND LORY P. Direct inhibition of T-type calcium channels by the endogenous cannabinoid anandamide. *EMBO J* 20: 7033–7040, 2001.
61. COBB SR, BUHL EH, HALASY K, PAULSEN O, AND SOMOGYI P. Synchronization of neuronal activity in hippocampus by individual GABAergic interneurons. *Nature* 378: 75–78, 1995.
62. COLLINS DR, PERTWEE RG, AND DAVIES SN. The action of synthetic cannabinoids on the induction of long-term potentiation in the rat hippocampal slice. *Eur J Pharmacol* 259: R7–R8, 1994.
63. COLLINS DR, PERTWEE RG, AND DAVIES SN. Prevention by the cannabinoid antagonist, SR141716A, of cannabinoid-mediated blockade of long-term potentiation in the rat hippocampal slice. *Br J Pharmacol* 115: 869–870, 1995.
64. COMPTON DR AND MARTIN BR. The effect of the enzyme inhibitor phenylmethylsulfonyl fluoride on the pharmacological effect of anandamide in the mouse model of cannabimimetic activity. *J Pharmacol Exp Ther* 283: 1138–1143, 1997.
65. COMPTON DR, RICE KC, DE COSTA BR, RAZDAN RK, MELVIN LS, JOHNSON MR, AND MARTIN BR. Cannabinoid structure-activity relationships: correlation of receptor binding and in vivo activities. *J Pharmacol Exp Ther* 265: 218–226, 1993.
66. CONSTOE PF, JONES BC, AND CHIN L. Delta-9-tetrahydrocannabinol, EEG and behavior: the importance of adaptation to the testing milieu. *Pharmacol Biochem Behav* 3: 173–177, 1975.
67. CONTOS JJ, ISHII I, AND CHUN J. Lysophosphatidic acid receptors. *Mol Pharmacol* 58: 1188–1196, 2000.
68. CRAVATT BF, DEMAREST K, PATRICELLI MP, BRACEY MH, GIANG DK, MARTIN BR, AND LICHTMAN AH. Supersensitivity to anandamide and enhanced endogenous cannabinoid signaling in mice lacking fatty acid amide hydrolase. *Proc Natl Acad Sci USA* 98: 9371–9376, 2001.
69. CRAVATT BF, GIANG DK, MAYFIELD SP, BOGER DL, LERNER RA, AND GILULA NB. Molecular characterization of an enzyme that degrades neuromodulatory fatty-acid amides. *Nature* 384: 83–87, 1996.
70. CRAVATT BF, PROSPERO-GARCIA O, SIUZDAK G, GILULA NB, HENRIKSEN SJ, BOGER DL, AND LERNER RA. Chemical characterization of a family of brain lipids that induce sleep. *Science* 268: 1506–1509, 1995.
71. D'AMBRA TE, ESTEP KG, BELL MR, EISSENSTAT MA, JOSEF KA, WARD SJ, HAYCOCK DA, BAIZMAN ER, CASIANO FM, BEGLIN NC, CHIPPARI SM, GREGO JD, KULLNIG RK, AND DALEY GT. Conformationally restrained analogues of pravadoline: nanomolar potent, enantioselective, (aminoalkyl)indole agonists of the cannabinoid receptor. *J Med Chem* 35: 124–135, 1992.
72. DAVIS GW AND MURPHEY RK. Retrograde signaling and the development of transmitter release properties in the invertebrate nervous system. *J Neurobiol* 25: 740–756, 1994.
73. DAY TA, RAKHSHAN F, DEUTSCH DG, AND BARKER EL. Role of fatty acid amide hydrolase in the transport of the endogenous cannabinoid anandamide. *Mol Pharmacol* 59: 1369–1375, 2001.
74. DEADWYLER SA, HAMPSON RE, BENNETT BA, EDWARDS TA, MU J, PACHECO MA, WARD SJ, AND CHILDERS SR. Cannabinoids modulate potassium current in cultured hippocampal neurons. *Receptors Channels* 1: 121–134, 1993.
75. DEADWYLER SA, HAMPSON RE, MU J, WHYTE A, AND CHILDERS S. Cannabinoids modulate voltage sensitive potassium A-current in hippocampal neurons via a cAMP-dependent process. *J Pharmacol Exp Ther* 273: 734–743, 1995.
76. DEAHL M. Cannabis and memory loss. *Br J Addict* 86: 249–252, 1991.
77. DE LAGO E, FERNANDEZ-RUIZ J, ORTEGA-GUTIERREZ S, VISO A, LOPEZ-RODRIGUEZ ML, AND RAMOS JA. UCM707, a potent and selective inhibitor of endocannabinoid uptake, potentiates hypokinetic and antinociceptive effects of anandamide. *Eur J Pharmacol* 449: 99–103, 2002.
78. DELMAS P, WANAVERBECQ N, ABOGADIE FC, MISTRY M, AND BROWN DA. Signaling microdomains define the specificity of receptor-mediated InsP(3) pathways in neurons. *Neuron* 34: 209–220, 2002.
79. DE PETROCELLIS L, BISOGNO T, DAVIS JB, PERTWEE RG, AND DI MARZO V. Overlap between the ligand recognition properties of the anandamide transporter and the VR1 vanilloid receptor: inhibitors of anandamide uptake with negligible capsaicin-like activity. *FEBS Lett* 483: 52–56, 2000.
80. DESARNAUD F, CADAS H, AND PIOMELLI D. Anandamide amidohydrolase activity in rat brain microsomes. Identification and partial characterization. *J Biol Chem* 270: 6030–6035, 1995.
81. DEUTSCH DG AND CHIN SA. Enzymatic synthesis and degradation of anandamide, a cannabinoid receptor agonist. *Biochem Pharmacol* 46: 791–796, 1993.
82. DEUTSCH DG, LIN S, HILL WA, MORSE KL, SALEHANI D, ARREAZA G, OMEIR RL, AND MAKRIYANNIS A. Fatty acid sulfonyl fluorides inhibit anandamide metabolism and bind to the cannabinoid receptor. *Biochem Biophys Res Commun* 231: 217–221, 1997.
83. DEVANE WA AND AXELROD J. Enzymatic synthesis of anandamide, an endogenous ligand for the cannabinoid receptor, by brain membranes. *Proc Natl Acad Sci USA* 91: 6698–6701, 1994.
84. DEVANE WA, DYSARZ FA, JOHNSON MR III, MELVIN LS, AND HOWLETT AC. Determination and characterization of a cannabinoid receptor in rat brain. *Mol Pharmacol* 34: 605–613, 1988.
85. DEVANE WA, HANUS L, BREUER A, PERTWEE RG, STEVENSON LA, GRIFFIN G, GIBSON D, MANDELBAUM A, ETINGER A, AND MECHOULAM R. Isolation and structure of a brain constituent that binds to the cannabinoid receptor. *Science* 258: 1946–1949, 1992.
86. DIAMOND JS. Neuronal glutamate transporters limit activation of NMDA receptors by neurotransmitter spillover on CA1 pyramidal cells. *J Neurosci* 21: 8328–8338, 2001.
87. DIANA MA, LEVENES C, MACKIE K, AND MARTY A. Short-term retrograde inhibition of GABAergic synaptic currents in rat Purkinje cells is mediated by endogenous cannabinoids. *J Neurosci* 22: 200–208, 2002.
88. DI MARZO V, BREIVOGEL CS, TAO Q, BRIDGEN DT, RAZDAN RK, ZIMMER AM, ZIMMER A, AND MARTIN BR. Levels, metabolism, and pharmacological activity of anandamide in CB(1) cannabinoid receptor knockout mice: evidence for non-CB(1), non-CB(2) receptor-mediated actions of anandamide in mouse brain. *J Neurochem* 75: 2434–2444, 2000.
89. DI MARZO V, FONTANA A, CADAS H, SCHINELLI S, CIMINO G, SCHWARTZ JC, AND PIOMELLI D. Formation and inactivation of endogenous cannabinoid anandamide in central neurons. *Nature* 372: 686–691, 1994.
90. DI MARZO V, GOPARAJU SK, WANG L, LIU J, BATKAI S, JARAI Z, FEZZA F, MIURA GI, PALMITER RD, SUGIURA T, AND KUNOS G. Leptin-regulated endocannabinoids are involved in maintaining food intake. *Nature* 410: 822–825, 2001.
91. DI MARZO V, LASTRES-BECKER I, BISOGNO T, DE PETROCELLIS L, MILONE A, DAVIS JB, AND FERNANDEZ-RUIZ JJ. Hypolocomotor effects in rats of capsaicin and two long chain capsaicin homologues. *Eur J Pharmacol* 420: 123–131, 2001.
92. DI MARZO V, MELCK D, BISOGNO T, AND DE PETROCELLIS L. Endocannabinoids: endogenous cannabinoid receptor ligands with neuromodulatory action. *Trends Neurosci* 21: 521–528, 1998.
93. DINH TP, CARPENTER D, LESLIE FM, FREUND TF, KATONA I, SENSI SL, KATHURIA S, AND PIOMELLI D. Brain monoglyceride lipase participating in endocannabinoid inactivation. *Proc Natl Acad Sci USA* 99: 10819–10824, 2002.
94. EGERTOVA M AND ELPHICK MR. Localisation of cannabinoid receptors in the rat brain using antibodies to the intracellular C-terminal tail of CB. *J Comp Neurol* 422: 159–171, 2000.
95. EGERTOVA M, GIANG DK, CRAVATT BF, AND ELPHICK MR. A new perspective on cannabinoid signalling: complementary localization of fatty acid amide hydrolase and the CB1 receptor in rat brain. *Proc R Soc Lond B Biol Sci* 265: 2081–2085, 1998.
96. EMPTAGE N, BLISS TV, AND FINE A. Single synaptic events evoke NMDA receptor-mediated release of calcium from internal stores in hippocampal dendritic spines. *Neuron* 22: 115–124, 1999.
97. EVANS DM, JOHNSON MR, AND HOWLETT AC. Ca^{2+}-dependent release from rat brain of cannabinoid receptor binding activity. *J Neurochem* 58: 780–782, 1992.
98. EVANS DM, LAKE JT, JOHNSON MR, AND HOWLETT AC. Endogenous cannabinoid receptor binding activity released from rat brain slices by depolarization. *J Pharmacol Exp Ther* 268: 1271–1277, 1994.
99. FACCI L, DAL TOSO R, ROMANELLO S, BURIANI A, SKAPER SD, AND LEON A. Mast cells express a peripheral cannabinoid receptor with dif-

ferential sensitivity to anandamide and palmitoylethanolamide. *Proc Natl Acad Sci USA* 92: 3376–3380, 1995.
100. Farooqui AA, Rammohan KW, and Horrocks LA. Isolation, characterization, and regulation of diacylglycerol lipases from the bovine brain. *Ann NY Acad Sci* 559: 25–36, 1989.
101. Farooqui AA, Taylor WA, and Horrocks LA. Characterization and solubilization of membrane bound diacylglycerol lipases from bovine brain. *Int J Biochem* 18: 991–997, 1986.
102. Farquhar-Smith WP, Egertova M, Bradbury EJ, McMahon SB, Rice AS, and Elphick MR. Cannabinoid CB(1) receptor expression in rat spinal cord. *Mol Cell Neurosci* 15: 510–521, 2000.
103. Feigenbaum JJ, Bergmann F, Richmond SA, Mechoulam R, Nadler V, Kloog Y, and Sokolovsky M. Nonpsychotropic cannabinoid acts as a functional *N*-methyl-D-aspartate receptor blocker. *Proc Natl Acad Sci USA* 86: 9584–9587, 1989.
104. Ferraro L, Tomasini MC, Cassano T, Bebe BW, Siniscalchi A, O'Connor WT, Magee P, Tanganelli S, Cuomo V, and Antonelli T. Cannabinoid receptor agonist WIN 55,212–2 inhibits rat cortical dialysate gamma-aminobutyric acid levels. *J Neurosci Res* 66: 298–302, 2001.
105. Fezza F, Bisogno T, Minassi A, Appendino G, Mechoulam R, and Di Marzo V. Noladin ether, a putative novel endocannabinoid: inactivation mechanisms and a sensitive method for its quantification in rat tissues. *FEBS Lett* 513: 294–298, 2002.
106. Finch EA and Augustine GJ. Local calcium signalling by inositol-1,4,5-trisphosphate in Purkinje cell dendrites. *Nature* 396: 753–756, 1998.
107. Fitzsimonds RM and Poo MM. Retrograde signaling in the development and modification of synapses. *Physiol Rev* 78: 143–170, 1998.
108. Fowler CJ and Jacobsson SO. Cellular transport of anandamide, 2-arachidonoylglycerol and palmitoylethanolamide-targets for drug development? *Prostaglandins Leukotrienes Essent Fatty Acids* 66: 193–200, 2002.
109. Fowler CJ, Janson U, Johnson RM, Wahlstrom G, Stenstrom A, Norstrom K, and Tiger G. Inhibition of anandamide hydrolysis by the enantiomers of ibuprofen, ketorolac, and flurbiprofen. *Arch Biochem Biophys* 362: 191–196, 1999.
110. Fowler CJ, Tiger G, and Stenstrom A. Ibuprofen inhibits rat brain deamidation of anandamide at pharmacologically relevant concentrations. Mode of inhibition and structure-activity relationship. *J Pharmacol Exp Ther* 283: 729–734, 1997.
111. Freund TF and Buzsaki G. Interneurons of the hippocampus. *Hippocampus* 6: 347–470, 1996.
112. Fride E and Mechoulam R. Pharmacological activity of the cannabinoid receptor agonist, anandamide, a brain constituent. *Eur J Pharmacol* 231: 313–314, 1993.
113. Galiegue S, Mary S, Marchand J, Dussossoy D, Carriere D, Carayon P, Bouaboula M, Shire D, Le Fur G, and Casellas P. Expression of central and peripheral cannabinoid receptors in human immune tissues and leukocyte subpopulations. *Eur J Biochem* 232: 54–61, 1995.
114. Gaoni Y and Mechoulam R. Isolation, structure and partial synthesis of an active constituent of hashish. *J Am Chem Soc* 86: 1646–1647, 1964.
115. Gardner EL and Vorel SR. Cannabinoid transmission and reward-related events. *Neurobiol Dis* 5: 502–533, 1998.
116. Gerdeman G and Lovinger DM. CB1 cannabinoid receptor inhibits synaptic release of glutamate in rat dorsolateral striatum. *J Neurophysiol* 85: 468–471, 2001.
117. Gerdeman GL, Ronesi J, and Lovinger DM. Postsynaptic endocannabinoid release is critical to long-term depression in the striatum. *Nat Neurosci* 5: 446–451, 2002.
118. Gessa GL, Casu MA, Carta G, and Mascia MS. Cannabinoids decrease acetylcholine release in the medial-prefrontal cortex and hippocampus, reversal by SR 141716A. *Eur J Pharmacol* 355: 119–124, 1998.
119. Giang DK and Cravatt BF. Molecular characterization of human and mouse fatty acid amide hydrolases. *Proc Natl Acad Sci USA* 94: 2238–2242, 1997.
120. Gifford AN and Ashby CR Jr. Electrically evoked acetylcholine release from hippocampal slices is inhibited by the cannabinoid receptor agonist, WIN 55212–2, and is potentiated by the cannabinoid antagonist, SR 141716A. *J Pharmacol Exp Ther* 277: 1431–1436, 1996.
121. Gifford AN, Bruneus M, Gatley SJ, and Volkow ND. Cannabinoid receptor-mediated inhibition of acetylcholine release from hippocampal and cortical synaptosomes. *Br J Pharmacol* 131: 645–650, 2000.
122. Gifford AN, Samiian L, Gatley SJ, and Ashby CR Jr. Examination of the effect of the cannabinoid receptor agonist, CP 55,940, on electrically evoked transmitter release from rat brain slices. *Eur J Pharmacol* 324: 187–192, 1997.
123. Gifford AN, Tang Y, Gatley SJ, Volkow ND, Lan R, and Makriyannis A. Effect of the cannabinoid receptor SPECT agent, AM 281, on hippocampal acetylcholine release from rat brain slices. *Neurosci Lett* 238: 84–86, 1997.
124. Giuffrida A, Beltramo M, and Piomelli D. Mechanisms of endocannabinoid inactivation: biochemistry and pharmacology. *J Pharmacol Exp Ther* 298: 7–14, 2001.
125. Giuffrida A, Parsons LH, Kerr TM, Rodriguez de Fonseca F, Navarro M, and Piomelli D. Dopamine activation of endogenous cannabinoid signaling in dorsal striatum. *Nat Neurosci* 2: 358–363, 1999.
126. Giuffrida A and Piomelli D. The endocannabinoid system: a physiological perspective on its role in psychomotor control. *Chem Phys Lipids* 108: 151–158, 2000.
127. Giuffrida A, Rodriguez de Fonseca F, Nava F, Loubet-Lescoulie P, and Piomelli D. Elevated circulating levels of anandamide after administration of the transport inhibitor, AM404. *Eur J Pharmacol* 408: 161–168, 2000.
128. Glass M and Dragunow M. Induction of the Krox 24 transcription factor in striosomes by a cannabinoid agonist. *Neuroreport* 6: 241–244, 1995.
129. Glass M, Dragunow M, and Faull RL. Cannabinoid receptors in the human brain: a detailed anatomical and quantitative autoradiographic study in the fetal, neonatal and adult human brain. *Neuroscience* 77: 299–318, 1997.
130. Glitsch M, Llano I, and Marty A. Glutamate as a candidate retrograde messenger at interneurone-Purkinje cell synapses of rat cerebellum. *J Physiol* 497: 531–537, 1996.
131. Gobel I, Trendelenburg AU, Cox SL, Meyer A, and Starke K. Electrically evoked release of [(3)H]norepinephrine from mouse cultured sympathetic neurons: release-modulating heteroreceptors. *J Neurochem* 75: 2087–2094, 2000.
132. Gonzalez S, Romero J, de Miguel R, Lastres-Becker I, Villanua MA, Makriyannis A, Ramos JA, and Fernandez-Ruiz JJ. Extrapyramidal and neuroendocrine effects of AM404, an inhibitor of the carrier-mediated transport of anandamide. *Life Sci* 65: 327–336, 1999.
133. Goparaju SK, Ueda N, Taniguchi K, and Yamamoto S. Enzymes of porcine brain hydrolyzing 2-arachidonoylglycerol, an endogenous ligand of cannabinoid receptors. *Biochem Pharmacol* 57: 417–423, 1999.
134. Goparaju SK, Ueda N, Yamaguchi H, and Yamamoto S. Anandamide amidohydrolase reacting with 2-arachidonoylglycerol, another cannabinoid receptor ligand. *FEBS Lett* 422: 69–73, 1998.
135. Gray JA, McNaughton N, James DT, and Kelly PH. Effect of minor tranquillisers on hippocampal theta rhythm mimicked by depletion of forebrain noradrenaline. *Nature* 258: 424–425, 1975.
136. Grundy RI, Rabuffetti M, and Beltramo M. Cannabinoids and neuroprotection. *Mol Neurobiol* 24: 29–51, 2001.
137. Hájos N and Freund TF. Pharmacological separation of cannabinoid sensitive receptors on hippocampal excitatory and inhibitory fibers. *Neuropharmacology* 43: 503–510, 2002.
138. Hájos N, Katona I, Naiem SS, MacKie K, Ledent C, Mody I, and Freund TF. Cannabinoids inhibit hippocampal GABAergic transmission and network oscillations. *Eur J Neurosci* 12: 3239–3249, 2000.
139. Hájos N, Ledent C, and Freund TF. Novel cannabinoid-sensitive receptor mediates inhibition of glutamatergic synaptic transmission in the hippocampus. *Neuroscience* 106: 1–4, 2001.
140. Hájos N, Papp EC, Acsady L, Levey AI, and Freund TF. Distinct interneuron types express m2 muscarinic receptor immunoreactivity on their dendrites or axon terminals in the hippocampus. *Neuroscience* 82: 355–376, 1998.
141. Hampson AJ, Bornheim LM, Scanziani M, Yost CS, Gray AT, Hansen

BM, LEONOUDAKIS DJ, AND BICKLER PE. Dual effects of anandamide on NMDA receptor-mediated responses and neurotransmission. *J Neurochem* 70: 671–676, 1998.
142. HAMPSON RE AND DEADWYLER SA. Role of cannabinoid receptors in memory storage. *Neurobiol Dis* 5: 474–482, 1998.
143. HANUS L, ABU-LAFI S, FRIDE E, BREUER A, VOGEL Z, SHALEV DE, KUSTANOVICH I, AND MECHOULAM R. 2-Arachidonyl glyceryl ether, an endogenous agonist of the cannabinoid CB1 receptor. *Proc Natl Acad Sci USA* 98: 3662–3665, 2001.
144. HANUS L, GOPHER A, ALMOG S, AND MECHOULAM R. Two new unsaturated fatty acid ethanolamides in brain that bind to the cannabinoid receptor. *J Med Chem* 36: 3032–3034, 1993.
145. HARRIS KD, HIRASE H, LEINEKUGEL X, HENZE DA, AND BUZSAKI G. Temporal interaction between single spikes and complex spike bursts in hippocampal pyramidal cells. *Neuron* 32: 141–149, 2001.
146. HELMCHEN F, IMOTO K, AND SAKMANN B. Ca^{2+} buffering and action potential-evoked Ca^{2+} signaling in dendrites of pyramidal neurons. *Biophys J* 70: 1069–1081, 1996.
147. HELMCHEN F, SVOBODA K, DENK W, AND TANK DW. In vivo dendritic calcium dynamics in deep-layer cortical pyramidal neurons. *Nat Neurosci* 2: 989–996, 1999.
148. HENRY DJ AND CHAVKIN C. Activation of inwardly rectifying potassium channels (GIRK1) by co-expressed rat brain cannabinoid receptors in *Xenopus* oocytes. *Neurosci Lett* 186: 91–94, 1995.
149. HERKENHAM M, GROEN BG, LYNN AB, DE COSTA BR, AND RICHFIELD EK. Neuronal localization of cannabinoid receptors and second messengers in mutant mouse cerebellum. *Brain Res* 552: 301–310, 1991.
150. HERKENHAM M, LYNN AB, DE COSTA BR, AND RICHFIELD EK. Neuronal localization of cannabinoid receptors in the basal ganglia of the rat. *Brain Res* 547: 267–274, 1991.
151. HERKENHAM M, LYNN AB, JOHNSON MR, MELVIN LS, DE COSTA BR, AND RICE KC. Characterization and localization of cannabinoid receptors in rat brain: a quantitative in vitro autoradiographic study. *J Neurosci* 11: 563–583, 1991.
152. HERKENHAM M, LYNN AB, LITTLE MD, JOHNSON MR, MELVIN LS, DE COSTA BR, AND RICE KC. Cannabinoid receptor localization in brain. *Proc Natl Acad Sci USA* 87: 1932–1936, 1990.
153. HERNANDEZ-TRISTAN R, AREVALO C, CANALS S, AND LERET ML. The effects of acute treatment with delta9-THC on exploratory behaviour and memory in the rat. *J Physiol Biochem* 56: 17–24, 2000.
154. HERZBERG U, ELIAV E, BENNETT GJ, AND KOPIN IJ. The analgesic effects of *R*(+)-WIN 55,212–2 mesylate, a high affinity cannabinoid agonist, in a rat model of neuropathic pain. *Neurosci Lett* 221: 157–160, 1997.
155. HEYSER CJ, HAMPSON RE, AND DEADWYLER SA. Effects of delta-9-tetrahydrocannabinol on delayed match to sample performance in rats: alterations in short-term memory associated with changes in task specific firing of hippocampal cells. *J Pharmacol Exp Ther* 264: 294–307, 1993.
156. HILLARD CJ, EDGEMOND WS, JARRAHIAN A, AND CAMPBELL WB. Accumulation of *N*-arachidonoylethanolamine (anandamide) into cerebellar granule cells occurs via facilitated diffusion. *J Neurochem* 69: 631–638, 1997.
157. HILLARD CJ, HARRIS RA, AND BLOOM AS. Effects of the cannabinoids on physical properties of brain membranes and phospholipid vesicles: fluorescence studies. *J Pharmacol Exp Ther* 232: 579–588, 1985.
158. HILLARD CJ, MANNA S, GREENBERG MJ, DICAMELLI R, ROSS RA, STEVENSON LA, MURPHY V, PERTWEE RG, AND CAMPBELL WB. Synthesis and characterization of potent and selective agonists of the neuronal cannabinoid receptor (CB1). *J Pharmacol Exp Ther* 289: 1427–1433, 1999.
159. HILLARD CJ, WILKISON DM, EDGEMOND WS, AND CAMPBELL WB. Characterization of the kinetics and distribution of *N*-arachidonylethanolamine (anandamide) hydrolysis by rat brain. *Biochim Biophys Acta* 1257: 249–256, 1995.
160. HIRSCH D, STAHL A, AND LODISH HF. A family of fatty acid transporters conserved from mycobacterium to man. *Proc Natl Acad Sci USA* 95: 8625–8629, 1998.
161. HOFFMAN AF AND LUPICA CR. Mechanisms of cannabinoid inhibition of GABA(A) synaptic transmission in the hippocampus. *J Neurosci* 20: 2470–2479, 2000.
162. HOFFMAN AF AND LUPICA CR. Direct actions of cannabinoids on synaptic transmission in the nucleus accumbens: a comparison with opioids. *J Neurophysiol* 85: 72–83, 2001.
163. HOHMANN AG, BRILEY EM, AND HERKENHAM M. Pre- and postsynaptic distribution of cannabinoid and mu opioid receptors in rat spinal cord. *Brain Res* 822: 17–25, 1999.
164. HOHMANN AG AND HERKENHAM M. Localization of cannabinoid CB(1) receptor mRNA in neuronal subpopulations of rat striatum: a double-label in situ hybridization study. *Synapse* 37: 71–80, 2000.
165. HOHMANN AG AND HERKENHAM M. Localization of central cannabinoid CB1 receptor messenger RNA in neuronal subpopulations of rat dorsal root ganglia: a double-label in situ hybridization study. *Neuroscience* 90: 923–931, 1999.
166. HOWLETT AC, BARTH F, BONNER TI, CABRAL G, CASELLAS P, DEVANE WA, FELDER CC, HERKENHAM M, MACKIE K, MARTIN BR, MECHOULAM R, AND PERTWEE RG. International Union of Pharmacology. XXVII. Classification of Cannabinoid Receptors. *Pharmacol Rev* 54: 161–202, 2002.
167. HOWLETT AC, BIDAUT-RUSSELL M, DEVANE WA, MELVIN LS, JOHNSON MR, AND HERKENHAM M. The cannabinoid receptor: biochemical, anatomical and behavioral characterization. *Trends Neurosci* 13: 420–423, 1990.
168. HUANG CC, CHEN YL, LO SW, AND HSU KS. Activation of cAMP-dependent protein kinase suppresses the presynaptic cannabinoid inhibition of glutamatergic transmission at corticostriatal synapses. *Mol Pharmacol* 61: 578–585, 2002.
169. HUANG CC, LO SW, AND HSU KS. Presynaptic mechanisms underlying cannabinoid inhibition of excitatory synaptic transmission in rat striatal neurons. *J Physiol* 532: 731–748, 2001.
170. HUERTA PT AND LISMAN JE. Heightened synaptic plasticity of hippocampal CA1 neurons during a cholinergically induced rhythmic state. *Nature* 364: 723–725, 1993.
171. IRVING AJ, COUTTS AA, HARVEY J, RAE MG, MACKIE K, BEWICK GS, AND PERTWEE RG. Functional expression of cell surface cannabinoid CB(1) receptors on presynaptic inhibitory terminals in cultured rat hippocampal neurons. *Neuroscience* 98: 253–262, 2000.
172. ISHAC EJ, JIANG L, LAKE KD, VARGA K, ABOOD ME, AND KUNOS G. Inhibition of exocytotic noradrenaline release by presynaptic cannabinoid CB1 receptors on peripheral sympathetic nerves. *Br J Pharmacol* 118: 2023–2028, 1996.
173. IVERSEN L AND CHAPMAN V. Cannabinoids: a real prospect for pain relief? *Curr Opin Pharmacol* 2: 50–55, 2002.
174. JAGGAR SI, HASNIE FS, SELLATURAY S, AND RICE AS. The anti-hyperalgesic actions of the cannabinoid anandamide and the putative CB2 receptor agonist palmitoylethanolamide in visceral and somatic inflammatory pain. *Pain* 76: 189–199, 1998.
175. JARAI Z, WAGNER JA, VARGA K, LAKE KD, COMPTON DR, MARTIN BR, ZIMMER AM, BONNER TI, BUCKLEY NE, MEZEY E, RAZDAN RK, ZIMMER A, AND KUNOS G. Cannabinoid-induced mesenteric vasodilation through an endothelial site distinct from CB1 or CB2 receptors. *Proc Natl Acad Sci USA* 96: 14136–14141, 1999.
176. JARRAHIAN A, MANNA S, EDGEMOND WS, CAMPBELL WB, AND HILLARD CJ. Structure-activity relationships among *N*-arachidonylethanolamine (Anandamide) head group analogues for the anandamide transporter. *J Neurochem* 74: 2597–2606, 2000.
177. JENNINGS EA, VAUGHAN CW, AND CHRISTIE MJ. Cannabinoid actions on rat superficial medullary dorsal horn neurons in vitro. *J Physiol* 534: 805–812, 2001.
178. JESSELL TM AND KANDEL ER. Synaptic transmission: a bidirectional and self-modifiable form of cell-cell communication. *Cell* 72 *Suppl*: 1–30, 1993.
179. JOHNSTON D, HOFFMAN DA, COLBERT CM, AND MAGEE JC. Regulation of back-propagating action potentials in hippocampal neurons. *Curr Opin Neurobiol* 9: 288–292, 1999.
180. KARLSSON M, CONTRERAS JA, HELLMAN U, TORNQVIST H, AND HOLM C. cDNA cloning, tissue distribution, and identification of the catalytic triad of monoglyceride lipase. Evolutionary relationship to esterases, lysophospholipases, and haloperoxidases. *J Biol Chem* 272: 27218–27223, 1997.
181. KATAYAMA K, UEDA N, KATOH I, AND YAMAMOTO S. Equilibrium in the hydrolysis and synthesis of cannabimimetic anandamide demonstrated by a purified enzyme. *Biochim Biophys Acta* 1440: 205–214, 1999.
182. KATHMANN M, BAUER U, AND SCHLICKER E. CB1 receptor density and

CB1 receptor-mediated functional effects in rat hippocampus are decreased by an intracerebroventricularly administered antisense oligodeoxynucleotide. *Naunyn-Schmiedebergs Arch Pharmacol* 360: 421–427, 1999.
183. KATHMANN M, WEBER B, AND SCHLICKER E. Cannabinoid CB1 receptor-mediated inhibition of acetylcholine release in the brain of NMRI, CD-1 and C57BL/6J mice. *Naunyn-Schmiedebergs Arch Pharmacol* 363: 50–56, 2001.
184. KATHMANN M, WEBER B, ZIMMER A, AND SCHLICKER E. Enhanced acetylcholine release in the hippocampus of cannabinoid CB(1) receptor-deficient mice. *Br J Pharmacol* 132: 1169–1173, 2001.
185. KATHURIA S, GAETANI S, FEGLEY D, VALINO F, DURANTI A, TONTINI A, MOR M, TARZIA G, RANA GL, CALIGNANO A, GIUSTINO A, TATTOLI M, PALMERY M, CUOMO V, AND PIOMELLI D. Modulation of anxiety through blockade of anandamide hydrolysis. *Nat Med* 9: 76–81, 2003.
186. KATONA I, RANCZ EA, ACSADY L, LEDENT C, MACKIE K, HÁJOS N, AND FREUND TF. Distribution of CB1 cannabinoid receptors in the amygdala and their role in the control of GABAergic transmission. *J Neurosci* 21: 9506–9518, 2001.
187. KATONA I, SPERLAGH B, MAGLOCZKY Z, SANTHA E, KOFALVI A, CZIRJAK S, MACKIE K, VIZI ES, AND FREUND TF. GABAergic interneurons are the targets of cannabinoid actions in the human hippocampus. *Neuroscience* 100: 797–804, 2000.
188. KATONA I, SPERLAGH B, SIK A, KAFALVI A, VIZI ES, MACKIE K, AND FREUND TF. Presynaptically located CB1 cannabinoid receptors regulate GABA release from axon terminals of specific hippocampal interneurons. *J Neurosci* 19: 4544–4558, 1999.
189. KATSUKI H, IZUMI Y, AND ZORUMSKI CF. Noradrenergic regulation of synaptic plasticity in the hippocampal CA1 region. *J Neurophysiol* 77: 3013–3020, 1997.
190. KAWAGUCHI Y AND KUBOTA Y. GABAergic cell subtypes and their synaptic connections in rat frontal cortex. *Cereb Cortex* 7: 476–486, 1997.
191. KEITA MS, FRANKEL-KOHN L, BERTRAND N, LECANU L, AND MONMAUR P. Acetylcholine release in the hippocampus of the urethane anaesthetised rat positively correlates with both peak theta frequency and relative power in the theta band. *Brain Res* 887: 323–34, 2000.
192. KIRBY MT, HAMPSON RE, AND DEADWYLER SA. Cannabinoids selectively decrease paired-pulse facilitation of perforant path synaptic potentials in the dentate gyrus in vitro. *Brain Res* 688: 114–20, 1995.
193. KISS J, CSAKI A, BOKOR H, SHANABROUGH M, AND LERANTH C. The supramammillo-hippocampal and supramammillo-septal glutamatergic/aspartatergic projections in the rat: a combined [^{3}H]D-aspartate autoradiographic and immunohistochemical study. *Neuroscience* 97: 657–669, 2000.
194. KLEIN TW, NEWTON CA, AND FRIEDMAN H. Cannabinoids and the immune system. *Pain Res Manag* 6: 95–101, 2001.
195. KOBAYASHI M, OHNO M, SHIBATA S, YAMAMOTO T, AND WATANABE S. Concurrent blockade of beta-adrenergic and muscarinic receptors suppresses synergistically long-term potentiation of population spikes in the rat hippocampal CA1 region. *Brain Res* 777: 242–246, 1997.
196. KOMBIAN SB, MOUGINOT D, AND PITTMAN QJ. Dendritically released peptides act as retrograde modulators of afferent excitation in the supraoptic nucleus in vitro. *Neuron* 19: 903–912, 1997.
197. KOOS T AND TEPPER JM. Inhibitory control of neostriatal projection neurons by GABAergic interneurons. *Nat Neurosci* 2: 467–472, 1999.
198. KOUTEK B, PRESTWICH GD, HOWLETT AC, CHIN SA, SALEHANI D, AKHAVAN N, AND DEUTSCH DG. Inhibitors of arachidonoyl ethanolamide hydrolysis. *J Biol Chem* 269: 22937–22940, 1994.
199. KREITZER AC AND REGEHR WG. Cerebellar depolarization-induced suppression of inhibition is mediated by endogenous cannabinoids. *J Neurosci* 21: RC174, 2001.
200. KREITZER AC AND REGEHR WG. Retrograde inhibition of presynaptic calcium influx by endogenous cannabinoids at excitatory synapses onto Purkinje cells. *Neuron* 29: 717–727, 2001.
201. KRUSZKA KK AND GROSS RW. The ATP- and CoA-independent synthesis of arachidonoylethanolamide. A novel mechanism underlying the synthesis of the endogenous ligand of the cannabinoid receptor. *J Biol Chem* 269: 14345–14348, 1994.
202. KUJTAN PW, CARLEN PL, AND KAPUR BM. delta 9-Tetrahydrocannabinol and cannabidiol: dose-dependent effects on evoked potentials in the hippocampal slice. *Can J Physiol Pharmacol* 61: 420–426, 1983.
203. KURAHASHI Y, UEDA N, SUZUKI H, SUZUKI M, AND YAMAMOTO S. Reversible hydrolysis and synthesis of anandamide demonstrated by recombinant rat fatty-acid amide hydrolase. *Biochem Biophys Res Commun* 237: 512–515, 1997.
204. LANG W, QIN C, LIN S, KHANOLKAR AD, GOUTOPOULOS A, FAN P, ABOUZID K, MENG Z, BIEGEL D, AND MAKRIYANNIS A. Substrate specificity and stereoselectivity of rat brain microsomal anandamide amidohydrolase. *J Med Chem* 42: 896–902, 1999.
205. LARSON J AND LYNCH G. Induction of synaptic potentiation in hippocampus by patterned stimulation involves two events. *Science* 232: 985–988, 1986.
206. LASTRES-BECKER I, HANSEN HH, BERRENDERO F, DE MIGUEL R, PEREZ-ROSADO A, MANZANARES J, RAMOS JA, AND FERNANDEZ-RUIZ J. Alleviation of motor hyperactivity and neurochemical deficits by endocannabinoid uptake inhibition in a rat model of Huntington's disease. *Synapse* 44: 23–35, 2002.
207. LAWRENCE DK AND GILL EW. The effects of delta1-tetrahydrocannabinol and other cannabinoids on spin-labeled liposomes and their relationship to mechanisms of general anesthesia. *Mol Pharmacol* 11: 595–602, 1975.
208. LEDENT C, VALVERDE O, COSSU G, PETITET F, AUBERT JF, BESLOT F, BOHME GA, IMPERATO A, PEDRAZZINI T, ROQUES BP, VASSART G, FRATTA W, AND PARMENTIER M. Unresponsiveness to cannabinoids and reduced addictive effects of opiates in CB1 receptor knockout mice. *Science* 283: 401–404, 1999.
209. LENZ RA AND ALGER BE. Calcium dependence of depolarization-induced suppression of inhibition in rat hippocampal CA1 pyramidal neurons. *J Physiol* 521: 147–157, 1999.
210. LENZ RA, WAGNER JJ, AND ALGER BE. N- and L-type calcium channel involvement in depolarization-induced suppression of inhibition in rat hippocampal CA1 cells. *J Physiol* 512: 61–73, 1998.
211. LEVENES C, DANIEL H, SOUBRIE P, AND CREPEL F. Cannabinoids decrease excitatory synaptic transmission and impair long-term depression in rat cerebellar Purkinje cells. *J Physiol* 510: 867–879, 1998.
212. LICHTMAN AH, HAWKINS EG, GRIFFIN G, AND CRAVATT BF. Pharmacological activity of fatty acid amides is regulated, but not mediated, by fatty acid amide hydrolase in vivo. *J Pharmacol Exp Ther* 302: 73–79, 2002.
213. LITTLE PJ, COMPTON DR, JOHNSON MR, MELVIN LS, AND MARTIN BR. Pharmacology and stereoselectivity of structurally novel cannabinoids in mice. *J Pharmacol Exp Ther* 247: 1046–1051, 1988.
214. LLANO I, LERESCHE N, AND MARTY A. Calcium entry increases the sensitivity of cerebellar Purkinje cells to applied GABA and decreases inhibitory synaptic currents. *Neuron* 6: 565–574, 1991.
215. LOPEZ-RODRIGUEZ ML, VISO A, ORTEGA-GUTIERREZ S, LASTRES-BECKER I, GONZALEZ S, FERNANDEZ-RUIZ J, AND RAMOS JA. Design, synthesis and biological evaluation of novel arachidonic acid derivatives as highly potent and selective endocannabinoid transporter inhibitors. *J Med Chem* 44: 4505–4508, 2001.
216. LU XR, ONG WY, AND MACKIE K. A light and electron microscopic study of the CB1 cannabinoid receptor in monkey basal forebrain. *J Neurocytol* 28: 1045–1051, 1999.
217. LUJAN R, NUSSER Z, ROBERTS JD, SHIGEMOTO R, AND SOMOGYI P. Perisynaptic location of metabotropic glutamate receptors mGluR1 and mGluR5 on dendrites and dendritic spines in the rat hippocampus. *Eur J Neurosci* 8: 1488–1500, 1996.
218. LUJAN R, ROBERTS JD, SHIGEMOTO R, OHISHI H, AND SOMOGYI P. Differential plasma membrane distribution of metabotropic glutamate receptors mGluR1 alpha, mGluR2 and mGluR5, relative to neurotransmitter release sites. *J Chem Neuroanat* 13: 219–241, 1997.
219. LUO C, KUMAMOTO E, FURUE H, CHEN J, AND YOSHIMURA M. Anandamide inhibits excitatory transmission to rat substantia gelatinosa neurones in a manner different from that of capsaicin. *Neurosci Lett* 321: 17–20, 2002.
220. LYSAKOWSKI A, WAINER BH, BRUCE G, AND HERSH LB. An atlas of the regional and laminar distribution of choline acetyltransferase immunoreactivity in rat cerebral cortex. *Neuroscience* 28: 291–336, 1989.

221. MACKIE K AND HILLE B. Cannabinoids inhibit N-type calcium channels in neuroblastoma-glioma cells. *Proc Natl Acad Sci USA* 89: 3825–3829, 1992.
222. MACKIE K, LAI Y, WESTENBROEK R, AND MITCHELL R. Cannabinoids activate an inwardly rectifying potassium conductance and inhibit Q-type calcium currents in AtT20 cells transfected with rat brain cannabinoid receptor. *J Neurosci* 15: 6552–6561, 1995.
223. MAEJIMA T, HASHIMOTO K, YOSHIDA T, AIBA A, AND KANO M. Presynaptic inhibition caused by retrograde signal from metabotropic glutamate to cannabinoid receptors. *Neuron* 31: 463–475, 2001.
224. MAGEE JC AND JOHNSTON D. A synaptically controlled, associative signal for Hebbian plasticity in hippocampal neurons. *Science* 275: 209–213, 1997.
225. MAILLEUX P AND VANDERHAEGHEN JJ. Distribution of neuronal cannabinoid receptor in the adult rat brain: a comparative receptor binding radioautography and in situ hybridization histochemistry. *Neuroscience* 48: 655–668, 1992.
226. MAILLEUX P AND VANDERHAEGHEN JJ. Localization of cannabinoid receptor in the human developing and adult basal ganglia. Higher levels in the striatonigral neurons. *Neurosci Lett* 148: 173–176, 1992.
227. MAINGRET F, PATEL AJ, LAZDUNSKI M, AND HONORE E. The endocannabinoid anandamide is a direct and selective blocker of the background K(+) channel TASK-1. *EMBO J* 20: 47–54, 2001.
228. MALDONADO R AND RODRIGUEZ DE FONSECA F. Cannabinoid addiction: behavioral models and neural correlates. *J Neurosci* 22: 3326–3331, 2002.
229. MANNING BH, MERIN NM, MENG ID, AND AMARAL DG. Reduction in opioid- and cannabinoid-induced antinociception in rhesus monkeys after bilateral lesions of the amygdaloid complex. *J Neurosci* 21: 8238–8246, 2001.
230. MANZONI OJ AND BOCKAERT J. Cannabinoids inhibit GABAergic synaptic transmission in mice nucleus accumbens. *Eur J Pharmacol* 412: R3–R5, 2001.
231. MARSICANO G AND LUTZ B. Expression of the cannabinoid receptor CB1 in distinct neuronal subpopulations in the adult mouse forebrain. *Eur J Neurosci* 11: 4213–4225, 1999.
232. MARTIN LA AND ALGER BE. Muscarinic facilitation of the occurrence of depolarization-induced suppression of inhibition in rat hippocampus. *Neuroscience* 92: 61–71, 1999.
233. MARTIN LA, WEI DS, AND ALGER BE. Heterogeneous susceptibility of GABA(A) receptor-mediated IPSCs to depolarization-induced suppression of inhibition in rat hippocampus. *J Physiol* 532: 685–700, 2001.
234. MARTIN WJ, HOHMANN AG, AND WALKER JM. Suppression of noxious stimulus-evoked activity in the ventral posterolateral nucleus of the thalamus by a cannabinoid agonist: correlation between electrophysiological and antinociceptive effects. *J Neurosci* 16: 6601–6611, 1996.
235. MATSUDA LA, BONNER TI, AND LOLAIT SJ. Localization of cannabinoid receptor mRNA in rat brain. *J Comp Neurol* 327: 535–550, 1993.
236. MATSUDA LA, LOLAIT SJ, BROWNSTEIN MJ, YOUNG AC, AND BONNER TI. Structure of a cannabinoid receptor and functional expression of the cloned cDNA. *Nature* 346: 561–564, 1990.
237. MATSUZAKI M, CASELLA GA, AND RATNER M. delta 9-Tetrahydrocannabinol: EEG changes, bradycardia and hypothermia in the rhesus monkey. *Brain Res Bull* 19: 223–229, 1987.
238. MAZZARI S, CANELLA R, PETRELLI L, MARCOLONGO G, AND LEON A. *N*-(2-hydroxyethyl)hexadecanamide is orally active in reducing edema formation and inflammatory hyperalgesia by down-modulating mast cell activation. *Eur J Pharmacol* 300: 227–236, 1996.
239. MCALLISTER SD, GRIFFIN G, SATIN LS, AND ABOOD ME. Cannabinoid receptors can activate and inhibit G protein-coupled inwardly rectifying potassium channels in a *Xenopus* oocyte expression system. *J Pharmacol Exp Ther* 291: 618–626, 1999.
240. MCDONALD AJ AND MASCAGNI F. Localization of the CB1 type cannabinoid receptor in the rat basolateral amygdala: high concentrations in a subpopulation of cholecystokinin-containing interneurons. *Neuroscience* 107: 641–652, 2001.
241. MECHOULAM R. *The Pharmacohistory of Cannabis sativa.* Boca Raton, FL: CRC, 1986.
242. MECHOULAM R, BEN-SHABAT S, HANUS L, LIGUMSKY M, KAMINSKI NE, SCHATZ AR, GOPHER A, ALMOG S, MARTIN BR, COMPTON DR, PERTWEE RG, GRIFFIN G, BAYEWITCH M, BARG J, AND VOGEL Z. Identification of an endogenous 2-monoglyceride, present in canine gut, that binds to cannabinoid receptors. *Biochem Pharmacol* 50: 83–90, 1995.
243. MECHOULAM R, HANUS L, AND MARTIN BR. Search for endogenous ligands of the cannabinoid receptor. *Biochem Pharmacol* 48: 1537–1544, 1994.
244. MEGIAS M, EMRI Z, FREUND TF, AND GULYAS AI. Total number and distribution of inhibitory and excitatory synapses on hippocampal CA1 pyramidal cells. *Neuroscience* 102: 527–540, 2001.
245. MENG ID, MANNING BH, MARTIN WJ, AND FIELDS HL. An analgesia circuit activated by cannabinoids. *Nature* 395: 381–383, 1998.
246. MILES R, TOTH K, GULYAS AI, HÁJOS N, AND FREUND TF. Differences between somatic and dendritic inhibition in the hippocampus. *Neuron* 16: 815–823, 1996.
247. MILLER AS AND WALKER JM. Effects of a cannabinoid on spontaneous and evoked neuronal activity in the substantia nigra pars reticulata. *Eur J Pharmacol* 279: 179–185, 1995.
248. MILLER AS AND WALKER JM. Electrophysiological effects of a cannabinoid on neural activity in the globus pallidus. *Eur J Pharmacol* 304: 29–35, 1996.
249. MILLER AS AND WALKER JM. Local effects of cannabinoids on spontaneous activity and evoked inhibition in the globus pallidus. *Eur J Pharmacol* 352: 199–205, 1998.
250. MILLER LL AND BRANCONNIER RJ. Cannabis: effects on memory and the cholinergic limbic system. *Psychol Bull* 93: 441–456, 1983.
251. MISNER DL AND SULLIVAN JM. Mechanism of cannabinoid effects on long-term potentiation and depression in hippocampal CA1 neurons. *J Neurosci* 19: 6795–6805, 1999.
252. MOLDRICH G AND WENGER T. Localization of the CB1 cannabinoid receptor in the rat brain. An immunohistochemical study. *Peptides* 21: 1735–1742, 2000.
253. MONORY K, TZAVARA ET, LEXIME J, LEDENT C, PARMENTIER M, BORSODI A, AND HANOUNE J. Novel, not adenylyl cyclase-coupled cannabinoid binding site in cerebellum of mice. *Biochem Biophys Res Commun* 292: 231–235, 2002.
254. MORALES M AND BACKMAN C. Coexistence of serotonin 3 (5-HT_3) and CB1 cannabinoid receptors in interneurons of hippocampus and dentate gyrus. *Hippocampus* 12: 756–764, 2002.
255. MORALES M AND BLOOM FE. The 5-HT_3 receptor is present in different subpopulations of GABAergic neurons in the rat telencephalon. *J Neurosci* 17: 3157–3167, 1997.
256. MORISHITA W AND ALGER BE. Differential effects of the group II mGluR agonist, DCG-IV, on depolarization-induced suppression of inhibition in hippocampal CA1 and CA3 neurons. *Hippocampus* 10: 261–268, 2000.
257. MORISHITA W, KIROV SA, AND ALGER BE. Evidence for metabotropic glutamate receptor activation in the induction of depolarization-induced suppression of inhibition in hippocampal CA1. *J Neurosci* 18: 4870–4882, 1998.
258. MORISSET V, AHLUWALIA J, NAGY I, AND URBAN L. Possible mechanisms of cannabinoid-induced antinociception in the spinal cord. *Eur J Pharmacol* 429: 93–100, 2001.
259. MORISSET V AND URBAN L. Cannabinoid-induced presynaptic inhibition of glutamatergic EPSCs in substantia gelatinosa neurons of the rat spinal cord. *J Neurophysiol* 86: 40–48, 2001.
260. MUNRO S, THOMAS KL, AND ABU-SHAAR M. Molecular characterization of a peripheral receptor for cannabinoids. *Nature* 365: 61–65, 1993.
261. MURPHY LL, MUNOZ RM, ADRIAN BA, AND VILLANUA MA. Function of cannabinoid receptors in the neuroendocrine regulation of hormone secretion. *Neurobiol Dis* 5: 432–446, 1998.
262. NAKAMURA T, BARBARA JG, NAKAMURA K, AND ROSS WN. Synergistic release of Ca^{2+} from IP_3-sensitive stores evoked by synaptic activation of mGluRs paired with backpropagating action potentials. *Neuron* 24: 727–737, 1999.
263. NAKAMURA T, NAKAMURA K, LASSER-ROSS N, BARBARA JG, SANDLER VM, AND ROSS WN. Inositol 1,4,5-trisphosphate (IP_3)-mediated Ca^{2+} release evoked by metabotropic agonists and backpropagating action potentials in hippocampal CA1 pyramidal neurons. *J Neurosci* 20: 8365–8376, 2000.
264. NAKAZI M, BAUER U, NICKEL T, KATHMANN M, AND SCHLICKER E. Inhibition of serotonin release in the mouse brain via presynaptic cannabinoid CB1 receptors. *Naunyn-Schmiedebergs Arch Pharmacol* 361: 19–24, 2000.

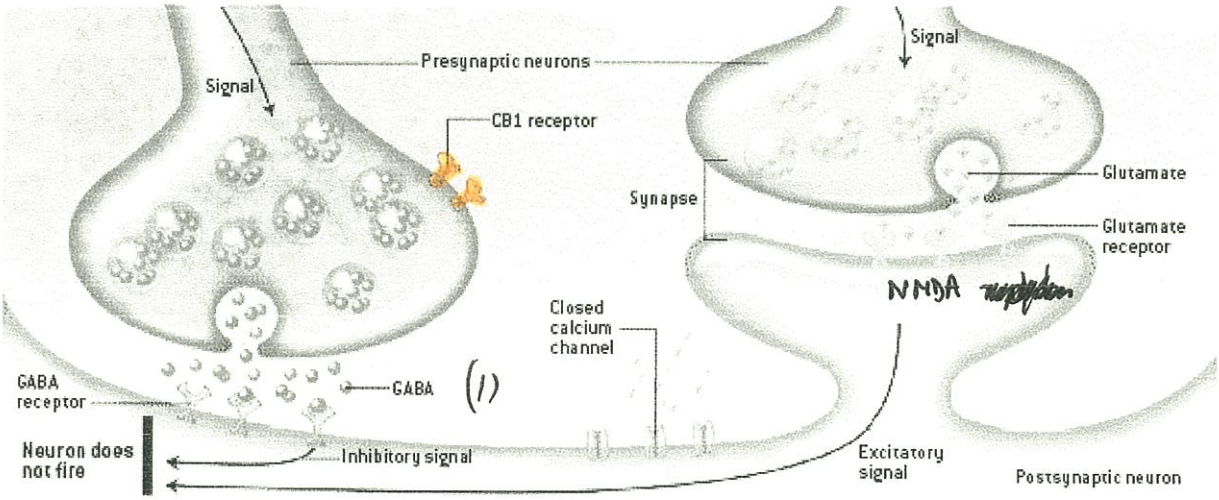
265. Natarajan V, Schmid PC, Reddy PV, and Schmid HH. Catabolism of *N*-acylethanolamine phospholipids by dog brain preparations. *J Neurochem* 42: 1613–1619, 1984.
266. Natarajan V, Schmid PC, Reddy PV, Zuzarte-Augustin ML, and Schmid HH. Biosynthesis of *N*-acylethanolamine phospholipids by dog brain preparations. *J Neurochem* 41: 1303–1312, 1983.
267. Natarajan V, Schmid PC, and Schmid HH. *N*-acylethanolamine phospholipid metabolism in normal and ischemic rat brain. *Biochim Biophys Acta* 878: 32–41, 1986.
268. Nava F, Carta G, Colombo G, and Gessa GL. Effects of chronic Delta(9)-tetrahydrocannabinol treatment on hippocampal extracellular acetylcholine concentration and alternation performance in the T-maze. *Neuropharmacology* 41: 392–399, 2001.
269. Nowicky AV, Teyler TJ, and Vardaris RM. The modulation of long-term potentiation by delta-9-tetrahydrocannabinol in the rat hippocampus, in vitro. *Brain Res Bull* 19: 663–672, 1987.
270. Nyiri G, Freund TF, and Somogyi P. Input-dependent synaptic targeting of alpha(2)-subunit-containing GABA(A) receptors in synapses of hippocampal pyramidal cells of the rat. *Eur J Neurosci* 13: 428–442, 2001.
271. Ohno-Shosaku T, Maejima T, and Kano M. Endogenous cannabinoids mediate retrograde signals from depolarized postsynaptic neurons to presynaptic terminals. *Neuron* 29: 729–738, 2001.
272. Ohno-Shosaku T, Shosaku J, Tsubokawa H, and Kano M. Cooperative endocannabinoid production by neuronal depolarization and group I metabotropic glutamate receptor activation. *Eur J Neurosci* 15: 953–961, 2002.
273. Ohno-Shosaku T, Tsubokawa H, Mizushima I, Yoneda N, Zimmer A, and Kano M. Presynaptic cannabinoid sensitivity is a major determinant of depolarization-induced retrograde suppression at hippocampal synapses. *J Neurosci* 22: 3864–3872, 2002.
274. O'Keefe J. A review of the hippocampal place cells. *Prog Neurobiol* 13: 419–439, 1979.
275. O'Keefe J and Recce ML. Phase relationship between hippocampal place units and the EEG theta rhythm. *Hippocampus* 3: 317–330, 1993.
276. Ong WY and Mackie K. A light and electron microscopic study of the CB1 cannabinoid receptor in primate brain. *Neuroscience* 92: 1177–1191, 1999.
277. Papp E, Leinekugel X, Henze DA, Lee J, and Buzsaki G. The apical shaft of CA1 pyramidal cells is under GABAergic interneuronal control. *Neuroscience* 102: 715–721, 2001.
278. Paton GS, Pertwee RG, and Davies SN. Correlation between cannabinoid mediated effects on paired pulse depression and induction of long term potentiation in the rat hippocampal slice. *Neuropharmacology* 37: 1123–1130, 1998.
279. Patricelli MP and Cravatt BF. Fatty acid amide hydrolase competitively degrades bioactive amides and esters through a nonconventional catalytic mechanism. *Biochemistry* 38: 14125–14130, 1999.
280. Paulsen O and Sejnowski TJ. Natural patterns of activity and long-term synaptic plasticity. *Curr Opin Neurobiol* 10: 172–179, 2000.
281. Pertwee RG. Cannabinoid receptors and pain. *Prog Neurobiol* 63: 569–611, 2001.
282. Petersen G, Chapman KD, and Hansen HS. A rapid phospholipase D assay using zirconium precipitation of anionic substrate phospholipids: application to *n*-acylethanolamine formation in vitro. *J Lipid Res* 41: 1532–1538, 2000.
283. Petersen G and Hansen HS. *N*-acylphosphatidylethanolamine-hydrolysing phospholipase D lacks the ability to transphosphatidylate. *FEBS Lett* 455: 41–44, 1999.
284. Pettit DA, Harrison MP, Olson JM, Spencer RF, and Cabral GA. Immunohistochemical localization of the neural cannabinoid receptor in rat brain. *J Neurosci Res* 51: 391–402, 1998.
285. Piomelli D. *Arachidonic Acid in Cell Signaling*. Heidelberg, Germany: Springer-Verlag, 1996.
286. Piomelli D, Beltramo M, Glasnapp S, Lin SY, Goutopoulos A, Xie XQ, and Makriyannis A. Structural determinants for recognition and translocation by the anandamide transporter. *Proc Natl Acad Sci USA* 96: 5802–5807, 1999.
287. Piomelli D, Giuffrida A, Calignano A, and Rodriguez de Fonseca F. The endocannabinoid system as a target for therapeutic drugs. *Trends Pharmacol Sci* 21: 218–224, 2000.
288. Pitler TA and Alger BE. Postsynaptic spike firing reduces synaptic $GABA_A$ responses in hippocampal pyramidal cells. *J Neurosci* 12: 4122–4132, 1992.
289. Pitler TA and Alger BE. Depolarization-induced suppression of GABAergic inhibition in rat hippocampal pyramidal cells: G protein involvement in a presynaptic mechanism. *Neuron* 13: 1447–1455, 1994.
290. Porcella A, Gessa GL, and Pani L. Delta9-tetrahydrocannabinol increases sequence-specific AP-1 DNA-binding activity and Fos-related antigens in the rat brain. *Eur J Neurosci* 10: 1743–1751, 1998.
291. Porter AC, Sauer JM, Knierman MD, Becker GW, Berna MJ, Bao J, Nomikos GG, Carter P, Bymaster FP, Leese AB, and Felder CC. Characterization of a novel endocannabinoid, virodhamine, with antagonist activity at the CB1 receptor. *J Pharmacol Exp Ther* 301: 1020–1024, 2002.
292. Power JM and Sah P. Nuclear calcium signaling evoked by cholinergic stimulation in hippocampal CA1 pyramidal neurons. *J Neurosci* 22: 3454–3462, 2002.
293. Rakhshan F, Day TA, Blakely RD, and Barker EL. Carrier-mediated uptake of the endogenous cannabinoid anandamide in RBL-2H3 cells. *J Pharmacol Exp Ther* 292: 960–967, 2000.
294. Ranck JB Jr. Studies on single neurons in dorsal hippocampal formation and septum in unrestrained rats. I. Behavioral correlates and firing repertoires. *Exp Neurol* 41: 461–531, 1973.
295. Reggio PH and Traore H. Conformational requirements for endocannabinoid interaction with the cannabinoid receptors, the anandamide transporter and fatty acid amidohydrolase. *Chem Phys Lipids* 108: 15–35, 2000.
296. Richardson JD, Aanonsen L, and Hargreaves KM. Antihyperalgesic effects of spinal cannabinoids. *Eur J Pharmacol* 345: 145–153, 1998.
297. Richardson JD, Kilo S, and Hargreaves KM. Cannabinoids reduce hyperalgesia and inflammation via interaction with peripheral CB1 receptors. *Pain* 75: 111–119, 1998.
298. Rinaldi-Carmona M, Barth F, Heaulme M, Shire D, Calandra B, Congy C, Martinez S, Maruani J, Neliat G, Caput D, Ferrara P, Soubrie P, Breliere JC, and LeFur G. SR141716A, a potent and selective antagonist of the brain cannabinoid receptor. *FEBS Lett* 350: 240–244, 1994.
299. Rinaldi-Carmona M, Pialot F, Congy C, Redon E, Barth F, Bachy A, Breliere JC, Soubrie P, and Le Fur G. Characterization and distribution of binding sites for [^{3}H]-SR 141716A, a selective brain (CB1) cannabinoid receptor antagonist, in rodent brain. *Life Sci* 58: 1239–1247, 1996.
300. Robbe D, Alonso G, Duchamp F, Bockaert J, and Manzoni OJ. Localization and mechanisms of action of cannabinoid receptors at the glutamatergic synapses of the mouse nucleus accumbens. *J Neurosci* 21: 109–116, 2001.
301. Robbe D, Kopf M, Remaury A, Bockaert J, and Manzoni OJ. Endogenous cannabinoids mediate long-term synaptic depression in the nucleus accumbens. *Proc Natl Acad Sci USA* 99: 8384–8388, 2002.
302. Rodriguez JJ, Mackie K, and Pickel VM. Ultrastructural localization of the CB1 cannabinoid receptor in mu-opioid receptor patches of the rat caudate putamen nucleus. *J Neurosci* 21: 823–833, 2001.
303. Rodriguez de Fonseca F, Navarro M, Gomez R, Escuredo L, Nava F, Fu J, Murillo-Rodriguez E, Giuffrida A, LoVerme J, Gaetani S, Kathuria S, Gall C, and Piomelli D. An anorexic lipid mediator regulated by feeding. *Nature* 414: 209–212, 2001.
304. Romero J, Berrendero F, Garcia-Gil L, de la Cruz P, Ramos JA, and Fernandez-Ruiz JJ. Loss of cannabinoid receptor binding and messenger RNA levels and cannabinoid agonist-stimulated [^{35}S]guanylyl-5′*O*-(thio)-triphosphate binding in the basal ganglia of aged rats. *Neuroscience* 84: 1075–1083, 1998.
305. Romero J, Berrendero F, Garcia-Gil L, Ramos JA, and Fernandez-Ruiz JJ. Cannabinoid receptor and WIN-55,212–2-stimulated [^{35}S]GTP gamma S binding and cannabinoid receptor mRNA levels in the basal ganglia and the cerebellum of adult male rats chronically exposed to delta 9-tetrahydrocannabinol. *J Mol Neurosci* 11: 109–119, 1998.
306. Romero J, Fernandez-Ruiz JJ, Vela G, Ruiz-Gayo M, Fuentes JA, and Ramos JA. Autoradiographic analysis of cannabinoid receptor binding and cannabinoid agonist-stimulated [^{35}S]GTP gamma S binding

in morphine-dependent mice. *Drug Alcohol Depend* 50: 241–249, 1998.
307. ROMERO J, HILLARD CJ, CALERO M, AND RABANO A. Fatty acid amide hydrolase localization in the human central nervous system: an immunohistochemical study. *Brain Res* 100: 85–93, 2002.
308. ROUSE ST, MARINO MJ, POTTER LT, CONN PJ, AND LEVEY AI. Muscarinic receptor subtypes involved in hippocampal circuits. *Life Sci* 64: 501–509, 1999.
309. SAGRATELLA S, SCOTTI DE CAROLIS A, AND LONGO VG. EEG interaction between delta 8-tetrahydrocannabinol and some sedative-anxiolytic drugs. Is the anxiogenic effect of Cannabis related to an action on the GABAergic system? *Adv Biochem Psychopharmacol* 41: 203–209, 1986.
310. SALAMONE JD, CERVONE K, MAKRIYANNIS A, AND GOUTOPOULOS A. Behavioral effects of anandamide and the amidase inhibitor AM374. *Proc Internet World Cong for Biomed Sci 6th 2000* (http://www.uclm.es/inabis2000/symposia/files/099).
311. SALIO C, FISCHER J, FRANZONI MF, AND CONRATH M. Pre- and postsynaptic localizations of the CB1 cannabinoid receptor in the dorsal horn of the rat spinal cord. *Neuroscience* 110: 755–764, 2002.
312. SALIO C, FISCHER J, FRANZONI MF, MACKIE K, KANEKO T, AND CONRATH M. CB1-cannabinoid and mu-opioid receptor co-localization on postsynaptic target in the rat dorsal horn. *Neuroreport* 12: 3689–3692, 2001.
313. SANUDO-PENA MC, TSOU K, AND WALKER JM. Motor actions of cannabinoids in the basal ganglia output nuclei. *Life Sci* 65: 703–713, 1999.
314. SANUDO-PENA MC AND WALKER JM. Role of the subthalamic nucleus in cannabinoid actions in the substantia nigra of the rat. *J Neurophysiol* 77: 1635–1638, 1997.
315. SASAKI T AND CHANG MC. *N*-arachidonylethanolamine (anandamide) formation from *N*-arachidonylphosphatidylethanolamine in rat brain membranes. *Life Sci* 61: 1803–1810, 1997.
316. SCHAFFER JE AND LODISH HF. Expression cloning and characterization of a novel adipocyte long chain fatty acid transport protein. *Cell* 79: 427–436, 1994.
317. SCHLICKER E, TIMM J, ZENTNER J, AND GOTHERT M. Cannabinoid CB1 receptor-mediated inhibition of noradrenaline release in the human and guinea-pig hippocampus. *Naunyn-Schmiedebergs Arch Pharmacol* 356: 583–589, 1997.
318. SCHMID HH, SCHMID PC, AND NATARAJAN V. *N*-acylated glycerophospholipids and their derivatives. *Prog Lipid Res* 29: 1–43, 1990.
319. SCHMID PC, PARIA BC, KREBSBACH RJ, SCHMID HH, AND DEY SK. Changes in anandamide levels in mouse uterus are associated with uterine receptivity for embryo implantation. *Proc Natl Acad Sci USA* 94: 4188–4192, 1997.
320. SCHWEITZER P. Cannabinoids decrease the K(+) M-current in hippocampal CA1 neurons. *J Neurosci* 20: 51–58, 2000.
321. SEGAL M. Central implantation of cannabinoids: induction of epileptiform discharges. *Eur J Pharmacol* 27: 40–45, 1974.
322. SELLEY DE, STARK S, SIM LJ, AND CHILDERS SR. Cannabinoid receptor stimulation of guanosine-5′-*O*-(3-[^{35}S]thio)triphosphate binding in rat brain membranes. *Life Sci* 59: 659–668, 1996.
323. SHEN M, PISER TM, SEYBOLD VS, AND THAYER SA. Cannabinoid receptor agonists inhibit glutamatergic synaptic transmission in rat hippocampal cultures. *J Neurosci* 16: 4322–4334, 1996.
324. SIPE JC, CHIANG K, GERBER AL, BEUTLER E, AND CRAVATT BF. A missense mutation in human fatty acid amide hydrolase associated with problem drug use. *Proc Natl Acad Sci USA* 99: 8394–8399, 2002.
325. SMART D AND JERMAN JC. Anandamide: an endogenous activator of the vanilloid receptor. *Trends Pharmacol Sci* 21: 134, 2000.
326. STANTON PK AND SARVEY JM. Depletion of norepinephrine, but not serotonin, reduces long-term potentiation in the dentate gyrus of rat hippocampal slices. *J Neurosci* 5: 2169–2176, 1985.
327. STELLA N AND PIOMELLI D. Receptor-dependent formation of endogenous cannabinoids in cortical neurons. *Eur J Pharmacol* 425: 189–196, 2001.
328. STELLA N, SCHWEITZER P, AND PIOMELLI D. A second endogenous cannabinoid that modulates long-term potentiation. *Nature* 388: 773–778, 1997.
329. STRAIKER AJ, BORDEN CR, AND SULLIVAN JM. G-protein alpha subunit isoforms couple differentially to receptors that mediate presynaptic inhibition at rat hippocampal synapses. *J Neurosci* 22: 2460–2468, 2002.
330. SUGIURA T, KONDO S, SUKAGAWA A, NAKANE S, SHINODA A, ITOH K, YAMASHITA A, AND WAKU K. 2-Arachidonoylglycerol: a possible endogenous cannabinoid receptor ligand in brain. *Biochem Biophys Res Commun* 215: 89–97, 1995.
331. SUGIURA T, KONDO S, SUKAGAWA A, TONEGAWA T, NAKANE S, YAMASHITA A, ISHIMA Y, AND WAKU K. Transacylase-mediated and phosphodiesterase-mediated synthesis of *N*-arachidonoylethanolamine, an endogenous cannabinoid-receptor ligand, in rat brain microsomes. Comparison with synthesis from free arachidonic acid and ethanolamine. *Eur J Biochem* 240: 53–62, 1996.
332. SUGIURA T, KONDO S, SUKAGAWA A, TONEGAWA T, NAKANE S, YAMASHITA A, AND WAKU K. Enzymatic synthesis of anandamide, an endogenous cannabinoid receptor ligand, through *N*-acylphosphatidylethanolamine pathway in testis: involvement of Ca(2+)-dependent transacylase and phosphodiesterase activities. *Biochem Biophys Res Commun* 218: 113–117, 1996.
333. SULLIVAN JM. Mechanisms of cannabinoid-receptor-mediated inhibition of synaptic transmission in cultured hippocampal pyramidal neurons. *J Neurophysiol* 82: 1286–1294, 1999.
334. SZABO B, DORNER L, PFREUNDTNER C, NORENBERG W, AND STARKE K. Inhibition of GABAergic inhibitory postsynaptic currents by cannabinoids in rat corpus striatum. *Neuroscience* 85: 395–403, 1998.
335. SZABO B, WALLMICHRATH I, MATHONIA P, AND PFREUNDTNER C. Cannabinoids inhibit excitatory neurotransmission in the substantia nigra pars reticulata. *Neuroscience* 97: 89–97, 2000.
336. SZALLASI A AND BLUMBERG PM. Vanilloid (Capsaicin) receptors and mechanisms. *Pharmacol Rev* 51: 159–212, 1999.
337. TAKAHASHI KA AND LINDEN DJ. Cannabinoid receptor modulation of synapses received by cerebellar Purkinje cells. *J Neurophysiol* 83: 1167–1180, 2000.
338. TERRANOVA JP, MICHAUD JC, LE FUR G, AND SOUBRIE P. Inhibition of long-term potentiation in rat hippocampal slices by anandamide and WIN55212–2: reversal by SR141716 A, a selective antagonist of CB1 cannabinoid receptors. *Naunyn-Schmiedebergs Arch Pharmacol* 352: 576–579, 1995.
339. TERSIGNI TJ AND ROSENBERG HC. Local pressure application of cannabinoid agonists increases spontaneous activity of rat substantia nigra pars reticulata neurons without affecting response to iontophoretically-applied GABA. *Brain Res* 733: 184–192, 1996.
340. THOMAS EA, CRAVATT BF, DANIELSON PE, GILULA NB, AND SUTCLIFFE JG. Fatty acid amide hydrolase, the degradative enzyme for anandamide and oleamide, has selective distribution in neurons within the rat central nervous system. *J Neurosci Res* 50: 1047–1052, 1997.
341. THOMPSON GA. *The Regulation of Membrane Lipid Metabolism* (2nd ed.). Boca Raton, FL: CRC, 1992.
342. TOGNETTO M, AMADESI S, HARRISON S, CREMINON C, TREVISANI M, CARRERAS M, MATERA M, GEPPETTI P, AND BIANCHI A. Anandamide excites central terminals of dorsal root ganglion neurons via vanilloid receptor-1 activation. *J Neurosci* 21: 1104–1109, 2001.
343. TOTH K, FREUND TF, AND MILES R. Disinhibition of rat hippocampal pyramidal cells by GABAergic afferents from the septum. *J Physiol* 500: 463–474, 1997.
344. TRENDELENBURG AU, COX SL, SCHELB V, KLEBROFF W, KHAIRALLAH L, AND STARKE K. Modulation of (3)H-noradrenaline release by presynaptic opioid, cannabinoid and bradykinin receptors and beta-adrenoceptors in mouse tissues. *Br J Pharmacol* 130: 321–330, 2000.
345. TSOU K, BROWN S, SANUDO-PENA MC, MACKIE K, AND WALKER JM. Immunohistochemical distribution of cannabinoid CB1 receptors in the rat central nervous system. *Neuroscience* 83: 393–411, 1998.
346. TSOU K, MACKIE K, SANUDO-PENA MC, AND WALKER JM. Cannabinoid CB1 receptors are localized primarily on cholecystokinin-containing GABAergic interneurons in the rat hippocampal formation. *Neuroscience* 93: 969–975, 1999.
347. TSOU K, NOGUERON MI, MUTHIAN S, SANUDO-PENA MC, HILLARD CJ, DEUTSCH DG, AND WALKER JM. Fatty acid amide hydrolase is located preferentially in large neurons in the rat central nervous system as revealed by immunohistochemistry. *Neurosci Lett* 254: 137–140, 1998.
348. TURKANIS SA AND KARLER R. Electrophysiologic properties of the cannabinoids. *J Clin Pharmacol* 21: 449S–463S, 1981.
349. TWITCHELL W, BROWN S, AND MACKIE K. Cannabinoids inhibit N- and

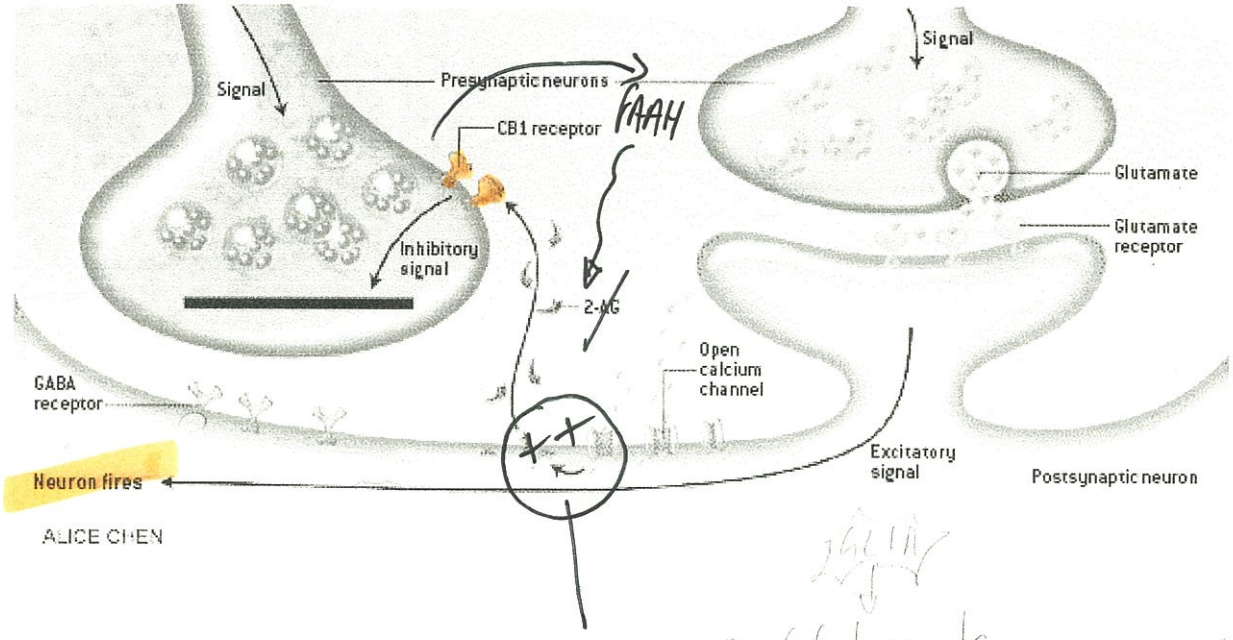
P/Q-type calcium channels in cultured rat hippocampal neurons. *J Neurophysiol* 78: 43–50, 1997.
350. UEDA N, KURAHASHI Y, YAMAMOTO S, AND TOKUNAGA T. Partial purification and characterization of the porcine brain enzyme hydrolyzing and synthesizing anandamide. *J Biol Chem* 270: 23823–23827, 1995.
351. UEDA N, YAMAMOTO K, YAMAMOTO S, TOKUNAGA T, SHIRAKAWA E, SHINKAI H, OGAWA M, SATO T, KUDO I, INOUE K, TAKIZAWA H, NAGANO T, HIROBE M, MATSUKI N, AND SAITO H. Lipoxygenase-catalyzed oxygenation of arachidonylethanolamide, a cannabinoid receptor agonist. *Biochim Biophys Acta* 1254: 127–134, 1995.
352. UEDA N, YAMANAKA K, TERASAWA Y, AND YAMAMOTO S. An acid amidase hydrolyzing anandamide as an endogenous ligand for cannabinoid receptors. *FEBS Lett* 454: 267–270, 1999.
353. UEDA N, YAMANAKA K, AND YAMAMOTO S. Purification and characterization of an acid amidase selective for *N*-palmitoylethanolamine, a putative endogenous anti-inflammatory substance. *J Biol Chem* 276: 35552–35557, 2001.
354. VALJENT E, PAGES C, ROGARD M, BESSON MJ, MALDONADO R, AND CABOCHE J. Delta 9-tetrahydrocannabinol-induced MAPK/ERK and Elk-1 activation in vivo depends on dopaminergic transmission. *Eur J Neurosci* 14: 342–352, 2001.
355. VAN SICKLE MD, OLAND LD, HO W, HILLARD CJ, MACKIE K, DAVISON JS, AND SHARKEY KA. Cannabinoids inhibit emesis through CB1 receptors in the brainstem of the ferret. *Gastroenterology* 121: 767–774, 2001.
356. VARDARIS RM AND WEISZ DJ. Delta9-tetrahydrocannabinol and the hippocampus: effects on CA1 field potentials in rats. *Brain Res Bull* 2: 181–187, 1977.
357. VARMA N, CARLSON GC, LEDENT C, AND ALGER BE. Metabotropic glutamate receptors drive the endocannabinoid system in hippocampus. *J Neurosci* 21: RC188, 2001.
358. VAUGHAN CW, CONNOR M, BAGLEY EE, AND CHRISTIE MJ. Actions of cannabinoids on membrane properties and synaptic transmission in rat periaqueductal gray neurons in vitro. *Mol Pharmacol* 57: 288–295, 2000.
359. VAUGHAN CW, MCGREGOR IS, AND CHRISTIE MJ. Cannabinoid receptor activation inhibits GABAergic neurotransmission in rostral ventromedial medulla neurons in vitro. *Br J Pharmacol* 127: 935–940, 1999.
360. VINCENT P AND MARTY A. Neighboring cerebellar Purkinje cells communicate via retrograde inhibition of common presynaptic interneurons. *Neuron* 11: 885–893, 1993.
361. VINCENT P AND MARTY A. Fluctuations of inhibitory postsynaptic currents in Purkinje cells from rat cerebellar slices. *J Physiol* 494: 183–199, 1996.
362. VIZI ES. Role of high-affinity receptors and membrane transporters in nonsynaptic communication and drug action in the central nervous system. *Pharmacol Rev* 52: 63–89, 2000.
363. VIZI ES, KATONA I, AND FREUND TF. Evidence for presynaptic cannabinoid CB(1) receptor-mediated inhibition of noradrenaline release in the guinea pig lung. *Eur J Pharmacol* 431: 237–244, 2001.
364. WAGNER JJ AND ALGER BE. Increased neuronal excitability during depolarization-induced suppression of inhibition in rat hippocampus. *J Physiol* 495: 107–112, 1996.
365. WALKER JM, HUANG SM, STRANGMAN NM, TSOU K, AND SANUDO-PENA MC. Pain modulation by release of the endogenous cannabinoid anandamide. *Proc Natl Acad Sci USA* 96: 12198–12203, 1999.
366. WALKER JM, STRANGMAN NM, AND HUANG SM. Cannabinoids and pain. *Pain Res Manag* 6: 74–79, 2001.
367. WALLMICHRATH I AND SZABO B. Analysis of the effect of cannabinoids on GABAergic neurotransmission in the substantia nigra pars reticulata. *Naunyn-Schmiedebergs Arch Pharmacol* 365: 326–334, 2002.
368. WATABE AM, CARLISLE HJ, AND O'DELL TJ. Postsynaptic induction and presynaptic expression of group 1 mGluR-dependent LTD in the hippocampal CA1 region. *J Neurophysiol* 87: 1395–1403, 2002.
369. WEISZ DJ, GUNNELL DL, TEYLER TJ, AND VARDARIS RM. Changes in hippocampal CA1 population spikes following administration of delta-9-THC. *Brain Res Bull* 8: 155–162, 1982.
370. WESTLAKE TM, HOWLETT AC, BONNER TI, MATSUDA LA, AND HERKENHAM M. Cannabinoid receptor binding and messenger RNA expression in human brain: an in vitro receptor autoradiography and in situ hybridization histochemistry study of normal aged and Alzheimer's brains. *Neuroscience* 63: 637–652, 1994.
371. WIGSTROM H AND GUSTAFSSON B. Facilitated induction of hippocampal long-lasting potentiation during blockade of inhibition. *Nature* 301: 603–604, 1983.
372. WILKISON DM AND PONTZER NJ. The actions of THC on the intact hippocampus: a comparison of dentate and CA1 responses. *Brain Res Bull* 19: 63–67, 1987.
373. WILLIAMS JH, ERRINGTON ML, LYNCH MA, AND BLISS TV. Arachidonic acid induces a long-term activity-dependent enhancement of synaptic transmission in the hippocampus. *Nature* 341: 739–742, 1989.
374. WILSON RI, KUNOS G, AND NICOLL RA. Presynaptic specificity of endocannabinoid signaling in the hippocampus. *Neuron* 31: 453–462, 2001.
375. WILSON RI AND NICOLL RA. Endogenous cannabinoids mediate retrograde signalling at hippocampal synapses. *Nature* 410: 588–592, 2001.
376. YLINEN A, SOLTESZ I, BRAGIN A, PENTTONEN M, SIK A, AND BUZSAKI G. Intracellular correlates of hippocampal theta rhythm in identified pyramidal cells, granule cells, and basket cells. *Hippocampus* 5: 78–90, 1995.
377. YOSHIDA T, HASHIMOTO K, ZIMMER A, MAEJIMA T, ARAISHI K, AND KANO M. The cannabinoid CB1 receptor mediates retrograde signals for depolarization-induced suppression of inhibition in cerebellar Purkinje cells. *J Neurosci* 22: 1690–1697, 2002.
378. ZILBERTER Y. Dendritic release of glutamate suppresses synaptic inhibition of pyramidal neurons in rat neocortex. *J Physiol* 528: 489–496, 2000.
379. ZILBERTER Y, KAISER KM, AND SAKMANN B. Dendritic GABA release depresses excitatory transmission between layer 2/3 pyramidal and bitufted neurons in rat neocortex. *Neuron* 24: 979–988, 1999.
380. ZIMMER A, ZIMMER AM, HOHMANN AG, HERKENHAM M, AND BONNER TI. Increased mortality, hypoactivity, and hypoalgesia in cannabinoid CB1 receptor knockout mice. *Proc Natl Acad Sci USA* 96: 5780–5785, 1999.
381. ZYGMUNT PM, PETERSSON J, ANDERSSON DA, CHUANG H, SORGARD M, DI MARZO V, JULIUS D, AND HOGESTATT ED. Vanilloid receptors on sensory nerves mediate the vasodilator action of anandamide. *Nature* 400: 452–457, 1999.

RETROGRADE SIGNALING

Researchers have found that endogenous cannabinoids (endocannabinoids) participate in retrograde signaling, a previously unknown form of communication in the brain. Rather than flowing forward in the usual way from a presynaptic (neurotransmitter-emitting) neuron to a postsynaptic (recipient) one, endocannabinoids work backward, traveling from the postsynaptic cell to the presynaptic one. The endocannabinoid 2-AG released from a postsynaptic cell can, for example, cause a presynaptic cell to decrease its secretion of the inhibitory neurotransmitter GABA onto the postsynaptic cell (*diagrams*).



If GABA from a presynaptic neuron hits a postsynaptic cell at the same time as excitatory signals (such as those carried by the neurotransmitter glutamate) reach the same cell (*above*), the GABA can block the postsynaptic cell from firing. If, however, changes in calcium levels in the postsynaptic neuron trigger the production of 2-AG (*below*), this endocannabinoid will travel back to its receptor (CB1) on the GABA-producing neuron. In a process known as depolarized-induced suppression of inhibition (DSI), it will then prevent the release of GABA and thus allow the excitatory signals to activate the postsynaptic cell.



(1) ~~choleckystokinin ?~~

↓

~~Cholecystokinine~~

Toxicité
Excès de 2-AG.

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POTENTIAL THERAPEUTIC EFFECTS OF CANNABINOIDS ON ANXIETY, A REVIEW

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A brief review of the effects of cannabinoid agonists, antagonists and other cannabinoids in preclinical animal models of anxiety will be presented. These studies lead to the conclusion that CB_1 antagonists, are anxiolytic. Agonists, on the other hand seem to have biphasic effects. Low doses seem to be anxiolytic, while high doses anxiogenic. Studies of cannabidiol (CBD) and cannabichromene also strongly suggest that they have anxiolytic properties. In addition, data on fatty acid hydrolase (FAAH) inhibitors, seem to be anxiolytic.

Following this a review of human studies with smoked *Cannabis* and CBD will be presented. These data extend the conclusion from animal studies. It seems that low doses of smoked *Cannabis* have anxiolytic properties at low doses. CBD has anxiolytic properties as well. For CBD, data will be presented from brain scanning studies. Tracer uptake after, CBD adminstration, was increased relative to placebo in the left parahippocampal gyrus and the left fusiform gyrus compared with placebo. Tracer uptake decreased in the CBD relative to placebo in the left amygdala-hippocampal complex and uncus, the hypothalamus and left superior portion of the posterior cingulate gyrus.

The brain area which showed increased activity in relation to placebo was the left parahippocampal gyrus. Deactivation of this area of the brain has been associated with panic attacks induced by lactate, anxiety induced by combat related images and autobiographical memory scripts. It seems that anxiety is associated with reduced parahippocampal activity, consistent with the findings that CBD increases activity in this brain area. Because activity in the CBD after CBD decreased relative to placebo, these data fit well since there is a large amount of data linking amygdala activation in a large variety of anxiety states. Similarly, the hypothalamus involved in various anxiety states, particularly in imaging studies, which have shown increases in hypothalamic activity in anxiety induced in normal volunteers and panic patients. These data are consistent with the anxiolytic effect of CBD. In regard to the posterior cingulate gyrus, increased brain activity is associated when viewing anxiety-provoking videos and provoked obsessions in obsessive patients. OCD patients, untreated, have increased metabolism in the brain area, which decreases with treatment and symptom remission.

In sum it seems clear that cannabinoids can be anxiolytic. We suggest that further clinical research should be conducted to confirm and extend these findings.

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Research Activities of the Group of Prof. Dr. Beat Lutz

Our group investigates the molecular basis of behaviour, in particular the ability to learn, to remember and to extinguish memories. Many aspects of behaviour depend on the ability to learn from experience. At the cellular level, this ability is reflected by the characteristics of the brain to undergo experience-dependent changes in synaptic connectivity and strength. Under pathophysiological conditions of the brain, dysfunctional learning and memory is often observed. Dysfunctional learning and memory can even lead to psychiatric disorders. Cognitive deficits are also a hallmark of neurodegenerative disorders. Understanding memory processing at the molecular level may point to targets of therapeutic interventions in order to ameliorate such disorders.

How do we learn and remember?
In the first research focus, we aim to get insights into mechanisms underlying learning and memory, we use mice as a model system. Applying the so-called Cre/loxP technology, mutants with tissue- and cell type-specific inactivation of genes that are implicated in synaptic plasticity are generated and are analysed in different behavioural tests, including learning and memory tests. Properties of brain physiology are investigated with molecular, cell biological and electrophysiological methods. Genes of interest are related
- to the endocannabinoid system (e.g. the cannabinoid receptor type 1; CB1) (ref. 1),
- to the Ca2+/cAMP signalling system (e.g. CREB-binding protein) (ref. 2), and
- to intracellular kinases/phosphatases (e.g. Dyrk1A) (refs. 3, 4).

RNA interference is used as an additional tool to dissect cellular pathways necessary for synaptic plasticity.

What are the functions of the endocannabinoid system?
The second research focus resides in investigations on the various physiological and pathophysiological roles of the endocannabinoid system (refs. 5, 6). This recently discovered modulatory system appears to have important roles in maintaining the body's homeostasis. This is investigated regarding
(i) the extinction of aversive memories (refs 1, 4),
(ii) the control of excitability of neurons and neuroprotective properties (ref. 7-10),
(iii) feeding behaviour and energy metabolism (refs. 11, 12),
(iv) protection against inflammatory processes in the gastrointestinal tract (ref. 13), and
(v) the control of proliferation and differentiation processes of neural progenitor cells in the embryonic and adult brain.

References
1. Marsicano G, Wotjak CW, Azad SC, Bisogno T, Rammes G, Song T, Hofmann C, Zieglgänsberger W, Di Marzo V, Lutz B (2002) The endogenous cannabinoid system controls extinction of aversive memories. Nature 418:530-535.
2. Zhang Z, Hofmann C, Casanova E, Schütz G, Lutz B (2004) Generation of a conditional allele of the CBP gene in mouse. Genesis 40:82-89.
3. Sitz JH, Tigges M, Baumgärtel K, Khaspekov LG, Lutz B (2004) Dyrk1A potentiates steroid hormone-induced transcription via the chromatin remodeling factor Arip4. Mol Cell Biol 24:5821-5834.
4. Cannich A, Wotjak C, Kamprath C, Lutz B, Marsicano G (2004). CB1 cannabinoid receptors modulate kinase and phosphatase activity during extinction of conditioned fear in mice. Learning & Memory, Learning & Memory 11:625-632.
5. Lutz B (2002) Molecular biology of cannabinoid receptors. Prostaglandins Leukot Essent Fatty Acids 66:123-142. (Review)
6. Lutz B (2004) On-demand activation of the endocannabinoid system in the control of neuronal excitability and epileptiform seizures. Biochem Pharm 68:1691-1998. (Review)
7. Marsicano G, Goodenough S, Monory K, Hermann H, Eder M, Cannich A, Azad SC, Grazia Cascio M, Ortega Gutiérrez S, van der Stelt M, Luz López-Rodriguez M, Casanova E, Schütz G, Zieglgänsberger W, Di Marzo V, Behl C, Lutz B (2003) CB1 cannabinoid receptors mediate on-demand defense against excitotoxicity. Science 302:84-88.
8. Khaspekov LG, Brenz Verca MS, Frumkina LE, Hermann H, Marsicano G, Lutz B (2004) Involvement of brain-derived neurotrophic factor in cannabinoid receptor-dependent protection against excitotoxicity. Eur J Neurosci 19:1691-1698.
9. Bayatti N, Hermann H, Lutz B, Behl C. CRH-mediated induction of BDNF expression is inhibited by the activation of the cannabinoid system. Endocrinology 146:1205-1213.
10. Marsicano G, Moosmann B, Hermann H, Behl C, Lutz B (2002) Neuroprotective properties of cannabinoids against oxidative stress: role of the cannabinoid receptor CB1. J Neurochem 80:448-456.
11. Cota D, Marsicano G, Tschöp M, Grübler Y, Flachskamm C, Schubert M, Auer D, Thöne-Reinecke C, Ortmann S, Cervino C, Linthorst A, Pasquali R, Lutz B, Stalla GK, Pagotto U (2003) Decreased fat mass in mice deficient for cannabinoid receptor 1 is due to decreased orexigenic drive and impaired adipocyte differentiation. J Clin Invest 112:423-431.
12. Marsicano G, Cota D, Stalla GK, Pasquali R, Pagotto U, Lutz B (2003) Cannabinoids in energy balance and prospectives in the therapy of obesity. Curr Med Chem CNS Agents 3:81-87. (Review)
13. Massa F, Marsicano G, Hermann H, Cannich A, Monory K, Cravatt BF, Ferri G-L, Sibaev A, Storr M, Lutz B (2004) The endogenous cannabinoid system protects against colonic inflammation. J Clin Invest 113:1202-1209.

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Reduced anxiety-like behaviour induced by genetic and pharmacological inhibition of the endocannabinoid-degrading enzyme fatty acid amide hydrolase (FAAH) is mediated by CB1 receptors

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Abstract

Anandamide and 2-arachidonoyl glycerol, referred to as endocannabinoids (eCBs), are the endogenous agonists for the cannabinoid receptor type 1 (CB1). Several pieces of evidence support a role for eCBs in the attenuation of anxiety-related behaviours, although the precise mechanism has remained uncertain. The fatty acid amid hydrolase (FAAH), an enzyme responsible for the degradation of eCBs, has emerged as a promising target for anxiety-related disorders, since FAAH inhibitors are able to increase the levels of anandamide and thereby induce anxiolytic-like effects in rodents. The present study adopted both genetic and pharmacological approaches and tested the hypothesis that FAAH-deficient ($FAAH^{-/-}$) mice as well as C57BL/6N mice treated with an FAAH inhibitor (URB597) would express reduced anxiety-like responses. Furthermore, as it is known that anandamide can bind several other targets than CB1 receptors, we investigated whether FAAH inhibition reduces anxiety via CB1 receptors. $FAAH^{-/-}$ mice showed reduced anxiety both in the elevated plus maze and in the light-dark test. These genotype-related differences were prevented by the CB1 receptor antagonist rimonabant (3 mg/kg). Moreover, URB597 (1 mg/kg) induced an anxiolytic-like effect in C57BL/6N mice exposed to the elevated plus maze, which was prevented by rimonabant (3 mg/kg). The present work provides genetic and pharmacological evidence supporting the inhibition of FAAH as an important mechanism for the alleviation of anxiety. In addition, it indicates an increased activation of CB1 receptors as a mechanism underlying the effects of FAAH inhibition in two models of anxiety.
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Keywords: Endocannabinoids; Anandamide; Fatty acid amide hydrolase; CB1 receptor; Anxiety; FAAH knock out mice

1. Introduction

The herb *Cannabis sativa* may induce a diversity of emotional responses ranging from anxiolytic and relaxing effects to the induction of panic attacks (Hall and Solowij, 1998). Divergences have also been observed in both humans and rodents after the administration of Δ^9-tetrahydrocannabinol (Δ^9-THC), the main active compound of marijuana, or its synthetic counterparts (Berrendero and Maldonado, 2002; Marco et al., 2004; Patel and Hillard, 2006; Zuardi et al., 1982).

The mechanisms underlying these bidirectional effects remain to be determined. In the brain, cannabinoids activate the type 1 cannabinoid (CB1) receptor, which is densely expressed in a number of regions related to the modulation of fear and anxiety (Mackie, 2005; Pacher et al., 2006). However, several variables are likely to interfere with the activity of these compounds on experimental anxiety (for a review, see Viveros et al., 2005). First, the effect may depend on the dose administered. In general, low doses tend to be anxiolytic and high doses tend to be anxiogenic (Marco et al., 2004). Second, the characteristics of the experimental environment,

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such as the intensity of illumination, may influence the effects of CB1 activation or blockade (Haller et al., 2004a; Naidu et al., 2007). Third, the various protocols employed for measuring anxiety may generate diverse aversive states, with which cannabinoids may interfere in opposite ways (Viveros et al., 2005). Finally, the previous history of the subjects, such as exposure to stresses or drug treatments, may also be of relevance to the response to cannabinoids (Rodgers et al., 2005).

An alternative approach to the direct activation of CB1 receptors is the enhancement of the availability of the endogenous ligands, referred to as endocannabinoids (eCBs), of which anandamide and 2-arachidonoylglycerol (2-AG) are the mostly investigated (Piomelli, 2003). Their actions are terminated by a putative uptake process, followed by degradation by fatty acid amide hydrolase (FAAH) and by monoacylglycerol lipase (McKinney and Cravatt, 2005), respectively. Specific inhibitors of FAAH have been developed that significantly increase the brain levels of anandamide, but not 2-AG, thereby potentiating the effects of anandamide (Kathuria et al., 2003). FAAH inhibitors induce analgesia, enhance memory extinction and attenuate anxiety via an increased activation of CB1 receptors (Kathuria et al., 2003; Patel and Hillard, 2006; Varvel et al., 2007). However, anandamide is a promiscuous neuromodulator that may bind to other sites in the brain, implying that alternative mechanisms, apart from the activation of CB1 receptors, may be involved in its actions (Ross, 2003). For example, the transient receptor potential vanilloid type 1 channel (TRPV1) is activated by anandamide and located in several brain regions related to emotions (Cristino et al., 2006). TRPV1 was shown to have a role on the modulation of both anxiety and conditioned fear (Marsch et al., 2007). Therefore, increasing the endogenous levels of anandamide may induce effects that are not only CB1-mediated, but also dependent on other receptors.

Apart from the pharmacological studies mentioned above, genetic approaches have also proved to be very useful for the study of the endocannabinoid system. Cravatt et al. (2001) generated mouse mutants lacking the FAAH gene ($FAAH^{-/-}$ mice). These animals have a 10–15-fold increase in the levels of anandamide and an enhanced response to the injection of this endocannabinoid, altered nociceptive responses and enhanced memory extinction (Cravatt et al., 2001; Varvel et al., 2007). Despite these results, the response of $FAAH^{-/-}$ mice in models of anxiety has remained uncertain. Furthermore, behavioural changes not related to an increased activation of the CB1 receptor have also been observed after genetic inhibition of FAAH (Wise et al., 2007).

Therefore, the aim of this study was to test the notion that $FAAH^{-/-}$ mice might show a reduced anxiety-like behaviour as compared to their wild-type (WT) littermates. The elevated plus maze (EPM) and the light dark test (LDT) were employed as animal models. Furthermore, in order to mimic these experiments pharmacologically, studies were conducted with injections of an FAAH inhibitor in C57BL/6N mice. Finally, to test whether there was an involvement of CB1 receptors, we investigated the effects of rimonabant in blocking the behavioural changes observed after genetic or pharmacological inhibition of FAAH.

2. Materials and methods

2.1. Subjects

The animals used in this study were male C57BL/6N mice, FAAH knock out ($FAAH^{-/-}$) mice and their WT littermates ($FAAH^{+/+}$), with a weight of 20–30 g and age of 3–4 months. The generation of $FAAH^{-/-}$ mice was described previously (Cravatt et al., 2001). Mutant mice were backcrossed 6 times into C57BL/6N background and heterozygous breedings were utilized. Tail biopsy and ear clip sampling were performed at an age of 6–10 weeks, and genotyping by PCR was performed as described (Cravatt et al., 2001). C57BL/6N mice were bred in the same animal facility as FAAH-deficient mice and were also tailed and ear marked. All animals were housed in a temperature- and humidity-controlled room where food and water were available ad libitum. Light phase was from 7:00 to 19:00. One week prior to behavioural analysis, mice were separated and single housed.

2.2. Drugs

Powdered diazepam (Sigma) and rimonabant (SR141716; NIMH Chemical Synthesis and Drug Supply Program) were suspended in 5% polyoxyethylene-sorbitan monooleate (Tween 80) followed by 0.9% NaCl solution (saline). URB597 (Cayman) was dissolved in a mixture of DMSO, ethanol and saline solution (1:1:18). All drugs were injected intraperitoneally (i.p.) in a volume of 10 ml/kg body weight.

2.3. Apparatus

All experiments were conducted between 9:00 and 13:00 h. The room was illuminated in a way that the intensity of light at the location of the apparatus was 200-lx (Luxmeter LX1108, Voltcraft, Germany). The experiments were recorded by a camera connected to a computer, where the program Smart (Panlab, Madrid) automatically evaluated the position of the animals and the distance moved.

The elevated plus maze (EPM) consisted of a cross-shaped plastic apparatus, elevated 100 cm from the floor, with 2 opposite open arms and 2 opposite enclosed arms. The floor of the arms was made of white plastic, 35 cm long and 6 cm wide and connected by a central platform of 6 × 6 cm. Walls in black plastic of 20 cm height surrounded the enclosed arms. The animals were placed into the centre of the apparatus, facing the enclosed arms, and were allowed to explore it during 5 min (Carobrez and Bertoglio, 2005). The percentage of time and entries in the open arms were calculated in relation to the total values for the open and enclosed arms through the following formula: % open arms = 100 × open/(open + enclosed).

The light dark test (LDT) was performed in a box (38 cm wide, with walls of 26 cm height) divided in a lit compartment (in white plastic, 26 cm in length; without a roof) and a dark compartment (in black plastic, 13 cm in length; with a roof). Lit and dark compartments were directly connected by a small entrance (5 × 5 cm). The animals were placed in the middle of the lit compartment and allowed to explore the apparatus during 5 min, starting with the first entry to the dark compartment (Bourin and Hascoet, 2003). The percentage of time spent in the lit was calculated as time (s) in this compartment divided by the total time, as follows: % time in the lit = 100 × time in the lit/300.

The open-field consisted of a squared transparent plastic apparatus (40 × 40 × 40 cm). The animals were placed in the centre and allowed to explore it during 5 min (Sousa et al., 2006).

2.4. Procedures

To avoid interferences in the baseline levels of anxiety or exploratory activity, the animals were not exposed twice to the tests – different batches of animals were used in each experiment. The behaviour of WT and $FAAH^{-/-}$ mice

was investigated in the open-field, in the EPM and in the LDT. First, experiments were conducted with these animals without any treatment. In another series of experiments, $FAAH^{-/-}$ and WT mice received either vehicle or rimonabant (3 mg/kg) injections 30 min before the tests. In the experiments with C57BL/6N mice, the animals were treated with vehicle or URB597 (1 mg/kg) 2 h before the tests (Naidu et al., 2007) with no further treatment or with rimonabant (3 mg/kg) or vehicle injection 30 min before the tests.

2.5. Statistics

The percentage of entries and time spent in the open arms as well as the number of entries in the enclosed arms of the EPM, the percentage of time spent in the lit compartment in the LDT and the total distance moved in the open field were analyzed by *t*-test or 2-way analysis of variance (ANOVA), as appropriate. Whenever an interaction between factors was found, post-hoc comparisons were performed with Bonferroni test. In addition, when a reduction in the number of entries in the enclosed arms of the EPM was detected, the percentage of entries in the open arms was re-analyzed by analysis of covariance (ANCOVA), with the number of entries in the enclosed arms as a co-variable. The data are presented as mean ± SEM, and the level of significance was set at a p-value of 0.05.

3. Results

3.1. Pharmacological validation of the animal models of anxiety

As positive controls for the EPM and the LDT, wild-type male C57BL/6N mice received an injection of the anxiolytic diazepam (2 mg/kg) or its vehicle 30 min prior to the experiments. The effect of diazepam on locomotion was evaluated in an open-field, where no significant change in the total distance moved was observed (Table 1). In the EPM, diazepam induced an increase in the percentage of entries (vehicle 14.76 ± 6.68% and diazepam 66.75 ± 11.01%; $t_{14} = 4.03$, $p = 0.0012$; $n = 8$/group) and in the time spent (vehicle 10.90 ± 5.69% and diazepam 70.92 ± 12.61%; $t_{14} = 4.33$, $p = 0.0007$) in the open arms. Its effect on the total number of entries in the enclosed arms is presented in Table 2. In the LDT, diazepam induced an increase in the percentage of time spent in the lit compartment (vehicle 15.84 ± 2.57% and diazepam 37.75 ± 7.76%; $t_{15} = 2.75$, $p = 0.01$; $n = 8, 9$).

3.2. Anxiolytic-like effects after genetic inactivation of FAAH

In the first experiment, we aimed to analyze the phenotypes of WT and $FAAH^{-/-}$ in the EPM. Therefore, mice were exposed to the apparatus without receiving any injection. As depicted in Fig. 1, $FAAH^{-/-}$ mice entered more often ($t_{18} = 2.21$; $p = 0.04$; upper panel) and spent significantly more time ($t_{18} = 2.39$; $p = 0.02$; lower panel) in the open arms, consistent with a phenotype characterized by a reduced anxiety-like behaviour. WT and $FAAH^{-/-}$ did not differ regarding the distance moved in the open field (Table 1) or the number of entries in the enclosed arms of the EPM (Table 2). Next, as presented in Fig. 2, it was tested whether the difference between the genotypes is prevented by the CB1 receptor antagonist rimonabant (3 mg/kg). For the percentage of entries in the open arms, 2-way ANOVA did not reveal any interaction between genotype and drug factors ($F_{1,36} = 1.1$; NS). However, there was a significant effect of genotype ($F_{1,36} = 5.28$; $p = 0.02$) and drug ($F_{1,36} = 18$; $p = 0.0001$). Since there was no interaction between these factors, no post-hoc test was performed. However, for the percentage of time spent in the open arms, there was an borderline interaction between the genotype and drug factors ($F_{1,36} = 3.83$; $p = 0.05$). There was no genotype effect ($F_{1,36} = 2.15$; NS), but a significant drug effect ($F_{1,36} = 4.97$; $p = 0.03$). In this case, a post-hoc analysis was performed revealing that the vehicle-treated $FAAH^{-/-}$ mice spent significantly more time in the open arms as compared to all the other groups. As for the number of entries in the enclosed arms, there was no interaction between factors, neither a genotype effect (Table 2). However, rimonabant markedly reduced this parameter, regardless the genotype (Table 2). File (1992) proposed that, in these cases, the percentage of entries in the open arms should be re-analyzed by analysis of co-variance (ANCOVA), considering the number of entries in the enclosed arms as a co-variable. Accordingly, we performed a 2-way ANCOVA and the result of this analysis was similar to the former 2-way ANOVA. The interaction between factors did not change markedly, and there was still a tendency towards a significance ($F_{1,35} = 3.59$; $p = 0.06$). Again, no genotype effect was seen ($F_{1,35} = 2.41$; N.S.) and

Table 1
Total distance moved during 5 min assessed in the open field for the different mice and treatments employed in the present study ($n = 7$–8/group)

Experimental groups	Distance moved, Mean ± S.E.M.	Statistics (t test or 2-way ANOVA)
Vehicle (Veh)	1378 ± 107.7	$t(14) = 0.9032$; N.S.
Diazepam (2 mg/kg)	1196 ± 169.7	
WT	912.6 ± 163.3	$t(12) = 1.218$; N.S.
$FAAH^{-/-}$	1154 ± 112.9	
WT + Veh	616.5 ± 145.1	Interaction: F(1,28) = 0.92; N.S.
$FAAH^{-/-}$ + Veh	964.9 ± 93.6	Phenotype: F(1,28) = 2.63; N.S.
WT + Rimonabant (3 mg/kg)	566.5 ± 149.1	Drug: F(1,28) = 1.77; N.S.
$FAAH^{-/-}$ + Rimonabant	655.8 ± 143.7	
Veh + Veh	1683 ± 106.8	Interaction: F(1,28) = 2.03; N.S.
URB597 (1 mg/kg) + Veh	1570 ± 139.2	Veh × URB: F(1,28) = 0.43; N.S.
Veh + Rimonabant (3 mg/kg)	1587 ± 171.6	Veh × Rimonabant: F(1,28) = 0.59; N.S.
URB597 + Rimonabant	1890 ± 158.3	

Table 2
Total number of entries in the enclosed arms of the EPM ($n = 6$–10/group)

Experimental groups	Number of entries in the enclosed arms, Mean ± S.E.M.	Statistics (t test or 2-way ANOVA)
Vehicle (Veh)	13.25 ± 1.51	$t(14) = 1.91$; N.S.
Diazepam (2 mg/kg)	9.38 ± 1.36	
WT	12.90 ± 1.17	$t(18) = 1.31$; N.S.
$FAAH^{-/-}$	16.10 ± 16.15	
WT + Veh	10.4 ± 2.47	Interaction: F(1,36) = 0.73; N.S.
$FAAH^{-/-}$ + Veh	10.5 ± 2.11	Phenotype: F(1,36) = 0.70; N.S.
WT + Rimonabant (3 mg/kg)	2.9 ± 1.23	Drug: F(1,36) = 13.04; p = 0.0009
$FAAH^{-/-}$ + Rimonabant	4.3 ± 1.5	
Veh	11.33 ± 1.05	$t(10) = 0.90$; N.S.
URB597 (1 mg/kg)	10.01 ± 1.03	
Veh + Veh	13.50 ± 2.52	Interaction: F(1,28) = 1.11; N.S.
URB597 (1 mg/kg) + Veh	15.50 ± 0.87	Veh × URB: F(1,28) = 0.04; N.S.
Veh + Rimonabant (3 mg/kg)	17.38 ± 2.78	Veh × Rimonabant: F(1,28) = 0.38; N.S.
URB597 + Rimonabant	14.50 ± 2.58	

a drug effect was still present ($F_{1,35} = 6.45$; $p = 0.02$). Thus, although rimonabant significantly reduced entries into the enclosed arms, this effect may have a modest contribution to the changes in the percentage of time in the open arms. No changes in distance moved were found in the open field (Table 1).

To further explore the phenotype of the $FAAH^{-/-}$ mice, in a next series of experiments the WT and $FAAH^{-/-}$ mice were compared in another model of anxiety, the LDT. WT and $FAAH^{-/-}$ mice were exposed to the apparatus without receiving any injection. Supporting the results obtained in the EPM, a reduced anxiety-like behaviour was detected in $FAAH^{-/-}$ mice (Fig. 3). They spent significantly more time in the lit compartment as compared to the WT group ($t_{25} = 2.06$; $p = 0.04$). Finally, it was tested whether this difference is also prevented by rimonabant, as observed in the EPM. As depicted in Fig. 4, no interaction between the genotype and drug factors were found for the percentage of time spent in the lit compartment ($F_{1,34} = 2.73$; NS). However, there was a significant difference between the genotypes ($F_{1,34} = 6.31$; $p = 0.02$), further indicating a reduced anxiety-like behaviour in the $FAAH^{-/-}$ mice. There was also a significant drug effect ($F_{1,34} = 4.42$; $p = 0.04$), indicating that rimonabant was able to reduce the exploration of the lit compartment.

3.3. Anxiolytic-like effects after pharmacological inhibition of FAAH

In order to further strengthen the results obtained with $FAAH^{-/-}$ mice, we have also adopted a pharmacological approach to inhibit the FAAH enzyme. C57BL/6N mice were treated with vehicle or the FAAH inhibitor URB597 (1 mg/kg) and exposed to the EPM two hours later (Fig. 5). A significant increase both in the number of entries ($t_{10} = 2.88$; $p = 0.01$) and in the time spent ($t_{10} = 1.4$; $p = 0.0058$) in the open arms was detected. Next, to check for the involvement of CB1 receptors, the effect of rimonabant (3 mg/kg) was investigated (Fig. 6). A 2-way ANOVA was performed considering the following experimental factors: treatment with URB597 or its respective vehicle (URB factor) and treatment with rimonabant or its respective vehicle (rimonabant factor). For the percentage of entries in the open arms (Fig. 6, upper panel), a significant interaction between these factors was detected ($F_{1,28} = 5.37$; $p = 0.04$). There was no significant effect neither for the URB factor ($F_{1,28} = 0.21$; NS) nor for the rimonabant factor ($F_{1,28} = 0.03$; NS). Post-hoc analysis did not found any difference between particular groups. For the percentage of time spent in the open arms (Fig. 6, lower panel), a significant interaction between these factor was observed ($F_{1,28} = 4.42$; $p = 0.04$). Furthermore, there was an significant effect of URB ($F_{1,28} = 4.59$; $p = 0.04$), but not of the rimonabant factor ($F_{1,28} = 0.89$; NS). Post-hoc analysis revealed a significant increase in the time spent in the open arms only for the group treated with vehicle-URB597, as compared to the vehicle-vehicle group. Altogether, these pharmacological data mimic those observed with the $FAAH^{-/-}$ mice, as they indicate that FAAH inhibition induces an anxiolytic-like effect mediated by the CB1 receptor. Neither drug changed in locomotion in the open field (Table 1). In addition, as presented in Table 2, there were no changes in the total number of entries in the enclosed arms of the EPM.

Finally, we tested the effect of URB597 (1 mg/kg) in the LDT. C57BL/6N mice were treated with vehicle or URB597 (1 mg/kg) and exposed to the test 2 h later (Fig. 7). Contrary to the EPM, no significant effect was observed in this model of anxiety ($t_{20} = 1.4$; NS).

4. Discussion

The present study shows that $FAAH^{-/-}$ mice exhibit reduced anxiety-like behaviour in two experimental models. Since this phenotype was reversed after injection of the CB1 antagonist rimonabant, it is likely that elevated levels of eCBs acting via CB1 receptors are responsible for the

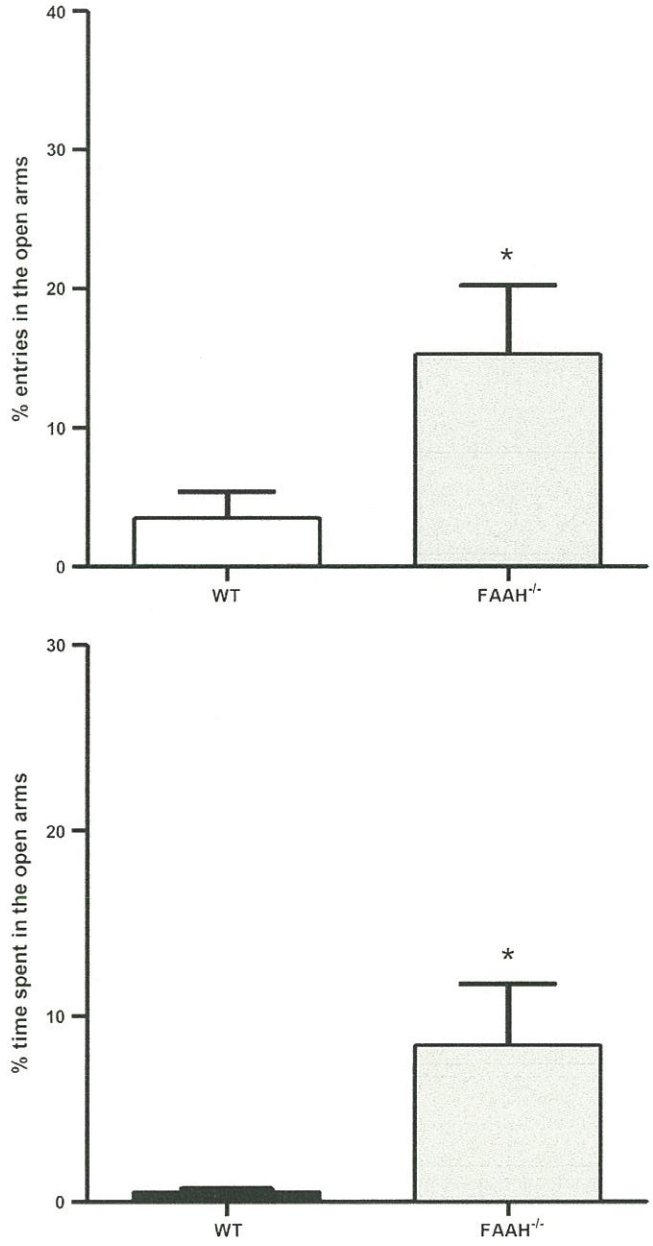

Fig. 1. Behaviour of FAAH$^{-/-}$ mice in the elevated plus maze. FAAH$^{-/-}$ mice entered more frequently (upper panel) and spent a longer time (lower panel) in the open arms as compared to their WT littermates, indicating a reduced anxiety-like behaviour (*$p < 0.05$; $n = 10$/group).

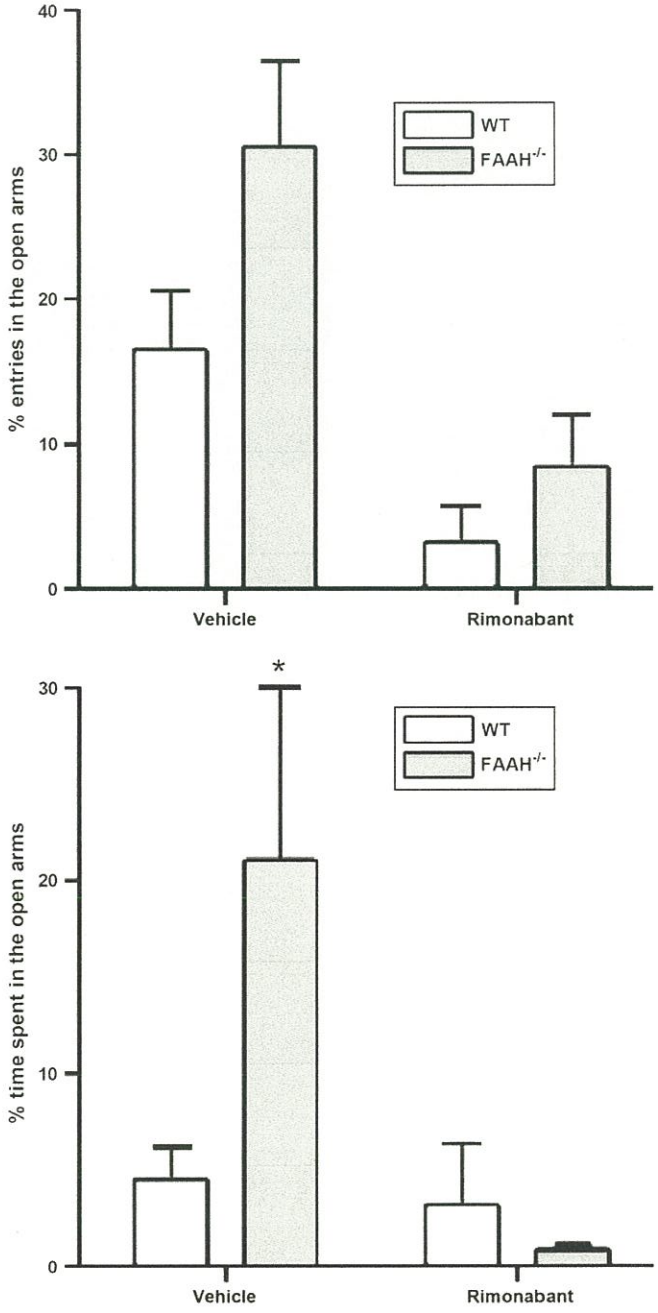

Fig. 2. Effects of the CB1 antagonist rimonabant on anxiety-like behaviour of WT and FAAH$^{-/-}$ mice in the elevated plus maze. Rimonabant (3 mg/kg) treatment in FAAH$^{-/-}$ mice was able to prevent the increase in the number of entries (upper panel) and in the time spent (lower panel) in the open arms, indicating increased signalling via CB1 receptors in FAAH$^{-/-}$ mice (*$p < 0.05$ compared to all the other groups; $n = 10$/group).

decreased anxiety in animals lacking FAAH. Furthermore, an anxiolytic-like effect was observed in C57BL/6N mice after injection of the FAAH inhibitor URB597, an effect blocked by rimonabant, and, thus, also mediated by CB1 receptors. No differences in spontaneous locomotion were observed in the open field, indicating that these results are not secondary to altered motor control in FAAH knock out or in drug-treated animals.

Significant differences between FAAH$^{-/-}$ mice and their WT littermates were observed in several experiments, supporting the consistency of this phenotype. These differences were observed both when the animals were exposed to the tests without any treatment and when they received vehicle injections. However, the baseline levels of anxiety were dissimilar between non-treated and vehicle-treated animals. One reason for this observation could be the stress caused by intraperitoneal injections. This procedure may have a major impact in the basal levels of anxiety, and even saline or "sham" injection may change baseline behaviour as compared to non-treated

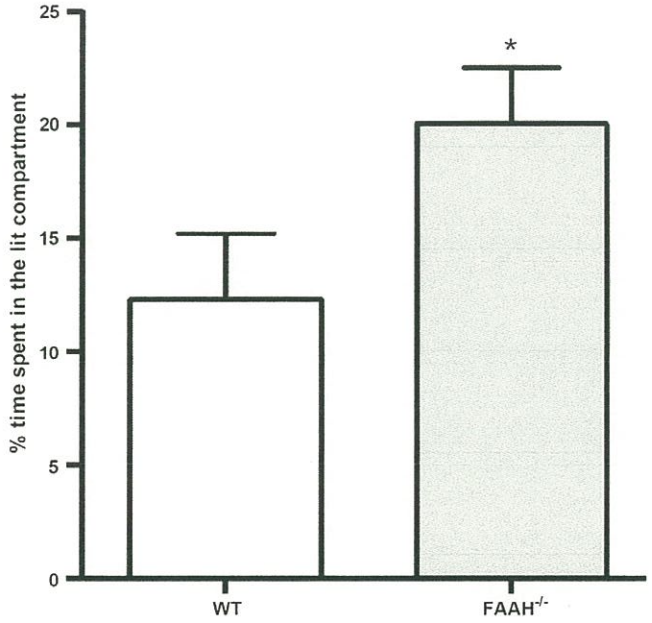

Fig. 3. Behaviour of FAAH$^{-/-}$ mice in the light-dark test. FAAH$^{-/-}$ mice spent a longer time in the lit compartment as compared to their WT littermates, indicating reduced anxiety-like behaviour (*$p < 0.05$; $n = 13$; 14).

animals (Carobrez and Bertoglio, 2005; Lapin, 1995). In addition, painful stimuli may increase the release of anandamide in brain regions that are also related to anxiety (Walker et al., 1999). This may also explain the differences observed in the present study.

The phenotype of FAAH$^{-/-}$ mice is congruent with previous pharmacological experiments demonstrating attenuation of anxiety without motor impairment by the FAAH-inhibitor URB597 in rats and mice (Kathuria et al., 2003; Patel and Hillard, 2006). Similar properties were reported in rats for the anandamide transporter inhibitor AM404 (Bortolato et al., 2006). Altogether, the available pharmacological and genetic evidence suggest that enhancing the activity of the endocannabinoid system would be a valuable strategy for the treatment of anxiety. Moreover, it supports the importance of this system in reducing the impact of aversive encounters of

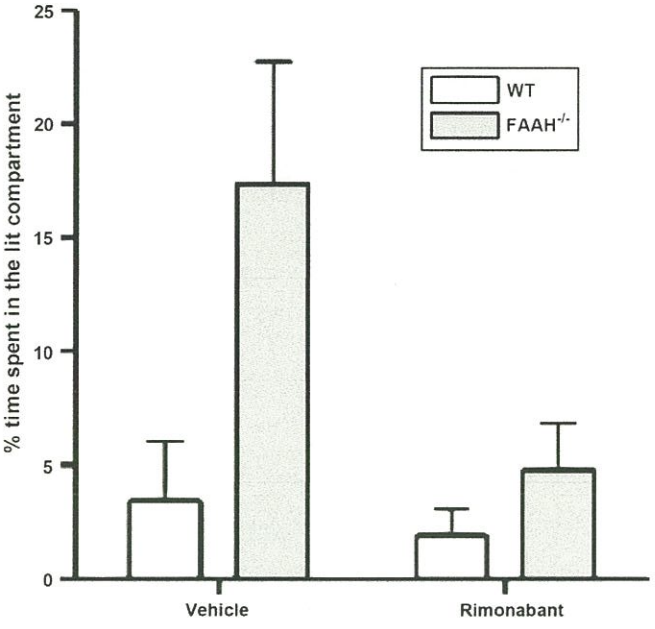

Fig. 4. Effects of the CB1 antagonist rimonabant on anxiety-like behaviour of WT and FAAH$^{-/-}$ mice in the light-dark test. Rimonabant (3 mg/kg) treatment reduced the time spent in the lit compartment in both groups of mice ($n = 10$; 9; 10; 9).

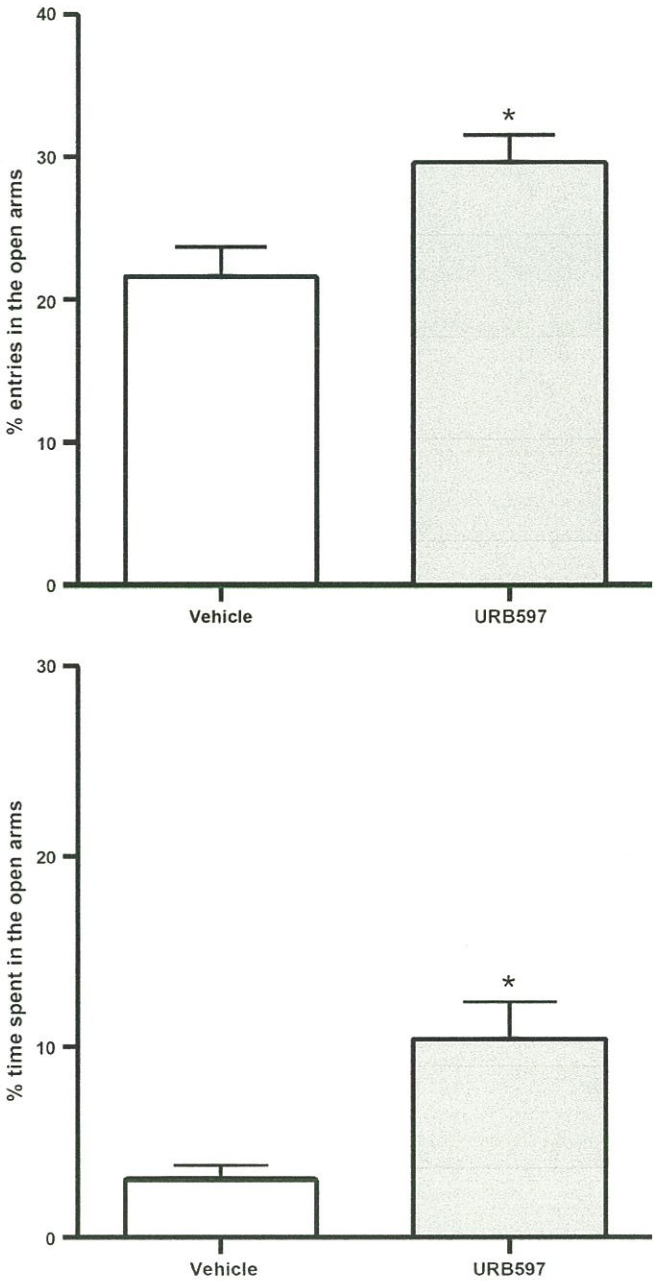

Fig. 5. Effect of pharmacological inhibition of FAAH on the behaviour of C57BL/6N mice in the elevated plus maze. Animals treated with URB597 (1 mg/kg) entered the open arms more frequently (upper panel) and spent a longer time (lower panel) in the open arms as compared to the vehicle-treated group, indicating an anxiolytic-like effect of URB597 (*$p < 0.05$; $n = 6$/group).

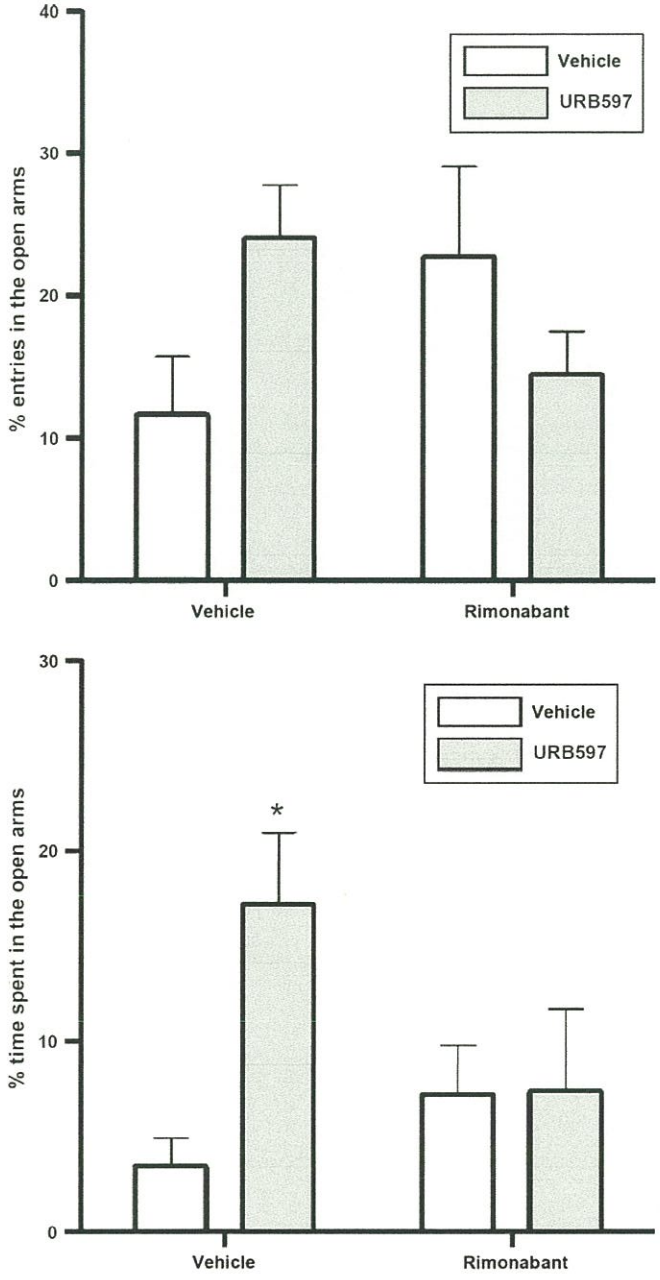

Fig. 6. Effect of the CB1 antagonist rimonabant on the anxiolytic-like activity of URB597 in C57BL/6N mice. Rimonabant (3 mg/kg) treatment was able to prevent the increase in the number of entries (upper panel) and in the time spent (lower panel) in the open arms induced by URB597 (1 mg/kg), indicating an involvement of CB1 receptors in the effect of URB597 ($^*p < 0.05$ compared to all the other groups; $n = 8$/group).

either conditioned or unconditioned nature (Hill et al., 2006; Kamprath et al., 2006; Marsicano et al., 2002). Indeed, increased levels of eCBs were observed during aversive stimuli in key regions related to fear and anxiety responses, where this system may act on-demand to oppose the impact of stress (Marsicano et al., 2002). Based on this assumption, it is reasonable to suppose that an increased activation of CB1 receptors (due to higher levels of eCBs) can protect the $FAAH^{-/-}$ mice more efficiently from the aversive stimuli, as compared to wild-type mice.

The role of CB1 receptors in the modulation of anxiety is also supported by experiments with Δ^9-THC, which may induce anxiolytic effect in mice exposed to the LDT (Berrendero and Maldonado, 2002). However, anxiogenic effects have also been reported with this drug (Onaivi et al., 1990; Patel and Hillard, 2006). Generally, low doses of cannabinoids tend to be anxiolytic and higher doses tend to act on the opposite direction (Viveros et al., 2005). One possible explanation for this phenomenon is the presence of CB1 receptor in pre-synaptic terminals in several brain regions, modulating both excitatory (glutamate) and inhibitory (γ-aminobutyric acid, GABA) neurotransmissions (see Monory et al., 2006; and references therein). Therefore, cannabinoids interfere with systems that exert opposing activities on anxiety responses (Millan, 2003). Importantly, GABAergic terminals contain high levels of CB1 receptors, while glutamatergic terminals contain low levels (Marsicano and Lutz, 1999; Monory et al., 2006). Hence, low concentrations of cannabinoid drugs might be able to effectively activate a significant portion of CB1 receptors on glutamatergic terminals, while a much higher concentration would be needed to activate the same fraction of CB1 receptors on GABAergic terminals. For a better understanding of this subject, the molecular mechanisms and the brain regions underlying the behavioural effects of cannabinoids should be further investigated. One possible site for their anxiolytic activity could be the hippocampus, since this action is prevented by blockade of neurogenesis in this structure (Jiang et al., 2005). Another candidate is the dorsolateral periaqueductal gray, since injections of CB1 receptor agonists into this structure induce anxiolytic-like effects (Moreira et al., 2007). Understanding the mechanism by which the eCBs modulate these systems may help to clarify their role on anxiety-related behaviours.

The phenotype in $FAAH^{-/-}$ mice was reversed by rimonabant both in the EPM and in the LDT. This drug also blocked the effect of URB597 in C57BL/6N mice exposed to the EPM. Thus, an enhancement of CB1 receptor-mediated signalling, presumably due to higher levels of anandamide, seems to be responsible for the reduction in anxiety-related behaviours. However, it should be noted that in $FAAH^{-/-}$ and WT mice rimonabant reduced the number of entries in the enclosed arms of the EPM. To check whether this effect would be interfering with the percentage of time spent in the open arms, we re-analyzed this data by 2-way ANCOVA with the number of entries in the enclosed arms a co-variable. The result of this analysis was similar to the 2-way ANOVA. Thus, it may be concluded that although rimonabant markedly reduced the entries in the enclosed arms, this may have a minor contribution to the effects on the percentage of time spent in the open arms (File, 1992). In C57BL/6N mice, this drug did not change enclosed arms entries. Also, it failed to interfere with total distances moved by $FAAH^{-/-}$, WT or C57BL/6N mice in the open field. Consistent with our study, Kathuria et al. (2003) also showed that rimonabant blocked the anxiolytic effect induced by pharmacological inhibition of FAAH in rats, also

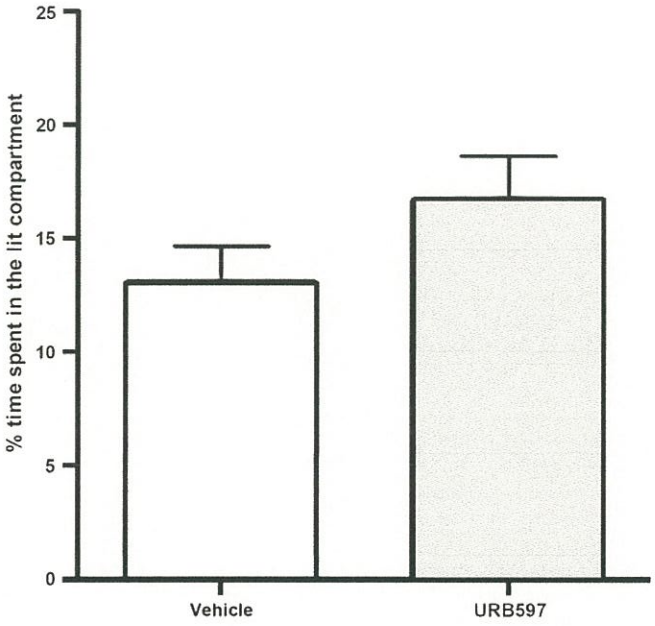

Fig. 7. Effect of the pharmacological blockade of FAAH on the behaviour of C57BL/6N mice in the light-dark test The animals treated with URB597 (1 mg/kg) did not spent a longer time in the lit compartment as compared to the vehicle treated group ($n = 10$; 12).

pointing to an involvement of CB1 receptors in the anxiolytic. These results are relevant because anandamide is a promiscuous neurotransmitter that may bind to other sites in the brain (Ross, 2003; Wise et al., 2007). In our study, apart from blocking the phenotype of $FAAH^{-/-}$ mice, rimonabant also inhibited the exploration of aversive environments in WT mice. This means that this CB1 antagonist induced an anxiogenic-like effect, in accordance with the possible existence of a modulatory tonus by endocannabinoids on anxiety. However, the same was not observed in C57BL/6N mice, in which rimonabant selectively prevented the anxiolytic effect of URB597, without any reduction in basal levels in vehicle-treated mice. These diverse responses have been observed after blockade of the CB1 receptors with either rimonabant or AM251. While in some cases, these drugs may induce no modification on measures of anxiety (Kathuria et al., 2003), others studies show anxiogenic effects (Navarro et al., 1997; Patel and Hillard, 2006; Rodgers et al., 2005). Studies with CB1-deficient mice also showed a complex behaviour. These mutants may have either increased levels of anxiety or no phenotype, depending on the environmental stimuli (Haller et al., 2004a; Martin et al., 2001).

Apart from CB1 receptors, an alternative site mediating some effects of anandamide is the TRPV1 receptor (Ross, 2003), which is also located in brain regions related to anxiety (Cristino et al., 2006). However, it is unlikely that an increased activation of this receptor would contribute to the anxiolytic-like phenotype of $FAAH^{-/-}$ mice. One reason is that the phenotype was blocked by rimonabant, as discussed above. Another reason is that, contrary to CB1 receptors, the pharmacological blockade of TRPV1 receptors induces anxiolytic, rather than anxiogenic responses (Kasckow et al., 2004). Also, TRPV1-deficient mice exhibit a phenotype characterized by reduced, rather than increased, levels of anxiety (Marsch et al., 2007). Altogether, these data support the notion that anandamide might balance anxiety by exerting actions in opposite directions via the activation of CB1 and TRPV1 receptors (Marsch et al., 2007).

At the time when the present experiments were being conducted, another study showed no phenotype of the $FAAH^{-/-}$ mice in the EPM (Naidu et al., 2007). Although the present results are apparently discrepant with the cited investigation, differences in genetic background (C57BL/6N versus C57BL/6J), animal housing as well in experimental context might explain these discrepancies. In fact, in the study by Naidu et al. (2007), the FAAH inhibitor URB597 was ineffective when the experiments were conducted under low light environment, although an anxiolytic-like effect was detected when illumination over the open arms of the EPM was increased. These differences may arise because the stress levels of the subjects and the experimental context are critical factors for detecting anxiolytic responses in the EPM (Mechiel Korte and De Boer, 2003). Such parameters might be particularly relevant in manipulations involving eCBs, which are supposed to act on-demand to counteract aversive responses (Marsicano et al., 2002; Kamprath et al., 2006). In line with this view, CB1 knock-out mice have a phenotype characterized by an increased anxiety-like behaviour, which is observed only when the experiments are conducted under high light intensity (Haller et al., 2004a). Therefore, different experimental environments may explain some apparent discrepancies in studies with the endocannabinoid system.

Finally, we adopted a pharmacological approach to further strengthen the results obtained with $FAAH^{-/-}$ mice. In accordance with previous data (Kathuria et al., 2003; Naidu et al., 2007; Patel and Hillard, 2006), the FAAH inhibitor induced an anxiolytic-like effect in rodents exposed to the EPM, which was prevented by rimonabant. However, while Rutkowska et al. (2006) detected an anxiolytic effect in mice exposed to the LDT after treatment with the FAAH inhibitor AACOCF3, no significant effect of URB597 was observed in this model in the present study, even though diazepam was effective as a positive control. A possible explanation for the discrepancy between the EPM and the LDT is that these models may generate different levels of aversion. While the LDT is based on the aversion for a lit environment, the EPM is based on the aversion for an open environment (Bourin and Hascoet, 2003; Carobrez and Bertoglio, 2005). It is possible that these models have different sensitivities to detect interventions in the endocannabinoid system. In addition to this lack of effect in the LDT, the effect of URB597 in the EPM was not as efficacious as that observed with the reference compound diazepam. One possible advantage of URB597 over diazepam could be its low potential to induce sedation and memory impairment. Also, the observation that it does not induce conditioned place-preference (Gobbi et al., 2005) suggests its low potential to be addictive. However, to date, it is not fully clear which side effects a long-term deficiency of FAAH activity

may cause because of the accumulation of other classes of lipids, such as N-acyl taurines (Saghatelian et al., 2006). Furthermore, a potential problem in humans is the recent finding of a second FAAH gene (FAAH-2), which is not present in rodents. FAAH-2 is, however, also inhibited by URB597 (Wei et al., 2006).

In conclusion, the present study shows that $FAAH^{-/-}$ mice have a phenotype characteristic of a reduced anxiety-like behaviour, as revealed by two different behavioural models. Furthermore, it suggests that this phenotype is due to an increased activation of CB1 receptors, possibly reflecting the increased levels of anandamide after FAAH inhibition. Finally, it further supports the anxiolytic-like activity of the FAAH inhibitor URB597. These results point to the enhancement of eCBs activity as a promising strategy for the alleviation of anxiety-related disorders.

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References

Berrendero, F., Maldonado, R., 2002. Involvement of the opioid system in the anxiolytic-like effects induced by Delta(9)-tetrahydrocannabinol. Psychopharmacology (Berl.) 163, 111–117.

Bortolato, M., Campolongo, P., Mangieri, R.A., Scattoni, M.L., Frau, R., Trezza, V., La Rana, G., Russo, R., Calignano, A., Gessa, G.L., Cuomo, V., Piomelli, D., 2006. Anxiolytic-like properties of the anandamide transport inhibitor AM404. Neuropsychopharmacology 31, 2652–2659.

Bourin, M., Hascoet, M., 2003. The mouse light/dark box test. European Journal of Pharmacology 463, 55–65.

Carobrez, A.P., Bertoglio, L.J., 2005. Ethological and temporal analyses of anxiety-like behavior: the elevated plus-maze model 20 years on. Neuroscience and Biobehavioral Reviews 29, 1193–1205.

Cravatt, B.F., Demarest, K., Patricelli, M.P., Bracey, M.H., Giang, D.K., Martin, B.R., Lichtman, A.H., 2001. Supersensitivity to anandamide and enhanced endogenous cannabinoid signalling in mice lacking fatty acid amide hydrolase. Proceedings of the National Academy of Sciences of the USA 98, 9371–9376.

Cristino, L., de Petrocellis, L., Pryce, G., Baker, D., Guglielmotti, V., Di Marzo, V., 2006. Immunohistochemical localization of cannabinoid type 1 and vanilloid transient receptor potential vanilloid type 1 receptors in the mouse brain. Neuroscience 139, 1405–1415.

File, S.E., 1992. Behavioral detection of anxiolytic action. In: Elliot, J.M., Heal, D.J., Marsden, C.A. (Eds.), Experimental Approaches to Anxiety and Depression. John Wiley & Sons, New York, pp. 25–44.

Gobbi, G., Bambico, F.R., Mangieri, R., Bortolato, M., Campolongo, P., Solinas, M., Cassano, T., Morgese, M.G., Debonnel, G., Duranti, A., Tontini, A., Tarzia, G., Mor, M., Trezza, V., Goldberg, S.R., Cuomo, V., Piomelli, D., 2005. Antidepressant-like activity and modulation of brain monoaminergic transmission by blockade of anandamide hydrolysis. Proceedings of the National Academy of Sciences of the USA 102, 18620–18625.

Hall, W., Solowij, N., 1998. Adverse effects of Cannabis. Lancet 352, 1611–1616.

Haller, J., Varga, B., Ledent, C., Freund, T.F., 2004a. Context dependent effects of CB1 cannabinoid gene disruption on anxiety-like and social behaviour in mice. European Journal of Neuroscience 19, 1906–1912.

Hill, M.N., Kambo, J.S., Sun, J.C., Gorzalka, B.B., Galea, L.A., 2006. Endocannabinoids modulate stress-induced suppression of hippocampal cell proliferation and activation of defensive behaviours. European Journal of Neuroscience 24, 1845–1849.

Jiang, W., Zhang, Y., Xiao, L., Van Cleemput, J., Ji, S.P., Bai, G., Zhang, X., 2005. Cannabinoids promote embryonic and adult hippocampus neurogenesis and produce anxiolytic- and antidepressant-like effects. Journal of Clinical Investigation 115, 3104–3116.

Kamprath, K., Marsicano, G., Tang, J., Monory, K., Bisogno, T., Di Marzo, V., Lutz, B., Wotjak, C.T., 2006. Cannabinoid CB1 receptor mediates fear extinction via habituation-like processes. Journal of Neuroscience 26, 6677–6686.

Kasckow, J.W., Mulchahey, J.J., Geracioti, T.D., 2004. Effects of the vanilloid agonists olvanil and antagonist capsazepine on rat behaviours. Progress in Neuropharmacology and Biological Psychiatry 28, 291–295.

Kathuria, S., Gaetani, S., Fegley, D., Valino, F., Duranti, A., Tontini, A., Mor, M., Tarzia, G., La Rana, G., Calignano, A., Giustino, A., Tattoli, M., Palmery, M., Cuomo, V., Piomelli, D., 2003. Modulation of anxiety through blockade of anandamide hydrolysis. Nature Medicine 9, 76–81.

Lapin, I.P., 1995. Only controls: effect of handling, sham injections, and intraperitoneal injection of saline on behaviour of mice in an elevated plus-maze. Journal of Pharmacology and Toxicological Methods 34, 73–77.

Mackie, K., 2005. Distribution of cannabinoid receptors in the central and peripheral nervous system. Handbook of Experimental Pharmacology 168, 299–325.

Marco, E.M., Perez-Alvarez, L., Borcel, E., Rubio, M., Guaza, C., Ambrosio, E., File, S.E., Viveros, M.P., 2004. Involvement of 5-HT1A receptors in behavioural effects of the cannabinoid receptor agonist CP 55,940 in male rats. Behavioural Pharmacology 15, 21–27.

Marsch, R., Foeller, E., Rammes, G., Bunck, M., Kossl, M., Holsboer, F., Zieglgansberger, W., Landgraf, R., Lutz, B., Wotjak, C.T., 2007. Reduced anxiety, conditioned fear, and hippocampal long-term potentiation in transient receptor potential vanilloid type 1 receptor-deficient mice. Journal of Neuroscience 27, 832–839.

Marsicano, G., Lutz, B., 1999. Expression of the cannabinoid receptor CB1 in distinct neuronal subpopulations in the adult mouse forebrain. European Journal of Neuroscience 11, 4213–4225.

Marsicano, G., Wotjak, C.T., Azad, S.C., Bisogno, T., Rammes, G., Cascio, M.G., Hermann, H., Tang, J., Hofmann, C., Zieglgansberger, W., Di Marzo, V., Lutz, B., 2002. The endogenous cannabinoid system controls the extinction of aversive memories. Nature 418, 530–534.

Martin, M., Ledent, C., Parmentier, M., Maldonado, R., Valverde, O., 2001. Involvement of CB1 cannabinoid receptors in emotional behaviour. Psychopharmacology (Berl.) 159, 379–387.

McKinney, M.K., Cravatt, B.F., 2005. Structure and function of fatty acid amide hydrolase. Annual Reviews of Biochemistry 74, 411–432.

Mechiel Korte, S., De Boer, S.F., 2003. A robust animal model of state anxiety: fear-potentiated behaviour in the elevated plus-maze. European Journal of Pharmacology 463, 163–175.

Millan, M.J., 2003. The neurobiology and control of anxious states. Progress in Neurobiology 70, 83–244.

Monory, K., Massa, F., Egertova, M., Eder, M., Blaudzun, H., Westenbroek, R., Kelsch, W., Jacob, W., Marsch, R., Ekker, M., Long, J., Rubenstein, J.L., Goebbels, S., Nave, K.A., During, M., Klugmann, M., Wolfel, B., Dodt, H.U., Zieglgansberger, W., Wotjak, C.T., Mackie, K., Elphick, M.R., Marsicano, G., Lutz, B., 2006. The endocannabinoid system controls key epileptogenic circuits in the hippocampus. Neuron 51, 455–466.

Moreira, F.A., Aguiar, D.C., Guimaraes, F.S., 2007. Anxiolytic-like effects of cannabinoids injected into the rat dorsolateral periaqueductal gray. Neuropharmacology 52, 958–965.

Naidu, P.S., Varvel, S.A., Ahn, K., Cravatt, B.F., Martin, B.R., Lichtman, A.H., 2007. Evaluation of fatty acid amide hydrolase inhibition in murine models of emotionality. Psychopharmacology (Berl.) 192, 61–70.

Navarro, M., Hernandez, E., Munoz, R.M., del Arco, I., Villanua, M.A., Carrera, M.R., Rodriguez de Fonseca, F., 1997. Acute administration of the CB1 cannabinoid receptor antagonist SR 141716A induces anxiety-like responses in the rat. Neuroreport 8, 491–496.

Onaivi, E.S., Green, M.R., Martin, B.R., 1990. Pharmacological characterization of cannabinoids in the elevated plus maze. Journal of Pharmacology and Experimental Therapeutics 253, 1002–1009.

Pacher, P., Batkai, S., Kunos, G., 2006. The endocannabinoid system as an emerging target of pharmacotherapy. Pharmacological Reviews 58, 389–462.

Patel, S., Hillard, C.J., 2006. Pharmacological evaluation of cannabinoid receptor ligands in a mouse model of anxiety: further evidence for an anxiolytic role for endogenous cannabinoid signalling. Journal of Pharmacology and Experimental Therapeutics 318, 304–311.

Piomelli, D., 2003. The molecular logic of endocannabinoid signalling. Nature Reviews Neuroscience 4, 873–884.

Rodgers, R.J., Evans, P.M., Murphy, A., 2005. Anxiogenic profile of AM-251, a selective cannabinoid CB1 receptor antagonist, in plus-maze-naive and plus-maze-experienced mice. Behavioural Pharmacology 16, 405–413.

Ross, R.A., 2003. Anandamide and vanilloid TRPV1 receptors. British Journal of Pharmacology 140, 790–801.

Rutkowska, M., Jamontt, J., Gliniak, H., 2006. Effects of cannabinoids on the anxiety-like response in mice. Pharmacological Reports 58, 200–206.

Saghatelian, A., McKinney, M.K., Bandell, M., Patapoutian, A., Cravatt, B.F., 2006. A FAAH-regulated class of N-acyl taurines that activates TRP ion channels. Biochemistry 45, 9007–9015.

Sousa, N., Almeida, O.F., Wotjak, C.T., 2006. A hitchhiker's guide to behavioral analysis in laboratory rodents. Genes Brain and Behavior 5 (Suppl. 2), 5–24.

Varvel, S.A., Wise, L.E., Niyuhire, F., Cravatt, B.F., Lichtman, A.H., 2007. Inhibition of fatty-acid amide hydrolase accelerates acquisition and extinction rates in a spatial memory task. Neuropsychopharmacology 32, 1032–1041.

Viveros, M.P., Marco, E.M., File, S.E., 2005. Endocannabinoid system and stress and anxiety responses. Pharmacology Biochemistry and Behavior 81, 331–842.

Walker, J.M., Huang, S.M., Strangman, N.M., Tsou, K., Sanudo-Pena, M.C., 1999. Pain modulation by release of the endogenous cannabinoid anandamide. Proceeding of the National Academy Sciences of the USA 96, 12198–12203.

Wei, B.Q., Mikkelsen, T.S., McKinney, M.K., Lander, E.S., Cravatt, B.F., 2006. A second fatty acid amide hydrolase with variable distribution among placental mammals. Journal of Biological Chemistry 281, 36569–36578.

Wise, L.E., Shelton, C.C., Cravatt, B.F., Martin, B.R., Lichtman, A.H., 2007. Assessment of anandamide's pharmacological effects in mice deficient of both fatty acid amide hydrolase and cannabinoid CB1 receptors. European Journal of Pharmacology 557, 44–48.

Zuardi, A.W., Shirakawa, I., Finkelfarb, E., Karniol, I.G., 1982. Action of cannabidiol on the anxiety and other effects produced by delta 9-THC in normal subjects. Psychopharmacology 76, 245–250.

Modulation of anxiety through blockade of anandamide hydrolysis

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The psychoactive constituent of cannabis, Δ⁹-tetrahydrocannabinol, produces in humans subjective responses mediated by CB1 cannabinoid receptors, indicating that endogenous cannabinoids may contribute to the control of emotion. But the variable effects of Δ⁹-tetrahydrocannabinol obscure the interpretation of these results and limit the therapeutic potential of direct cannabinoid agonists. An alternative approach may be to develop drugs that amplify the effects of endogenous cannabinoids by preventing their inactivation. Here we describe a class of potent, selective and systemically active inhibitors of fatty acid amide hydrolase, the enzyme responsible for the degradation of the endogenous cannabinoid anandamide. Like clinically used anti-anxiety drugs, in rats the inhibitors exhibit benzodiazepine-like properties in the elevated zero-maze test and suppress isolation-induced vocalizations. These effects are accompanied by augmented brain levels of anandamide and are prevented by CB1 receptor blockade. Our results indicate that anandamide participates in the modulation of emotional states and point to fatty acid amide hydrolase inhibition as an innovative approach to anti-anxiety therapy.

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Anandamide, the naturally occurring amide of arachidonic acid with ethanolamine, meets all key criteria of an endogenous cannabinoid substance[1]: it is released on demand by stimulated neurons[2,3]; it activates cannabinoid receptors with high affinity[1]; and it is rapidly eliminated through a two-step process consisting of carrier-mediated transport followed by intracellular hydrolysis[2,4]. Anandamide hydrolysis is catalyzed by the enzyme fatty acid amide hydrolase (FAAH), a membrane-bound serine hydrolase[5,6] that also cleaves other bioactive fatty acid ethanolamides such as oleoylethanolamide[7] and palmitoylethanolamide[8]. Mutant mice lacking the gene encoding FAAH (*Faah*) cannot metabolize anandamide[9] and, although fertile and generally normal, show signs of enhanced anandamide activity at cannabinoid receptors such as reduced pain sensation[9]. This is suggestive that drugs targeting FAAH may heighten the tonic actions of anandamide, while possibly avoiding the multiple and often unwanted effects produced by Δ⁹-tetrahydrocannabinol (Δ⁹-THC) and other direct-acting cannabinoid agonists[10,11]. To test this hypothesis, potent, selective and systemically active inhibitors of intracellular FAAH activity are needed. However, most current inhibitors of this enzyme lack the target selectivity and biological availability required for *in vivo* studies[12–14], whereas newer compounds, though promising, have not yet been characterized[15,16]. Thus, the therapeutic potential of FAAH inhibition remains essentially unexplored.

Lead identification and optimization

Despite its unusual catalytic mechanism[6], FAAH is blocked by a variety of serine hydrolase inhibitors, including compounds with activated carbonyls[16]. Therefore we examined whether esters of carbamic acid such as the anti-cholinesterase agent carbaryl (compound 1; Table 1) might inhibit FAAH activity in rat brain membranes. Although compound 1 was ineffective, its positional isomer 2 produced a weak inhibition of FAAH (half-maximal inhibitory concentration (IC_{50}) = 18.6 ± 0.7 μM; mean ± s.e.m., n = 3), which was enhanced by replacing the *N*-methyl substituent with a cyclohexyl group (compound 3; IC_{50} = 324 ± 31 nM). The aryl ester 4, the benzyloxyphenyl group of which can be regarded as an elongated bioisosteric variant of the naphthyl moiety of compound 2, inhibited the activity of FAAH with a potency (IC_{50} = 396 ± 63 nM) equivalent to that of compound 3. A conformational analysis of compound 4 revealed families of accessible conformers differing mainly in the torsion angle around the O–CH_2 bond, with substituents in anti or gauche conformations (data not shown). As the latter conformations more closely resembled the shape of the naphthyl derivative 3, we hypothesized that they might be responsible for the interac-

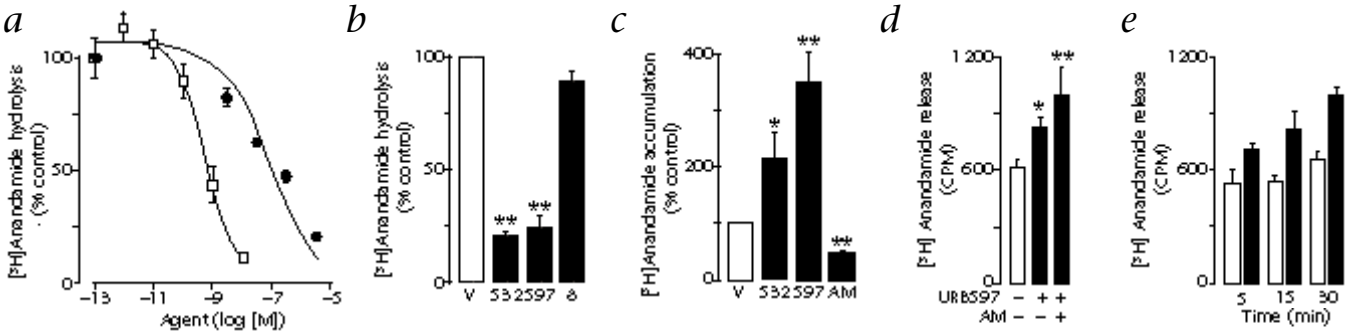
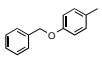
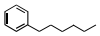
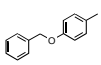


Fig. 1 The FAAH inhibitors URB532 and URB597 block [^{3}H]anandamide degradation in intact brain neurons. ***a***, Concentration-dependent inhibition of [^{3}H]anandamide hydrolysis by URB597 (open squares) and URB532 (filled circles) in primary cultures of rat cortical neurons. Baseline FAAH activity was 242.6 ± 3.9 cpm well min^{-1} (n = 4). ***b***, Unlike URB532 (3 μM) or URB597 (10 nM), the inactive analog 8 (10 μM) has no effect on [^{3}H]anandamide degradation. V, vehicle. ***c***, URB532 (3 μM) and URB597 (10 nM) promote accumulation of non-metabolized [^{3}H]anandamide in neurons, whereas the anandamide transport inhibitor AM404 (AM, 10 μM) reduces it. V, vehicle. ***d***, Release of non-metabolized [^{3}H]anandamide from neurons treated with URB597 (10 nM) during a 15-min incubation in the absence or presence of AM404 (AM, 10 μM). ***e***, Time course of [^{3}H]anandamide release from neurons treated with URB597 (10 nM). *P < 0.05; **P < 0.01 versus vehicle-treated neurons; ANOVA with Tukey's *post-hoc* test (n = 4–8). □, vehicle; ■, treatments. Error bars, s.e.m.

tion of compound 4 with the active site of FAAH. Testing this hypothesis led to the design of the biphenyl derivative 5 (IC_{50} = 63 ± 9 nM), which was further optimized by systematic modifications of the distal phenyl group, resulting in the potent inhibitor 6 (IC_{50} = 4.6 ± 1.6 nM; Table 1). The lead optimization process will be reported elsewhere.

Kinetic analyses and dialysis experiments indicate that compounds 4 and 6 may inhibit FAAH activity through an irreversible interaction with the enzyme (data not shown), possibly due to a nucleophilic attack of an active serine residue on the carbamate group. This mechanism sets the present compounds apart from the α-keto heterocycle derivatives described previously[16], which act as competitive FAAH inhibitors. A further indication of such a distinction is that in the α-keto heterocycle series potency is strongly dependent on the hydrophobicity of the flexible acyl chain, whereas in the carbamate series potency is modulated by the shape of the rigid aromatic moiety. Accordingly, when we replaced the biphenyl of compound 5 with a 5-phenylpentyl group, representing the most effective acyl chain in the α-keto heterocycle series, the inhibitory activity was lost (compound 7; Table 1).

Table 1 Structures of selected carbamate inhibitors of FAAH activity

	R	R_1	IC_{50} (nM)
1		CH_3	>100,000
2		CH_3	18,600 ± 708
3		c-C_6H_{11}	324 ± 31
4	 URB532	n-C_4H_9	396 ± 63
5		c-C_6H_{11}	63 ± 9
6	 URB597	c-C_6H_{11}	4.6 ± 1.6
7		c-C_6H_{11}	>100,000
8		p-FC_6H_4	>100,000

Values reported are the concentrations required to inhibit FAAH activity by 50% (IC_{50}, nM), and are expressed as the mean ± s.e.m. of at least three independent experiments. They were calculated from concentration-response curves, by using non-linear regression analysis as implemented in the Prism 2.0 software package.

Compounds 4 (URB532) and 6 (URB597) blocked the FAAH-catalyzed hydrolysis of exogenous [^{3}H]anandamide in primary cultures of intact cortical neurons, with IC_{50} values that paralleled those obtained in membrane preparations (URB532, 214 ± 79 nM; URB597, 0.50 ± 0.05 nM; n = 8; Fig. 1*a*). By contrast, compound 8, an analog of URB532 that does not inhibit FAAH in membranes (Table 1), had no such effect (Fig. 1*b*). Moreover, URB532 and URB597 selectively impaired the breakdown of [^{3}H]anandamide without reducing its carrier-mediated uptake, causing non-metabolized [^{3}H]anandamide to accumulate in, and eventually exit from, the neurons. Thus, after a 4-minute incubation with [^{3}H]anandamide, the intracellular content of non-metabolized [^{3}H]anandamide was higher in inhibitor-treated neurons than in control neurons (Fig. 1*c*). As expected, the anandamide transport blocker *N*-(4-hydroxyphenyl)arachidonamide (AM404) had an opposite effect, substantially reducing [^{3}H]anandamide internalization[4] (Fig. 1*c*). When neurons treated with URB597 were exposed for 4 minutes to [^{3}H]anandamide and then incubated for 15 minutes in an [^{3}H]anandamide-free solution, 42.6 ± 8.7% of the accumulated [^{3}H]anandamide was released back into the medium (n = 3; Fig. 1*d*). This process was linear with time (Fig. 1*e*) and was not inhibited by AM404 (Fig. 1*d*), indicating that it occurred through passive diffusion rather than reverse transport. No such time-dependent release was observed in control neurons, the medium of which contained only residual levels of [^{3}H]anandamide carried over from the pre-incubation period. These studies identify a new class of carbamate inhibitors of FAAH activity, which potently block anandamide breakdown in intact brain neurons.

Target selectivity

URB532 and URB597 inhibited FAAH, but did not affect the activities of three other serine hydrolases: electric eel acetyl-

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Table 2 Analysis of selected FAAH inhibitors *in vitro*

Compound	AChE	BCh	MGL	AT	CB1	CB2
URB532	>100, SI ≥333	>100, SI ≥333	>30, SI ≥100	>300, SI ≥1,000	>300, SI ≥1,000	>300, SI ≥1,00, SI
URB597	>100, SI ≥2,5000	≥100, SI ≥25,000	>30, SI ≥7,500	>30, SI ≥7,500	>100, SI ≥2,5000	>100, SI ≥2,5000

Values indicate the maximal concentrations of FAAH inhibitor tested on each target (in μM) and their corresponding selectivity index (SI). The SI is the ratio of maximal inhibitor concentration tested/IC_{50} for FAAH (from Table 1).

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cholinesterase, horse plasma butyryl cholinesterase and rat brain monoglyceride lipase (MGL; Table 2). The lack of MGL inhibition is particularly noteworthy in light of the proposed role of this enzyme in the biological inactivation of 2-arachidonoylglycerol[17] (2-AG), another endogenous cannabinoid present in brain[18–20]. Furthermore, URB532 and URB597 had no effect on anandamide transport in human astrocytoma cells or on the binding of a high-affinity ligand to CB1 and CB2 receptors (Table 2). In addition, URB532 (10 μM) did not significantly interact with a panel of 21 receptors, ion channels and neurotransmitter transporters, which included adenosine A_1, A_{2A} and A_{2B}; adrenergic α_{1A}, α_{2A}, β_1 and β_2; dopamine D_1 and D_2; glutamate *N*-methyl-(D)-aspartate; γ-amino-butyric acid $(GABA)_A$ agonist site; histamine H_1; opiate μ; muscarinic M_2; and brain nicotinic receptors (data not shown). This high selectivity for FAAH encouraged us to examine the effects of URB532 and URB597 in live animals.

FAAH inhibition *in vivo*

Intraperitoneal (i.p.) injection of either URB532 or URB597, but not the inactive analog 8, produced a profound, dose-dependent inhibition of brain FAAH activity (Fig. 2*a*). In six experiments, half-maximal inhibition was reached at 0.60 ± 0.09 mg kg^{-1} of URB532, and 0.150 ± 0.007 mg kg^{-1} of URB597. The discrepancy between potency ratios of URB532 and URB597 *in vitro* (100-fold) and *in vivo* (4-fold) presumably reflects differences in bioavailability or brain penetration of the two compounds. After injection of a maximal dose of URB597 (0.3 mg kg^{-1}, i.p.), FAAH inhibition was rapid in onset (<15 minutes), persistent (>6 hours; Fig. 2*b*) and accompanied by significant elevations in the brain levels of anandamide (Fig. 2*c*) and other fatty acid ethanolamides that are substrates for FAAH (in pmol g^{-1} of tissue at 2 hours after injection: oleoylethanolamide, vehicle, 137.0 ± 14.3, URB597 (0.3 mg kg^{-1}), 725.3 ± 28.6; palmitoylethanolamide, vehicle, 259.1 ± 15.0, URB597, 1,324 ± 395; n = 8–15). Parallel changes in FAAH activity and fatty acid ethanolamide levels were also measured in various peripheral tissues (data not shown). In agreement with the lack of MGL inhibition noted in our *in vitro* experiments (Table 2), URB597 did not change the brain content of 2-AG (Fig. 2*d*).

As previously observed in *Faah*$^{-/-}$ mice[9], FAAH inhibition was associated with increased sensitivity to the administration of exogenous anandamide. Accordingly, URB597 (0.3 mg kg^{-1}, i.p.) intensified and prolonged the decrease in body temperature elicited by a subthreshold dose of anandamide (5 mg kg^{-1}, i.p.), whereas it had no effect when injected alone (Figs. 2*e* and *f*). Underscoring the role of CB1 receptors in this response, the effect of anandamide plus URB597 was prevented by the CB1 antagonist SR141716A (rimonabant; Figs. 2*e* and *f*).

Pharmacological properties of FAAH inhibitors *in vivo*

Although URB532 and URB597 increased brain anandamide lev-

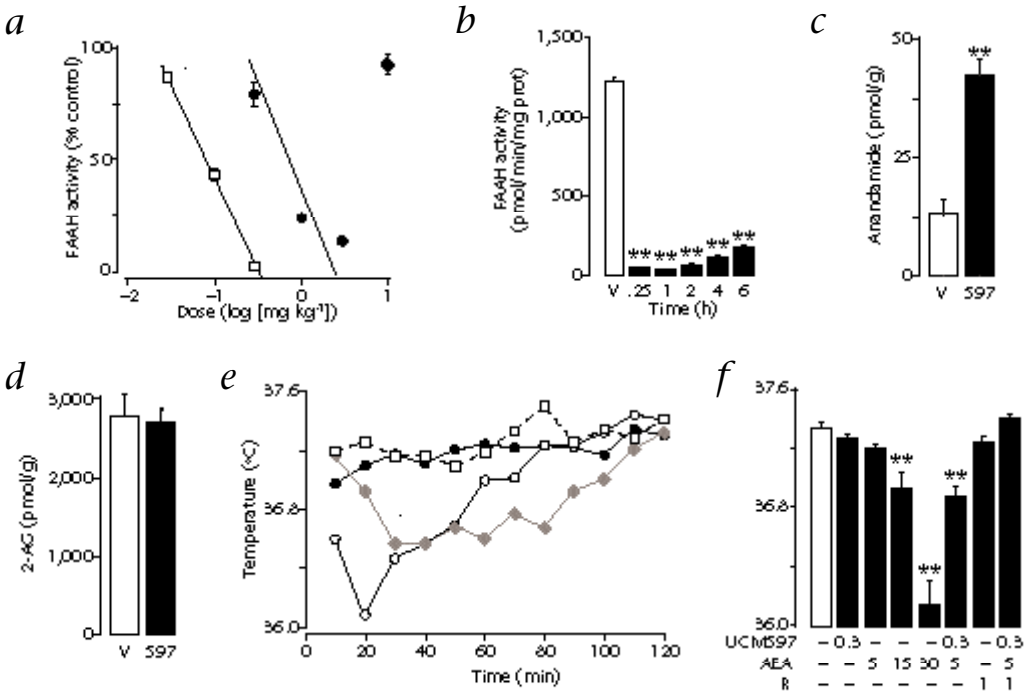

Fig. 2 *In vivo* inhibition of FAAH activity by URB532 and URB597. ***a***, Dose-dependent inhibition of brain FAAH activity by URB532 (●) and URB597 (□), but not by the inactive analog 8 (◆), after systemic (i.p.) administration in the rat. Baseline FAAH activity was 3,133 ± 59 cpm per mg of protein min^{-1} (n = 9). ***b***, Time course of the inhibition of brain FAAH activity after a single injection of URB597 (0.3 mg kg^{-1}, i.p.). ***c*** and ***d***, Brain levels of anandamide (*c*) and 2-AG (*d*) 2 h after injections of vehicle (V) or URB597 (0.3 mg kg^{-1}, i.p). *P < 0.05; **P < 0.01, ANOVA followed by Tukey's test; n = 4–8. ***e*** and ***f***, Enhancement of anandamide-induced hypothermia by URB597. *e*, Time course of the effects of URB597 (0.3 mg kg^{-1}, □), anandamide (5 mg kg^{-1}, ●; 5 mg kg^{-1}, ○) and anandamide (5 mg kg^{-1}) plus URB597 (0.3 mg kg^{-1}, 30 min before anandamide, ◆). Two-way ANOVA: F_{times} = 2.99, df = 12/416, P < 0.0005; $F_{treatments}$ = 52.25, df = 3/416, P < 0.0001; $F_{timesxtreatments}$ = 1.96, df = 36/416, P < 0.001. *f*, Effects of vehicle, URB597 (0.3 mg kg^{-1}), anandamide (AEA, 5–30 mg kg^{-1}), anandamide (5 mg kg^{-1}) plus URB597 (0.3 mg kg^{-1}), rimonabant (R, 1 mg kg^{-1}), and anandamide (5 mg kg^{-1}) plus URB597 (0.3 mg kg^{-1}) and rimonabant (1 mg kg^{-1}). One-way ANOVA: F = 27.22, df = 103, P < 0.0001. □, vehicle; ■, urb597. Error bars, s.e.m.

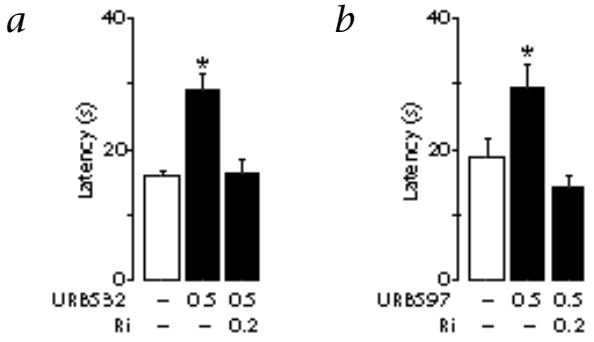

Fig. 3 Anti-nociceptive actions of URB532 and URB597. Shown are effects of URB532 (***a***) and URB597 (***b***) (both at 0.5 mg kg^{-1}, i.p.) on response latencies in the mouse hot-plate test, in the absence or presence of the CB1 antagonist rimonabant (Ri, 0.2 mg kg^{-1}, i.v.). FAAH inhibitors and rimonabant were injected 60 min and 40 min before tests, respectively. Rimonabant alone had no effect on response latencies (not shown). *$P < 0.05$; ANOVA followed by Dunnett's test ($n = 12$). □, vehicle; ■, urb597. Error bars, s.e.m.

els, they did not mimic the spectrum of pharmacological responses produced by exogenous anandamide. Systemic doses of URB532 (10 mg kg^{-1}, i.p.) or URB597 (0.3 mg kg^{-1}, i.p.) that maximally blocked FAAH activity produced no catalepsy (rigid immobility), hypothermia or hyperphagia (increased food intake), three typical signs of CB1 receptor activation[11] (data not shown). However, the compounds exerted moderate anti-nociceptive actions in the mouse hot-plate test, which measures response to noxious thermal stimuli. In this model, URB532 and URB597 significantly lengthened response latencies at a dose of 0.5 mg kg^{-1} (Figs. 3*a* and *b*), but not 0.1 mg kg^{-1} (data not shown). These effects were prevented by a dose of the CB1 antagonist rimonabant (0.2 mg kg^{-1}, intravenous (i.v.); Figs. 3*a* and *b*), which caused no hyperalgesia when administered alone (data not shown). Our results corroborate those obtained in *Faah*$^{-/-}$ mice[9], indicating that acute disruption of FAAH activity results in a mild CB1-mediated anti-nociception, but no hypothermia or catalepsy.

Anxiolytic effects of FAAH inhibitors

To identify intrinsic actions of anandamide that might be significantly magnified by FAAH inhibition, we turned, for three reasons, to the regulation of emotional reactivity. First, CB1 receptors are expressed at high levels in brain regions such as the amygdala which are implicated in the control of anxiety and fear[21–23]. Second, acute administration of cannabinoid drugs produces emotional responses in rodents[11] and humans[10,24]. Third, pharmacological[25,26] or genetic[27,45] thus, disruption of CB1 receptor activity elicits anxiety-like behaviors in rodents, suggestive of the existence of an intrinsic anxiolytic tone mediated by endogenous cannabinoids.

We used two pharmacologically validated animal models of anxiety: the elevated zero-maze test and the isolation-induced ultrasonic emission test. The zero maze consists of an elevated annular platform with two open and two closed quadrants and the test is based on the conflict between an animal's instinct to explore its environment and its fear of open spaces, where it may be attacked by predators[28,29]. Clinically used anxiolytic drugs such as the benzodiazepines increase the proportion of time spent in, and the number of entries made into, the open compartments. Similarly, URB532 (0.1–10 mg kg^{-1}, i.p.) and URB597 (0.05–0.1 mg kg^{-1}, i.p.) evoked anxiolytic-like responses at doses that corresponded to those required to inhibit FAAH activity *in vivo* ($F = 24.7$, df = 4/41, $P < 0.001$; $F = 7.7$, df = 2/27, $P < 0.01$; Figs. 4*a* and *b*). In keeping with an involvement of endogenous anandamide, the anxiolytic-like effects of both compounds were attenuated by a non-anxiogenic dose of rimonabant (2 mg kg^{-1}, i.p.; $F = 14.87$, df = 3/28, $P < 0.001$; $F = 15.2$, df = 3/28, $P < 0.001$; Figs. 4*c* and *d*). Moreover, the effects were apparently dissociated from overall changes in motor behavior. Indeed, although URB532 elicited in adult rats a modest decrease in ambulation (which was also antagonized by rimonabant; data not shown), it did so at doses that were higher than those needed to cause anxiolysis (≥10 mg kg^{-1}; $F = 3.57$, df = 2/22, $P < 0.05$; Fig. 4*e*). We confirmed this dissociation by testing URB532 and URB597 in the ultrasonic vocalization emission model, which measures the

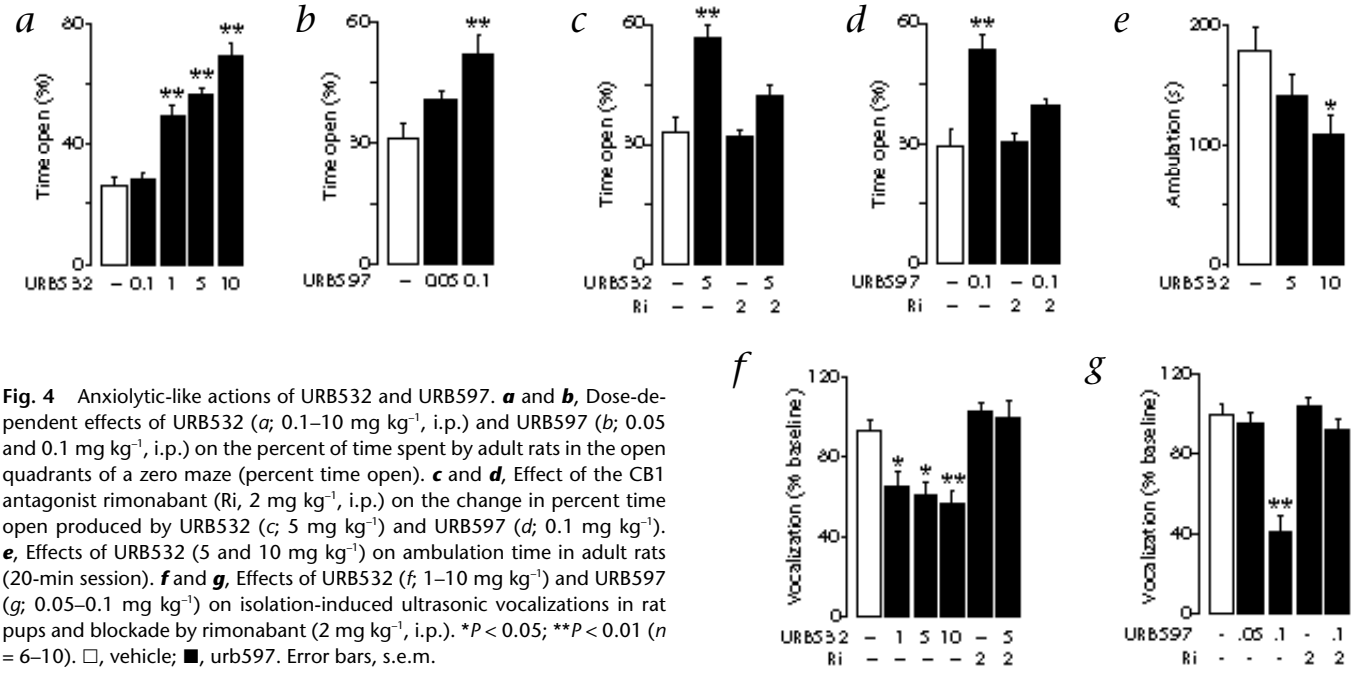

Fig. 4 Anxiolytic-like actions of URB532 and URB597. ***a*** and ***b***, Dose-dependent effects of URB532 (*a*; 0.1–10 mg kg^{-1}, i.p.) and URB597 (*b*; 0.05 and 0.1 mg kg^{-1}, i.p.) on the percent of time spent by adult rats in the open quadrants of a zero maze (percent time open). ***c*** and ***d***, Effect of the CB1 antagonist rimonabant (Ri, 2 mg kg^{-1}, i.p.) on the change in percent time open produced by URB532 (*c*; 5 mg kg^{-1}) and URB597 (*d*; 0.1 mg kg^{-1}). ***e***, Effects of URB532 (5 and 10 mg kg^{-1}) on ambulation time in adult rats (20-min session). ***f*** and ***g***, Effects of URB532 (*f*; 1–10 mg kg^{-1}) and URB597 (*g*; 0.05–0.1 mg kg^{-1}) on isolation-induced ultrasonic vocalizations in rat pups and blockade by rimonabant (2 mg kg^{-1}, i.p.). *$P < 0.05$; **$P < 0.01$ ($n = 6$–10). □, vehicle; ■, urb597. Error bars, s.e.m.

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number of stress-induced vocalizations emitted by rat pups removed from their nest[30–32]. As seen with anxiolytic drugs, the FAAH inhibitors reduced ultrasonic calls ($F = 10.8$, df = 5/33, $P < 0.001$; $F = 19.3$; df = 4/25, $P < 0.001$; Figs. 4*f* and *g*) at doses (URB532, 1 and 5 mg kg^{-1}; URB597, 0.1 mg kg^{-1}) that had no effect on pup movement (data not shown). These anxiolytic-like responses were blocked by rimonabant (2 mg kg^{-1}; Figs. 4*f* and *g*).

Discussion

We have developed a new class of agents that prevents anandamide inactivation by targeting the intracellular enzymatic activity of FAAH. URB597, the most potent member of this class, inhibited FAAH activity with an IC_{50} value of 4 nM in brain membranes and 0.5 nM in intact neurons, and an ID_{50} value of 0.15 mg kg^{-1} after systemic administration in the rat. This compound had much greater selectivity for FAAH than other cannabinoid-related targets, including cannabinoid receptors (selectivity index, >25,000) and MGL, an enzyme involved in the deactivation of the endogenous cannabinoid ester 2-AG (selectivity index, >7,500). Such target discrimination was matched by a lack of overt cannabimimetic effects *in vivo*. Thus, at doses that almost abolished FAAH activity and substantially raised brain anandamide levels, URB597 and its analog URB532 did not evoke catalepsy, reduce body temperature or stimulate feeding, three key symptoms of cannabinoid intoxication in the rodent[11].

Nevertheless, the compounds did elicit marked anxiolytic-like responses, which paralleled their ability to inactivate FAAH and were prevented by the CB1 receptor antagonist rimonabant. We interpret these findings to indicate that URB597 and URB532 may selectively modulate anxiety-like behaviors by enhancing the tonic actions of anandamide on a subset of CB1 receptors which may normally be engaged in controlling emotions. Forebrain sites that might be implicated in such actions include the basolateral amygdala, the anterior cingulate cortex and the prefrontal cortex, all key elements of an "emotion circuit"[33] that contains high densities of CB1 receptors[21,22]. CB1 receptors in these structures are localized to the axon terminals of a subpopulation of GABAergic interneurons, which also express the peptide cholecystokinin[23,34] (CCK). The anxiogenic properties of CCK[35] and the ability of CB1 agonists to inhibit K^+-evoked CCK release from hippocampal slices[36] indicate that interactions between this peptide and anandamide may participate in the control of anxiety.

In addition to their anxiolytic-like actions, URB597 and URB532 exerted modest anti-nociception in a model of acute pain, which also was sensitive to CB1 receptor blockade. These findings are similar to those reported for *Faah*$^{-/-}$ mice[9] and support the proposed roles of anandamide in the intrinsic modulation of pain[37]. However, as emotional states may strongly influence pain sensation, it is possible that anxiolysis might have contributed to the mild anti-nociceptive effects of the FAAH inhibitors.

URB597 and URB532 increased brain anandamide levels without modifying those of the second endogenous cannabinoid, 2-AG. It is therefore likely that the pharmacological actions of these compounds, which are sensitive to the CB1 antagonist rimonabant, are primarily due to anandamide accumulation. But the FAAH inhibitors also produced large elevations in the levels of two anandamide analogs, palmitoylethanolamide and oleoylethanolamide, whose biological effects are independent of CB1 receptors[7,8]. Thus, we cannot exclude the possibility that additional properties of URB597 and URB532, mediated by these fatty ethanolamides, remain to be discovered.

Our results define a novel class of inhibitors of FAAH activity, which enhance endogenous anandamide signaling without directly interacting with cannabinoid receptors. The behavioral profile of these agents—characterized by anxiolysis and mild analgesia—reveals a key role for anandamide in the regulation of emotional states and indicates a new mechanistic approach to anti-anxiety therapy.

Methods

Animals and cells. We used Wistar rats (200–350 g) and Swiss mice (20 g). All procedures met the National Institutes of Health guidelines for the care and use of laboratory animals and those of the Italian Ministry of Health (D.L. 116/92). We prepared cultures of cortical neurons from 18-day-old Wistar rat embryos and maintained them as described[38]. We purchased astrocytoma cells from American Type Culture Collection (Manassas, Virginia).

Chemicals. Anandamide and related lipids were synthesized in the laboratory[39]. SR141716A (rimonabant) was provided by RBI (Natick, Massachusetts) as part of the Chemical Synthesis Program of the National Institutes of Health. AM404 was from Tocris (Avonmouth, UK) and other drugs from Sigma.

Synthesis of inhibitors. *n*-Butylcarbamic acid 4-benzyloxyphenyl ester (URB532, compound 4) and 4-fluorophenylcarbamic acid 4-benzyloxyphenyl ester (compound 8) were obtained by treatment of 4-benzyloxyphenol with *n*-butylisocyanate and 4-fluorophenylisocyanate, respectively, with a catalytic amount of triethylamine in refluxing toluene. Similarly, cyclohexylcarbamic acid biphenyl-3-yl ester (compound 5), cyclohexylcarbamic acid 5-phenylpentyl ester (compound 7) and cyclohexylcarbamic acid 3′-carbamoyl-biphenyl-3-yl ester (URB597; compound 6) were synthesized by reacting cyclohexylisocyanate with 3-phenylphenol, 5-phenylpentan-1-ol and 3′-hydroxybiphenyl-3-carboxylic acid amide, respectively. The latter reactant was prepared as follows: 3-bromobenzoic acid amide, obtained by reaction of 3-bromobenzonitrile and sodium perborate, was coupled with methoxyphenylboronic acid to give 3′-methoxybiphenyl-3-carboxylic acid amide, which was hydrolyzed with BBr_3 to the desired 3′-hydroxybiphenyl-3-carboxylic acid amide. Detailed synthetic procedures and physicochemical data will be reported elsewhere.

Biochemical assays. We prepared cell fractions from brain homogenates and assayed membrane FAAH activity and cytosol MGL activity using anandamide[ethanolamine-^{3}H] (60 Ci/mmol; American Radiolabeled Chemicals (ARC), St. Louis, Missouri) and 2-mono-oleoyl-glycerol-[glycerol-1,2,3-^{3}H] (20 Ci/mmol; ARC, St. Louis, Missouri), respectively, as substrates[17]. We conducted [^{3}H]anandamide transport assays in human astrocytoma cells[40]; CB1 and CB2 binding assays in rat cerebellar membranes and CB2-overexpressing CHO cells (Receptor Biology-Perkin Elmer, Wellesley, Massachusetts), respectively, using [^{3}H]WIN-55212-2 (NEN-Dupont, Boston, Massachusetts, 40-60 Ci/mmol) as a ligand[1]; and cholinesterase assays with a commercial kit (Sigma) using purified enzymes (electric eel acetylcholinesterase type V-S and horse serum cholinesterase; both from Sigma). To measure anandamide transport and hydrolysis in cortical neurons, we preincubated cells with FAAH inhibitors for 10 min at 37 °C, before exposure to [^{3}H]anandamide for 4 min. In some experiments, we stopped the reactions with cold Tris-Krebs buffer containing 0.1% bovine serum albumin (Type V, fatty acid free, Sigma), removed the cells by trypsin-EDTA treatment and extracted lipids with chloroform/methanol (1/1, vol/vol). We measured non-metabolized [^{3}H]anandamide in the organic phase and metabolized [^{3}H]anandamide (as [^{3}H]ethanolamine) in the aqueous phase. In other experiments, after having exposed the neurons to [^{3}H]anandamide for 4 min, we rinsed the cells and measured [^{3}H]anandamide release as described above.

High-performance liquid chromatography/mass spectrometry. We extracted lipids with methanol-chloroform and fractionated them by column chromatography[39]. Anandamide and other fatty acid derivatives were quantified by high-performance liquid chromatography/mass spectrometry[39].

Body temperature and catalepsy. We administered compounds (in saline/Tween 80/polyethylene glycol, 90/5/5, i.p.) immediately before tests. We measured body temperature with a rectal probe connected to a digital

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thermometer (Physitemp Instruments, Clifton, New Jersey), and catalepsy as described[41].

Food intake. We administered URB597 (in DMSO/saline, 7:3, i.p.) 45 min before tests. We recorded food intake in free-feeding rats by using an automated system (Scipro Inc., New York, New York). After a 3-d acclimation, tests began at the onset of the dark phase and lasted for 24 h.

Anti-nociception. FAAH inhibitors (in polyethylene glycol/water, 1:1) and rimonabant (in saline) were tested in the mouse hot-plate assay, as described[4].

Anxiety and motor activity. We dissolved FAAH inhibitors and rimonabant in dimethylsulfoxide (DMSO)/saline (7:3 and 9:1, respectively). We administered FAAH inhibitors by i.p. injection 30 min before tests and rimonabant 30 min before FAAH inhibitors. The elevated zero-maze apparatus is described elsewhere[28,29]. We placed the rats in a closed quadrant and video-recorded them for 5-min periods. Results are expressed as percent time in open quadrant/total time (percent time open). Results were analyzed by one-way ANOVA followed by Tukey's test. We recorded motor activity in an Opto-Varimex cage (Columbus Instruments, Columbus, Ohio) linked to a computer and placed in a sound-attenuated room illuminated by a 20-W white light. The amount of time spent in ambulatory activity was analyzed using an Auto-Track software[42,43] (Columbus Instruments, Columbus, Ohio). Session duration was 20 min for adult rats and 60 s for 10-day-old pups. We analyzed data by overall one-way ANOVA followed by Tukey's test for individual between-group comparisons. We recorded 10-day-old pup ultrasonic vocalizations in a sound-attenuating chamber, as described[44]. Tests were conducted between 900 and 1400 h and lasted for 15 s. Drugs were administered after baseline value collection (15 s) and pups were tested again 30 min after drug administration. Data were expressed as percent change from baseline and analyzed by overall one-way ANOVA followed by Tukey's test for individual between-group comparisons.

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Competing interests statement

The authors declare competing financial interests: see the website http://www.nature.com/naturemedicine for details.

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1. Devane, W.A. *et al.* Isolation and structure of a brain constituent that binds to the cannabinoid receptor. *Science* **258**, 1946–1949 (1992).
2. Di Marzo, V. *et al.* Formation and inactivation of endogenous cannabinoid anandamide in central neurons. *Nature* **372**, 686–691 (1994).
3. Giuffrida, A. *et al.* Dopamine activation of endogenous cannabinoid signaling in dorsal striatum. *Nature Neurosci.* **2**, 358–363 (1999).
4. Beltramo, M. *et al.* Functional role of high-affinity anandamide transport, as revealed by selective inhibition. *Science* **277**, 1094–1097 (1997).
5. Cravatt, B.F. *et al.* Molecular characterization of an enzyme that degrades neuromodulatory fatty-acid amides. *Nature* **384**, 83–87 (1996).
6. Patricelli, M.P., Lovato, M.A. & Cravatt, B.F. Chemical and mutagenic investigations of fatty acid amide hydrolase: evidence for a family of serine hydrolases with distinct catalytic properties. *Biochemistry* **38**, 9804–9812 (1999).
7. Rodríguez de Fonseca, F. *et al.* An anorexic lipid mediator regulated by feeding. *Nature* **414**, 209–212 (2001).
8. Calignano, A., La Rana, G., Giuffrida, A. & Piomelli, D. Control of pain initiation by endogenous cannabinoids. *Nature* **394**, 277–281 (1998).
9. Cravatt, B.F. *et al.* Supersensitivity to anandamide and enhanced endogenous cannabinoid signaling in mice lacking fatty acid amide hydrolase. *Proc. Natl. Acad. Sci. USA* **98**, 9371–9376 (2001).
10. Hall, W. & Solowij, N. Adverse effects of cannabis. *Lancet* **352**, 1611–1616 (1998).
11. Chaperon, F. & Thiébot, M.H. Behavioral effects of cannabinoid agents in animals. *Crit. Rev. Neurobiol.* **13**, 243–281 (1999).
12. Koutek, B. *et al.* Inhibitors of arachidonoyl ethanolamide hydrolysis. *J. Biol. Chem.* **269**, 22937–22940 (1994).
13. Beltramo, M., di Tomaso, E. & Piomelli, D. Inhibition of anandamide hydrolysis in rat brain tissue by (E)-6-(bromomethylene) tetrahydro-3-(1-naphthalenyl)-2H-pyran-2-one. *FEBS Lett.* **403**, 263–267 (1997).
14. De Petrocellis, L. *et al.* Novel inhibitors of brain, neuronal, and basophilic anandamide amidohydrolase. *Biochem. Biophys. Res. Commun.* **231**, 82–88 (1997).
15. Deutsch, D.G. *et al.* Fatty acid sulfonyl fluorides inhibit anandamide metabolism and bind to the cannabinoid receptor. *Biochem. Biophys. Res. Commun.* **231**, 217–221 (1997).
16. Boger, D.L. *et al.* Exceptionally potent inhibitors of fatty acid amide hydrolase: the enzyme responsible for degradation of endogenous oleamide and anandamide. *Proc. Natl. Acad. Sci. USA* **97**, 5044–5049 (2000).
17. Dinh, T.P. *et al.* Brain monoglyceride lipase participating in endocannabinoid inactivation. *Proc. Natl. Acad. Sci. USA* **99**, 10819–10824 (2002).
18. Mechoulam, R. *et al.* Identification of an endogenous 2-monoglyceride, present in canine gut, that binds to cannabinoid receptors. *Biochem. Pharmacol.* **50**, 83–90 (1995).
19. Sugiura, T. *et al.* 2-Arachidonoylglycerol: a possible endogenous cannabinoid receptor ligand in brain. *Biochem. Biophys. Res. Commun.* **215**, 89–97 (1995).
20. Stella, N., Schweitzer, P. & Piomelli, D. A second endogenous cannabinoid that modulates long-term potentiation. *Nature* **388**, 773–778 (1997).
21. Herkenham, M. *et al.* Cannabinoid receptor localization in brain. *Proc. Natl. Acad. Sci. USA* **87**, 1932–1936 (1990).
22. Glass, M., Dragunow, M. & Faull, R.L. Cannabinoid receptors in the human brain: a detailed anatomical and quantitative autoradiographic study in the fetal, neonatal and adult human brain. *Neuroscience* **77**, 299–318 (1997).
23. Katona, I. *et al.* Distribution of CB1 cannabinoid receptors in the amygdala and their role in the control of GABAergic transmission. *J. Neurosci.* **21**, 9506–9518. (2001).
24. Robson, P. Therapeutic aspects of cannabis and cannabinoids. *Br. J. Psychiatry* **178**, 107–115 (2001).
25. Rodríguez de Fonseca, F., Carrera, M.R.A., Navarro, M., Koob, G.F. & Weiss, F. Activation of corticotropin-releasing factor in the limbic system during cannabinoid withdrawal. *Science* **276**, 2050–2054 (1997).
26. Arévalo, C., de Miguel, R. & Hernández-Tristán, R. Cannabinoid effects on anxiety-related behaviours and hypothalamic neurotransmitters. *Pharmacol. Biochem. Behav.* **70**, 123–131 (2001).
27. Martin, M., Ledent, C., Parmentier, M., Maldonado, R. & Valverde, O. Involvement of CB1 cannabinoid receptors in emotional behaviour. *Psychopharmacology (Berl)* **159**, 379–387 (2002).
28. Bickerdike, M.J., Marsden, C.A., Dourish, C.T. & Fletcher, A. The influence of 5-hydroxytryptamine re-uptake blockade on CCK receptor antagonist effects in the rat elevated zero-maze. *Eur. J. Pharmacol.* **271**, 403–411 (1994).
29. Shepherd, J.K., Grewal, S.S., Fletcher, A., Bill, D.J. & Dourish, C.T. Behavioural and pharmacological characterisation of the elevated "zero-maze" as an animal model of anxiety. *Psychopharmacology* **116**, 56–64 (1994).
30. Insel, T.R., Hill, J.L. & Mayor, R.B. Rat pup ultrasonic isolation calls: possible mediation by the benzodiazepine receptor complex. *Pharmacol. Biochem. Behav.* **24**, 1263–1267 (1986).
31. Miczek, K.A., Weerts, E.M., Vivian, J.A. & Barros, H.M. Aggression, anxiety and vocalizations in animals: $GABA_A$ and 5-HT anxiolytics. *Psychopharmacology* **121**, 38–56 (1995).
32. Winslow, J.T. & Insel, T.R. Infant rat separation is a sensitive test for novel anxiolytics. *Prog. Neuropsychopharmacol. Biol. Psychiatry* **15**, 745–757 (1991).
33. Cahill, L. & McGaugh, J.L. Mechanisms of emotional arousal and lasting declarative memory. *Trends Neurosci.* **21**, 294–299 (1998).
34. McDonald, A.J. & Mascagni, F. Localization of the CB1 type cannabinoid receptor in the rat basolateral amygdala: high concentrations in a subpopulation of cholecystokinin-containing interneurons. *Neuroscience* **107**, 641–652 (2001).
35. Fink, H., Rex, A., Voits, M. & Voigt, J.P. Major biological actions of CCK: a critical evaluation of research findings. *Exp. Brain Res.* **123**, 77–83 (1998).
36. Beinfeld, M.C. & Connolly, K. Activation of CB1 cannabinoid receptors in rat hippocampal slices inhibits potassium-evoked cholecystokinin release, a possible mechanism contributing to the spatial memory defects produced by cannabinoids. *Neurosci. Lett.* **301**, 69–71 (2001).
37. Iversen, L. & Chapman, V. Cannabinoids: a real prospect for pain relief? *Curr. Opin. Pharmacol.* **2**, 50–55 (2002).
38. Stella, N. & Piomelli, D. Receptor-dependent formation of endogenous cannabinoids in cortical neurons. *Eur. J. Pharmacol.* **425**, 189–196 (2001).
39. Giuffrida, A., Rodríguez de Fonseca, F. & Piomelli, D. Quantification of bioactive acylethanolamides in rat plasma by electrospray mass spectrometry. *Anal. Biochem.* **280**, 87–93 (2000).
40. Piomelli, D. *et al.* Structural determinants for recognition and translocation by the anandamide transporter. *Proc. Natl. Acad. Sci. USA* **96**, 5802–5807 (1999).
41. Tseng, A.H. & Craft, R.M. Sex differences in antinociceptive and motoric effects of cannabinoids. *Eur. J. Pharmacol.* **430**, 41–47 (2001).
42. Ali, M.M., Bawari, M., Misra, U.K. & Babu, G.N. Locomotor and learning deficits in adult rats exposed to monosodium-L-glutamate during early life. *Neurosci. Lett.* **284**, 57–60 (2000).
43. Wedzony, K. *et al.* WAY 100135, an antagonist of 5-HT1A serotonin receptors, attenuates psychotomimetic effects of MK-801. *Neuropsychopharmacology* **23**, 547–559 (2000).
44. Cuomo, V., Cagiano, R., Renna, G., De Salvia, M.A. & Racagni, G. Ultrasonic vocalization in rat pups: effects of early postnatal exposure to SCH 23390 (a DA1-receptor antagonist) and sulpiride (a DA2-receptor antagonist). *Neuropharmacology* **26**, 701–705 (1987).
45., Haller, J., Bakus, N., Szirmay, M., Ledent, C. & Freund, T.F. The effects of genetic and pharmacological blockade of the CB1 cannabinoid receptor on anxiety. *Eur. J. Neurosci.* (in the press).

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Original article

Treating depression with cannabinoids

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Abstract

Although a variety of drugs are available for the treatment of depression, therapy is not effective in all cases and finding alternative options is desirable. Results from animal studies, anecdotal experience reported by patients using cannabis and observations from clinical studies where cannabinoids were used in serious diseases suggest an anti-depressive potential of cannabinoid receptor agonists. From 2003 to 2006, 75 patients suffering from depression, stress and burnout syndrome were successfully treated in a practice for general medicine with the cannabis ingredient dronabinol, alone or in combination with other antidepressants. Two case studies will be presented. The presented observations suggest that dronabinol has an antidepressive potential that can readily be used in medical practice.
Key words: Depression, burnout, cannabinoid, cannabis, dronabinol

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Introduction

In several prospective studies, consumption of cannabis was associated with an increased risk of developing depression and anxiety, particularly when cannabis had been used during adolescence [1,2]. There appears to be less evidence for a correlation between depression and cannabis use during adulthood [3,4]. On the other hand, patients have, in numerous surveys and interviews, reported anti-depressant and anxiolytic effects of cannabis [5-11]. Patients suffering from a range of chronic illnesses have reported that they use cannabis not only to mitigate physical symptoms, such as pain, nausea and lack of appetite, but also to improve general well-being and to mitigate anxiety and depression [8-10,12].

In several clinical studies, during which subjective parameters were monitored, cannabinoids not only improved physical symptoms but also improved well-being and produced measurable antidepressant effects [13-15]. A study by Musty (2002) with healthy volunteers, smoking cannabis showed a positive correlation with the ratings on a scale of depression (MMPI), indicating an antidepressant effect [16]. These indications of a therapeutic potential of symptoms of depression encouraged the author to start administering dronabinol to select patients suffering from depression.

Experiences in Medical Practice

The author operates a practice for general medicine in downtown Vienna, where a large population of younger people lives and works. In the late 1990s I began administering dronabinol to individual younger patients, who were dissatisfied with available antidepressants because of side effects or lack of effectiveness. In Austria, the active ingredient of cannabis has been available for medical therapy since 1998. The majority of these early patients, who suffered from a reactive depression or burnout syndrome, was well aware of the therapeutic potential of cannabis and considered a trial with dronabinol reasonable.

Between 2003 and 2006 some 250 patients who suffered from a wide range of illnesses were treated in my practice with dronabinol. Some 75, or 30%, of them suffered from depression, a sense of being overwhelmed or from burnout syndrome. The initial dose of 2.5 mg dronabinol in capsules was raised, over a period of several days, to generally 5 or 7.5 mg per day. For almost 80% of the patients, use of the medication cor-

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related with swift improvement of the depressed mood or the sense of being overwhelmed. Only 20% of patients did not experience any significant mood brightening. To that group a combination therapy of dronabinol and a selective serotonin reabsorption inhibitor (SSRI), such as fluoxetine hydrocholoride at a dose of 20 mg per day or a serotonin noradrenalin reabsorption inhibitor (SNRI), such as milnacipran at 50 mg per day, was administered. That therapy generally resulted in rapid and satisfactory improvement of depression and the lack of drive.
Side effects were generally low. Effective daily doses of dronabinol ranged generally from 7.5 to 12.5 mg per day. Only few patients required a higher dosage, generally those also suffering from a sleeping disorder.

Case Reports

In the following two exemplary cases from a large number of successful treatments are presented.

Case 1

Ms. H. came to my practice six years ago, at the age of 48. She had a long psychiatric record with episodes of depression and the abuse of alcohol and drugs, particularly of benzodiazepines. A former teacher, she is now retired but continues to work as an actress.
At the onset of the therapy the patient was in a difficult situation. Her father had recently passed away; she was highly depressed, sometimes even suicidal. Heavy abuse of drugs, such as oxazepam, and of alcohol further complicated her situation. Following an extensive discussion a treatment with oral dronabinol of 5 – 7.5 mg per day was started.
After 6 years of using dronabinol Ms. H. is now very experienced with the use of the drug. Depending on her symptoms, she takes between 2 and 4 capsules of 2.5 mg per day. She is no longer addicted to benzodiazepines and does currently not drink alcohol. As supplementary therapy she takes 2.5 mg per day of olanzapin (an atypical neuroleptic), 25 mg of venlafaxin (an SNRI) and, if needed, trazodon, SSRI. She reports that the dronabinol therapy has improved her quality of life significantly. She feels more stable than before and the chronically reoccurring episodes of depression are less severe. Her speed of reaction when operating a vehicle is impaired. Before extended car trips she has thus periodically suspended dronabinol for typically one week, which has resulted in psychological withdrawal symptoms.

Case 2

Ms. F. first visited our practice at the age of 22 where she received treatment over a 12-month period. At that time, the patient suffered from stress related headaches, migraine, asthma, neurodermatitis and an instable emotional personal disorder. Most prominent was an acute depressive syndrome, for which Ms. F. had already received treatment in the psychiatric clinic at Vienna General Hospital.
After repeatedly dropping out of school and frequent job changes the patient tried, despite a lack of family contacts, to improve her dismal social and physical conditions. She was also rather unhappy with her having to consume up to ten prescription medications. In addition to anti-depressants, such as fluoxetine and mianserin, neuroleptics, such as prothipendyl, sedatives and anti-allergic agents, such as hydroxyzine, NSAR, such as diclofenac, proton pump inhibitors, such as rabeprazole, analgesics, such as propyphenazone and tramadol, she daily consumed anti-asthmatics, such as terbutaline sulfate as prescribed by several other physicians.
Because the patient did not want to continue this multidrug treatment she came to our practice in search for a more simple and natural treatment, involving no more than two drugs. Primary objective of the treatment was to improve her acutely depressive condition, which had not improved despite the use of multiple drugs. Following an extensive consultation the patient opted for a monotherapy with dronabinol. After several days the initial dose of 2.5 mg was raised to 7.5 mg daily. After several days of treatment we observed a significant improvement of her depressive condition and of the concurrently occurring illnesses.
During the first month of therapy the daily dronabinol dose was raised to 10 mg and 12 month after starting her therapy the physical and psycho-social condition of the patient had stabilized at that dose. Subsequently, the patient resumed relationships with her family, relocated to a different state and left our practice.

Conclusions

In summary, the experience presented here suggests that general practitioners are able to treat a large number of patients suffering from depression and burnout syndrome without significant complications. Most patients were not reimbursed for dronabinol by their health insurance, unlike for patients with physical illnesses, such as cancer or multiple sclerosis, where the local health insurance in Vienna pays for nearly 60% of the cost of dronabinol.
These findings agree with the results from patient interviews, observations from clinical studies on the impact of cannabinoid use on mood and the results from animal experiments. In the latter, exogenous cannabinoid receptor agonists [17,18] as well as the inhibition of the deactivation of the endocannabinoid anandamide [18,19] resulted in antidepressant effects. To date no clinical studies have studied primarily the effectiveness of cannabinoids for the treatment of depression. In my opinion, such studies are desirable and promising.

References

1. Hayatbakhsh MR, Najman JM, Jamrozik K, Mamun AA, Alati R, Bor W. Cannabis and anxiety and depression in young adults: a large prospec-

tive study. J Am Acad Child Adolesc Psychiatry 2007;46(3):408-417.
2. Wittchen HU, Fröhlich C, Behrendt S, Günther A, Rehm J, Zimmermann P, Lieb R, Perkonigg A. Cannabis use and cannabis use disorders and their relationship to mental disorders: a 10-year prospective-longitudinal community study in adolescents. Drug Alcohol Depend 2007;88 Suppl 1:S60-70.
3. Moore TH, Zammit S, Lingford-Hughes A, Barnes TR, Jones PB, Burke M, Lewis G. Cannabis use and risk of psychotic or affective mental health outcomes: a systematic review. Lancet 2007;370(9584):319-28.
4. Harder VS, Morral AR, Arkes J.Marijuana use and depression among adults: Testing for causal associations. Addiction 2006;101(10):1463-72.
5. Joy JE, Watson SJ, Benson JA (eds): Marijuana and Medicine: Assessing the Science Base. Institute of Medicine. Washington DC: National Academy Press, 1999.
6. Gieringer D. Medical use of cannabis: experience in California. In: Grotenhermen F, Russo E, eds. Cannabis and cannabinoids. Pharmacology, toxicology, and therapeutic potential. Binghamton/New York: Haworth Press, 2002.
7. Schnelle M, Grotenhermen F, Reif M, Gorter RW. Ergebnisse einer standardisierten Umfrage zur medizinischen Verwendung von Cannabisprodukten im deutschen Sprachraum. Forsch Komplementärmed 1999;6(Suppl 3):28-36.
8. Prentiss D, Power R, Balmas G, Tzuang G, Israelski DM. Patterns of marijuana use among patients with HIV/AIDS followed in a public health care setting. J Acquir Immune Defic Syndr 2004;35(1):38-45.
9. Amtmann D, Weydt P, Johnson KL, Jensen MP, Carter GT. Survey of cannabis use in patients with amyotrophic lateral sclerosis. Am J Hosp Palliat Care 2004;21(2):95-104.
10. Woolridge E, Barton S, Samuel J, Osorio J, Dougherty A, Holdcroft A. Cannabis use in HIV for pain and other medical symptoms. J Pain Symptom Manage 2005;29(4):358-67.
11. Ware MA, Adams H, Guy GW. The medicinal use of cannabis in the UK: results of a nationwide survey. Int J Clin Pract 2005;59(3):291-5.
12. Page SA, Verhoef MJ, Stebbins RA, Metz LM, Levy JC. Cannabis use as described by people with multiple sclerosis. Can J Neurol Sci 2003;30(3):201-5.
13. Regelson W, Butler JR, Schulz J, Kirk T, Peek L, Green ML, Zalis MO. Delta-9-tetrahydrocannabinol as an effective antidepressant and appetite-stimulating agent in advanced cancer patients. In: Braude MC, Szara S, eds. Pharmacology of marihuana. Vol 2. Raven Press, New York 1976. S. 763-776.
14. Beal JE, Olson R, Laubenstein L, Morales JO, Bellman P, Yangco B, Lefkowitz L, Plasse TF, Shepard KV. Dronabinol as a treatment for anorexia associated with weight loss in patients with AIDS. J Pain Symptom Manage 1995;10(2):89-97.
15. Maida V, Ennis M, Irani S, Corbo M, Dolzhykov M. Adjunctive nabilone in cancer pain and symptom management: a prospective observational study using propensity scoring. J Support Oncol 2008;6(3):119-24.
16. Musty RE. Cannabinoid therapeutic potential in motivational processes, psychological disorders and central nervous system disorders. In: Onaivi ES, Hrsg. Biology of Marijuana. From Gene to Behaviour. London/New York: Taylor & Francis, 2002.
17. Bambico FR, Katz N, Debonnel G, Gobbi G. Cannabinoids elicit antidepressant-like behavior and activate serotonergic neurons through the medial prefrontal cortex. J Neurosci 2007;27(43): 11700-11.
18. Hill MN, Gorzalka BB. Pharmacological enhancement of cannabinoid CB1 receptor activity elicits an antidepressant-like response in the rat forced swim test. Eur Neuropsychopharmacol 2005;15(6):593-9.
19. Gobbi G, Bambico FR, Mangieri R, Bortolato M, Campolongo P, Solinas M, Cassano T, Morgese MG, Debonnel G, Duranti A, Tontini A, Tarzia G, Mor M, Trezza V, Goldberg SR, Cuomo V, Piomelli D. Antidepressant-like activity and modulation of brain monoaminergic transmission by blockade of anandamide hydrolysis. Proc Natl Acad Sci U S A 2005;102(51):18620-5.

100 YEARS AGO

An interesting instance of that adaptability to changing tastes and conditions which is the mainspring of progress in industry as well as in science is afforded by a note in the *Journal* of the Society of Arts (July 18). For some years the demand for claret has greatly diminished in favour of the wines of Champagne, and has seriously affected the wine industry in the Bordeaux region. Several proprietors in the Médoc have, however, now commenced the production of sparkling wines by the same process as champagne is made, and their action has been the means of developing practically a new industry. It may at first seem strange that white wine should be able to be made in the Médoc, where only black grapes are grown, but as a matter of fact champagne is almost entirely made from black grapes, and the most celebrated vineyards in the Champagne district are all planted with them… It is stated that to the ordinary taster there is nothing but the label to distinguish the sparkling médoc from the best brands of champagne.

From *Nature* 31 July 1902.

50 YEARS AGO

The Psychology of the Occult. **This stimulating and highly provocative book is an attempt to describe and analyse the part "played by various types of psychological anomaly in the creation and perpetuation of occult beliefs and practices"… Mr. Rawcliffe is completely unmoved by the flood of modern propaganda in favour of the reality of so-called psychic phenomena… As for the physical phenomena of the séance room, Mr. Rawcliffe finds the evidence scarcely worth considering. To him the whole of the studies of the psychical research worker are mere examples of magic and superstition dressed up in modern garb and often presented behind a façade of statistical jargon which is intended to disguise the faulty character of the original data. In support of his position he has skilfully put together a mass of material in which the incompetence and credulity of not a few workers in this field are cruelly displayed. Yet he has omitted much that would have strengthened his case and, it must be added, a good deal that would have weakened it… however, this book remains a useful handbook for those who suspect that much of what passes for psychical research and which is often unfortunately supported by leading parapsychologists is scarcely worth the paper on which it is recorded.**

From *Nature* 2 August 1952.

surface, see inside the structure and sample the rocks. But exposed impact structures are typically incomplete, lacking their uppermost parts through the vagaries of erosion and deformation processes. The only way of 'seeing' virgin morphology is when a structure has been rapidly buried after it formed, and so has been preserved. Geophysicists then need to use seismic techniques to reveal the three-dimensional structure, as has been done for Silverpit. The development of multiple concentric rings at such a small diameter may not be unusual because, until recently, we have been unable to obtain images with this degree of detail in such a well-preserved example.

Finally, the real test that Silverpit was created by an impact will be to look for shock effects in the rocks that form it. Shock-generated features such as unusual microscopic mineral deformations and shatter cones (conical fracture systems formed by shock waves in rock) would be compelling evidence of an impact origin. Two wells that were drilled in the search for oil and gas do penetrate the structure, but unfortunately, few samples of the drill cuttings from them were taken. Given that impact structures are among the most productive hydrocarbon sites on the planet, we may get more rock samples if Silverpit shows exploration potential. ■

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1. Stewart, S. A. & Allen, P. J. *Nature* **418,** 520–523 (2002).
2. Grieve, R. A. F., Therriault, A. & Rupert, A. *Annu. Rev. Planet. Sci.* **28,** 305–338 (2000).
3. Spudis, P. D. *The Geology of Multi-Ring Impact Basins* 263 (Cambridge Univ. Press, 1993).
4. Baldwin, R. B. *Phys. Earth Planet. Inter.* **6,** 327–339 (1972).
5. Melosh, H. J. *Nature* **373,** 104–105 (1995).

Neurobiology

Never fear, cannabinoids are here

Pankaj Sah

Although we understand how fearful memories are stored in the brain, how they are extinguished remains a mystery. The answers may lie with the cannabinoid compounds our bodies produce.

Cannabinoids such as marijuana and hashish have been used for over a thousand years for medicinal and recreational purposes. The active 'ingredient' of these drugs is Δ^9-tetrahydrocannabinol, which produces effects on nerve cells in the brain by binding to a protein on the neuronal surface, the CB1 receptor[1]. But of course the receptor is not there simply to detect this externally derived compound: it also binds to 'endogenous' cannabinoids, which are produced naturally by the body. On page 530 of this issue, Marsicano and colleagues[2] propose a new role for this 'endocannabinoid' system — extinguishing fear-related memories in mice. The finding might have implications for treating anxiety disorders in humans.

We can form memories in several different ways, one of which is Pavlovian conditioning — the classic example being that of Pavlov's dogs, which learned to expect food whenever they heard a ringing tone. We all form these types of associations; for instance, we may associate a particular piece of music with our first love affair. But the connection need not always be pleasant. Imagine you are having a quiet walk in a park when you are threatened by an armed person. During the attack you are terrified; your heart races and your palms are sweaty. You run and escape. Later, you may find that entering the same park brings back in detail the memory of the attack, right down to the sweaty palms.

In the lab, the neuronal and molecular mechanisms underlying fearful memories are often studied in animals by using 'fear conditioning'. Here, a neutral — or conditioned — stimulus, which is typically a tone or a light, is paired with an aversive (unconditioned) stimulus, typically a small electric shock to the foot. After the two stimuli are paired a few times, the conditioned stimulus alone evokes the stereotypical features of the fearful response to the unconditioned stimulus, including changes in heart rate and blood pressure and freezing of ongoing movements. Repeated presentation of the conditioned stimulus alone leads to extinction of the fearful response — the animal learns that it need no longer fear a shock from the tone or light.

A large body of work has established that a small, almond-shaped region in the brain, the amygdala, is crucial in acquiring and, possibly, storing the memory of conditioned fear[3,4]. It is thought that, at the cellular and molecular level, this learned behaviour requires neurons in the basolateral part of the amygdala, and changes in the strength of their connection with other neurons ('synaptic plasticity') that depend on the NMDA receptor[5], which responds to the neurotransmitter glutamate.

The extinction of aversive memories also involves the basolateral amygdala, but the cellular and molecular details are less clear. Infusing antagonists of the NMDA receptor into this region blocks extinction, implying that these receptors are important here, too[6]. Yet their exact role is not known. It has been proposed that synaptic plasticity is

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again involved[6], but the possible sites of plasticity and the underlying physiology are not known, and NMDA-receptor-dependent plasticity has not yet been correlated with extinction. Moreover, it has been suggested that there are also NMDA-receptor-independent mechanisms of extinction[7].

Marsicano *et al.*[2] now propose just such a mechanism, which involves the endocannabinoids anandamide and 2-arachidonylglycerol, and their CB1 receptors. These receptors are some of the most abundant neuromodulatory receptors in the central nervous system and are expressed at high levels in the limbic system, cerebellum and basal ganglia[8]. The classical behavioural effects of exogenous cannabinoids — such as sedation and memory changes — have been correlated with the presence of CB1 receptors in the limbic system and striatum.

It has been difficult, however, to pin down the physiological role of endocannabinoids and how they are released in these regions. In studies that were the first to reveal such a role, the depolarization of neurons by repetitive activity led to the release of endocannabinoids[9], which diffused to the terminals of other neurons and inhibited neurotransmitter release. This effect was transient in the hippocampus and cerebellum[9] and long lasting in the striatum[10]. Yet these changes in neurotransmission have not been connected to any specific behavioural effects. So the study by Marsicano *et al.*[2] represents a leap forward in two areas of neurobiology, in that it clearly implicates the release of endocannabinoids in a well-known, simple learning task. It also links endocannabinoid release to synaptic plasticity.

After engineering mice to lack the CB1 receptor, Marsicano *et al.* first showed that although these animals could learn and later recall the association of a tone with a foot shock, they could not extinguish the memory. A drug that antagonizes the CB1 receptor similarly prevented extinction in wild-type mice. The authors then found that during the extinction protocol (exposure to the tone alone), the levels of both anandamide and 2-arachidonylglycerol were raised in the basolateral amygdala in mutant and normal mice. This implies that a process involving activation of the CB1 receptors by endocannabinoids is essential in the extinction of conditioned fear.

Next, in experiments with slices of normal mouse brains, the authors looked at neurons in the basolateral amygdala that can release GABA (an inhibitory neurotransmitter). They found that low-frequency stimulation of these neurons leads to a long-term reduction in the release of GABA, which in turn leads to less inhibition of the connecting 'pyramidal' neurons. This long-term 'depression' — a type of synaptic plasticity — was completely blocked by the CB1-receptor antagonist, and absent in CB1-deficient mice. These findings suggest that the endocannabinoids reduce GABA release in the basolateral amygdala, thereby helping to extinguish the fear-conditioned response. In mammals, the neurons that release GABA are largely interneurons, which can be divided into several populations on the basis of their expression of certain proteins and peptides (such as cholecystokinin). The role of endocannabinoids in reducing GABA release fits with the finding that CB1 receptors in the basolateral amygdala are present on the terminals of cholecystokinin-containing interneurons[11,12].

This is an entirely new cellular and molecular mechanism for extinction. But how does it tie in with the NMDA receptors? There seems little doubt that activation of these glutamate receptors in the basolateral amygdala is somehow required for extinction[6]. But Marsicano *et al.*'s brain-slice experiments were performed with blocked glutamate receptors, showing that the endocannabinoid-mediated synaptic plasticity they report does not need the NMDA receptors. So we have yet to find out how these receptors are involved in extinction.

It has been argued that the neuronal circuitry underlying fear conditioning has similarities to that responsible for fear-related clinical conditions, such as post-traumatic stress disorder[4]. Behavioural therapies for these conditions — including systematic desensitization and imagery therapies — share features with extinction. The finding that the endocannabinoids contribute to extinction raises the possibility that drugs that target these molecules and their receptors could be useful new treatments for anxiety disorders. Finally, there is much anecdotal evidence of patients using cannabis heavily in the early stages of psychiatric illness. This has often been thought to contribute to acute illness. But it seems possible that it may instead be a form of self-medication for the sometimes extreme anxiety that these people experience. ■

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1. Matsuda, L. A. *et al. Nature* **346**, 561–564 (1990).
2. Marsicano, G. *et al. Nature* **418**, 530–534 (2002).
3. LeDoux, J. E. *Annu. Rev. Neurosci.* **23**, 155–184 (2000).
4. Davis, M. & Whalen, P. J. *Mol. Psychiatry* **6**, 13–34 (2001).
5. Blair, H. T., Schafe, G. E., Bauer, E. P., Rodrigues, S. M. & LeDoux, J. E. *Learn. Mem.* **8**, 229–242 (2001).
6. Walker, D. L. & Davis, M. *Pharmacol. Biochem. Behav.* **71**, 379–392 (2002).
7. Santini, E., Muller, R. U. & Quirk, G. J. *J. Neurosci.* **21**, 9009–9017 (2001).
8. Herkenham, M. *et al. J. Neurosci.* **11**, 563–583 (1991).
9. Wilson, R. I. & Nicoll, R. A. *Science* **296**, 678–682 (2002).
10. Gerdeman, G. L., Ronesi, J. & Lovinger, D. M. *Nature Neurosci.* **5**, 446–451 (2002).
11. Katona, I. *et al. J. Neurosci.* **21**, 9506–9518 (2001).
12. McDonald, A. J. & Mascagni, F. *Neuroscience* **107**, 641–652 (2001).

Earth science

Core values

John Brodholt and Francis Nimmo

Calculating the age of the Earth's solid inner core has proved to be a tricky business. But the suggestion that there is more potassium in the core than had been thought could help to reconcile differing estimates.

Potassium is a relatively insignificant element in the Earth, languishing in sixteenth place in the league table of chemical abundance. But, right now, radioactive decay of a potassium isotope, ^{40}K, is responsible for about 10% of the heat lost by the Earth. As ^{40}K has a half-life of 1.25 billion years, its decay would have produced much more heat in the past — just after the Earth formed, 4.6 billion years ago, daily decay of ^{40}K would have produced more heat than the present total daily heat loss of the Earth. Heat within the Earth drives processes such as convection in the mantle layer and the generation of the planet's magnetic field. So knowing how potassium and other radioactive elements are concentrated in different parts of the Earth is fundamental to understanding these processes.

There is a large concentration of potassium in the Earth's crust, and a significant proportion remains in the mantle below. What is not known is how much is in the Earth's core. Although experimental results have been ambiguous, it has generally been thought that, because of the relatively large radius of potassium ions, not much of this element could be absorbed in the core. But new results from Gessman and Wood[1], reported in *Earth and Planetary Science Letters*, show that the amount of potassium in the core depends on the core's sulphur and oxygen content, and on the structure of the coexisting silicate melt. Their findings help to explain some of the ambiguities in the earlier data, and also enable them to estimate the maximum concentration of potassium in the Earth's core — a number that has important bearing on the age of both the inner core and the Earth's magnetic field.

Although the magnetic field is generated by fluid flow in the liquid outer core, it is generally accepted that the solid inner core also plays a fundamental role. This is because

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20. Ritchie, M. E. Scale-dependent foraging and patch choice in fractal environments. *Evol. Ecol.* **12**, 309–330 (1998).
21. Ritchie, M. E. & Olff, H. Spatial scaling laws yield a synthetic theory of biodiversity. *Nature* **400**, 557–560 (2000).
22. Lawler, G., Schramm, O. & Werner, W. The dimension of the planar brownian frontier is 4/3. *Math. Res. Lett.* **8**, 13–25 (2001).
23. Milne, B. T. Spatial aggregation and neutral models in fractal landscapes. *Am. Nat.* **139**, 32–57 (1992).
24. Kunin, W. E. Extrapolating species abundance across spatial scales. *Science* **281**, 1513–1515 (1998).
25. Mandelbrot, B. B. *The Fractal Geometry of Nature* (Freeman, New York, 1982).
26. Olff, H. & Ritchie, M. E. Fragmented nature: consequences for biodiversity. *Landscape Urban Planning* **858**, 1–10 (2001).
27. Kotler, N. B. & Wiens, J. A. Multiple scales of patchiness and patch structure: a hierarchical framework for the study of heterogeneity. *Oikos* **59**, 253–260 (1990).
28. Poole, A. & Gill, F. (eds) *The Birds of North America* (The Birds of North America, Philadelphia, 1996–2000).
29. Dunning, J. B. *CRC Handbook of Avian Body Masses* (CRC, Boca Raton, 1993).
30. Brown, J. H. in *Experimental Ecology* (eds Resetarits, W. J. Jr & Bernardo, J.) 71–95 (Oxford Univ. Press, New York, 1998).

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Competing interests statement

The authors declare that they have no competing financial interests.

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The endogenous cannabinoid system controls extinction of aversive memories

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Acquisition and storage of aversive memories is one of the basic principles of central nervous systems throughout the animal kingdom[1]. In the absence of reinforcement, the resulting behavioural response will gradually diminish to be finally extinct. Despite the importance of extinction[2], its cellular mechanisms are largely unknown. The cannabinoid receptor 1 (CB1)[3] and endocannabinoids[4] are present in memory-related brain areas[5,6] and modulate memory[7,8]. Here we show that the endogenous cannabinoid system has a central function in extinction of aversive memories. CB1-deficient mice showed strongly impaired short-term and long-term extinction in auditory fear-conditioning tests, with unaffected memory acquisition and consolidation. Treatment of wild-type mice with the CB1 antagonist SR141716A mimicked the phenotype of CB1-deficient mice, revealing that CB1 is required at the moment of memory extinction. Consistently, tone presentation during extinction trials resulted in elevated levels of endocannabinoids in the basolateral amygdala complex, a region known to control extinction of aversive memories[9]. In the basolateral amygdala, endocannabinoids and CB1 were crucially involved in long-term depression of GABA (γ-aminobutyric acid)-mediated inhibitory currents. We propose that endocannabinoids facilitate extinction of aversive memories through their selective inhibitory effects on local inhibitory networks in the amygdala.

To study the involvement of the endogenous cannabinoid system in memory processing, we generated CB1-deficient mice ($CB1^{-/-}$; see Supplementary Information). $CB1^{-/-}$ mice and $CB1^{+/+}$ littermates were tested in auditory fear conditioning, which is highly dependent on the amygdala[1] and enables the dissection of different phases of memory formation, including acquisition, consolidation and extinction. Mice were trained to associate a tone with a foot-shock (conditioning). After conditioning, animals froze when

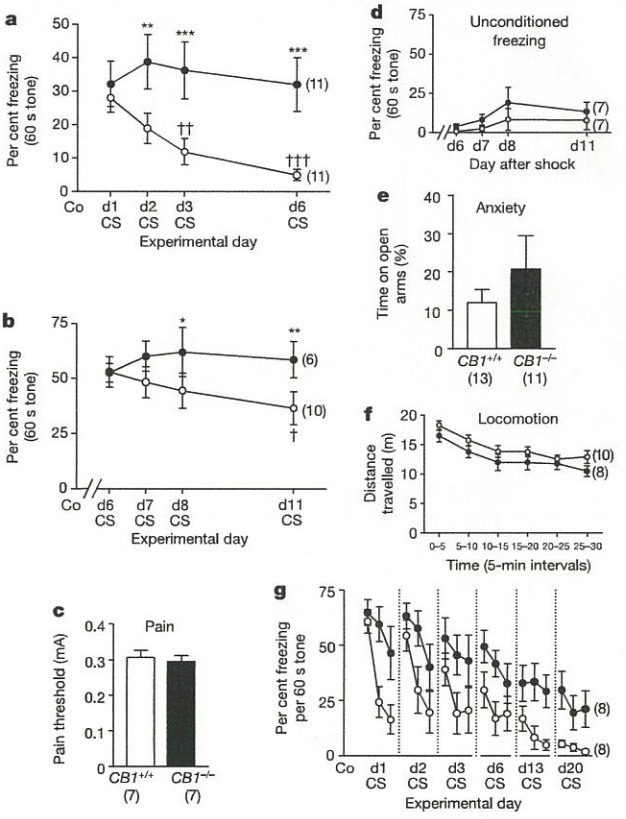

Figure 1 Impaired extinction of aversive memory in an auditory fear-conditioning task of $CB1^{-/-}$ mice (filled circles) as compared to their $CB1^{+/+}$ littermates (open circles). **a**, **b**, After conditioning (Co) animals were repeatedly exposed to 60 s tones (conditioned stimulus, CS) starting 24 h after conditioning (**a**) (d1) or after a 6-day consolidation period (**b**) (d6). **c**–**f**, $CB1^{-/-}$ and $CB1^{+/+}$ mice did not differ in their sensory-motor abilities, as assessed by sensitivity to rising electric foot-shock (**c**), unspecific freezing to a tone after shock application (**d**), anxiety-related behaviour on the elevated plus maze (**e**) and horizontal locomotion in an open field (**f**). **g**, $CB1^{-/-}$ mice showed memory extinction in response to a stronger extinction protocol (3 min tones until day 20; analysed in 60-s intervals), but still froze more than $CB1^{+/+}$ controls. Means ± s.e.m. are shown; number of animals are indicated in parentheses. Asterisk, $P < 0.05$; double asterisk, $P < 0.01$; triple asterisk, $P < 0.001$ (compared with $CB1^{+/+}$); dagger, $P < 0.05$; double dagger, $P < 0.01$; triple dagger, $P < 0.001$ (compared with day 1).

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re-exposed to the tone. This response served as an indicator of aversive memory, and is gradually extinguished on repeated tone presentations. As the amygdala has a crucial role for extinction of aversive memories[9,10], we studied amygdala-dependent memory performance in the absence of possible confounding influences of the hippocampus by re-exposing the mice to the tone in an environment different from the conditioning context[1]. In this environment, neither $CB1^{-/-}$ nor $CB1^{+/+}$ mice showed freezing without tone presentation 24 h after conditioning (data not shown). During the subsequent tone presentation, however, animals of both groups showed the same amount of freezing (Fig. 1a; d1, $P > 0.05$), pointing to an equally successful tone–foot-shock association. On repeated exposure to the tone, however, $CB1^{+/+}$ and $CB1^{-/-}$ mice differed significantly in their freezing behaviour (genotype: $F_{1,20} = 5.81$, $P < 0.05$; genotype × day interaction: $F_{3,60} = 4.86$, $P < 0.005$; Fig. 1a). In fact, $CB1^{+/+}$ mice ($F_{3,10} = 9.70$, $P < 0.0005$), but not $CB1^{-/-}$ ($F_{3,10} = 0.94$, $P = 0.433$), showed extinction of freezing.

The identical behavioural performance of the two genotypes on day 1 indicates that acquisition and early consolidation processes do not involve CB1. However, it is possible that memory consolidation processes were not completed 24 h after conditioning, leaving open a potential involvement of CB1 in later phases of memory consolidation. To test this hypothesis, new groups of animals remained undisturbed after conditioning for 6 days, and mice from these groups were then exposed to the 60-s tones (Fig. 1b). Again, $CB1^{-/-}$ and $CB1^{+/+}$ mice did not differ in their initial freezing response, but behaved in a significantly different way in the course of repeated tone presentations (genotype × day interaction: $F_{3,42} = 3.03$, $P < 0.05$). Whereas $CB1^{+/+}$ mice showed a decrease in freezing behaviour until day 11 ($F_{3,27} = 3.73$, $P < 0.05$), $CB1^{-/-}$ mice failed to extinguish the freezing response ($F_{3,15} = 1.03$, $P = 0.404$). A more detailed analysis of the freezing response in 20-s intervals confirmed the difference in extinction (genotype × 20-s bin interaction: $F_{11,154} = 2.60$, $P < 0.005$; Supplementary Information). These differences were due to altered short-term and long-term extinction in $CB1^{-/-}$ mice but not to increased spontaneous recovery of the freezing response (genotype: $F_{1,14} = 0.18$, $P = 0.675$; genotype × day interaction: $F_{2,28} = 1.61$, $P = 0.217$; Supplementary Information).

We next analysed whether the differences in memory extinction between the two genotypes could be attributed to alterations in sensory-motor abilities of $CB1^{-/-}$ mice, as cannabinoids are known to influence pain perception, emotionality and locomotion[4,11,12]. However, mice of either genotype showed the same pain sensitivity to a rising electric foot-shock defined as the shock intensity at which mice showed first signs of discomfort, that is, jumping and/or vocalization (Fig. 1c). Moreover, if the same animals were repeatedly exposed to the tone, there were no significant differences in freezing behaviour between the genotypes (genotype: $F_{1,12} = 1.61$, $P = 0.228$; genotype × day interaction: $F_{3,36} = 0.225$, $P = 0.878$; Fig. 1d), indicating that CB1 deficiency does not affect foot-shock-induced behavioural sensitization or unconditioned freezing to the tone. Anxiety-related behaviour was analysed on an elevated plus maze. Animals of either genotype spent the same relative time on open arms of the maze ($P > 0.05$, *t*-test and *U*-test; Fig. 1e), and made the same relative number of entries into open arms ($CB1^{+/+}$: 22.0 ± 4.0%; $CB1^{-/-}$: 21.1 ± 7.6%, $P > 0.05$, *t*-test and *U*-test). In contrast, $CB1^{-/-}$ mice showed reduced exploratory activity (number of closed-arm entries: 11.6 ± 1.1 in $CB1^{+/+}$ mice compared with 6.5 ± 1.2 in $CB1^{-/-}$ mice, $P < 0.01$, *t*-test). However, in an open-field locomotor activity test, no significant differences were found, including horizontal (Fig. 1f) and vertical locomotion, resting time, and time spent close to the walls of the box (data not shown).

The failure of $CB1^{-/-}$ mice to diminish their freezing response during a limited number of 60-s tone presentations (Fig. 1a, b) raises the question as to whether $CB1^{-/-}$ mice are able to extinguish aversive memories at all. Thus, conditioned $CB1^{-/-}$ and $CB1^{+/+}$ mice were exposed to a stronger extinction protocol (3 min tone, six exposures; Fig. 1g). Both $CB1^{+/+}$ ($F_{17,119} = 15.01$, $P < 0.000001$) and $CB1^{-/-}$ mice ($F_{17,119} = 7.59$, $P < 0.000001$) extinguished their freezing response over the course of repeated tone presentations. Nevertheless, extinction was still more pronounced in $CB1^{+/+}$ as compared with $CB1^{-/-}$ mice (genotype: $F_{1,14} = 5.30$, $P < 0.05$). Notably, the most marked differences between $CB1^{-/-}$

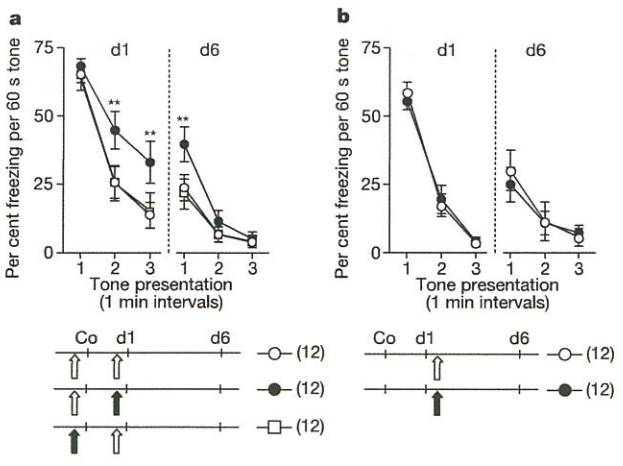

Figure 2 CB1 antagonist SR141716A impairs short-term and long-term extinction, but not acquisition and consolidation of aversive memories. **a**, Mice were treated with SR141716A (filled arrows) or vehicle (open arrows) 20 min before conditioning (Co) and the first extinction trial (d1; 3 min tone). **b**, Mice were treated with SR141716A or vehicle 10 min after the first extinction trial, as indicated. Freezing was analysed in 60-s intervals. Means ± s.e.m. are shown; number of animals are shown in parentheses. Double asterisk, $P < 0.01$ (compared with the two other groups).

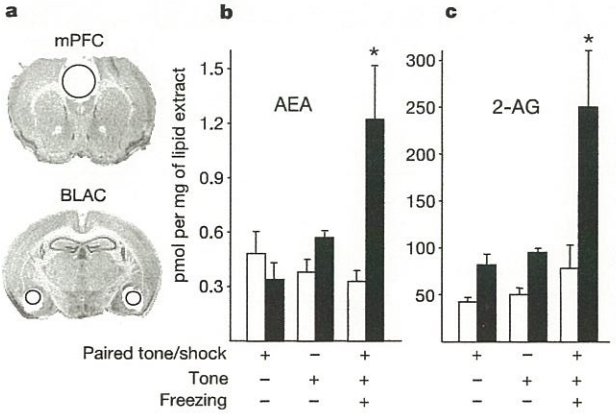

Figure 3 Re-exposure to the tone 24 h after conditioning causes increased endocannabinoid levels in the basolateral amygdala complex (BLAC) but not the medial prefrontal cortex (mPFC) of C57BL/6J mice. **a**, Micrographs of coronal brain sections showing representative examples of the dissected mPFC and BLAC. Circles indicate the size and positioning of tissue sampling. **b**, **c**, Anandamide (**b**, AEA) and 2-arachidonoylglycerol (**c**, 2-AG) levels of the three experimental groups (see text), which differed in conditioning procedure, re-exposure to the tone and resulting freezing response to the tone. Means ± s.e.m. are shown ($n = 4$ per group, 5 mice per n). Open bars, mPFC; filled bars, BLAC. Asterisk, $P < 0.05$ (compared with BLAC of the other groups).

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and $CB1^{+/+}$ mice were observed during acute tone presentation (short-term extinction). Therefore, $CB1^{-/-}$ mice might be primarily impaired in short-term extinction, with a resulting impairment in long-term extinction, assessed in the course of the subsequent extinction trials. Accordingly, spontaneous recovery was not different between the genotypes (genotype: $F_{1,14} = 1.73$, $P = 0.208$; genotype × day interaction: $F_{4,56} = 1.19$, $P = 0.323$; Supplementary Information).

Our behavioural data clearly indicate an involvement of the endogenous cannabinoid system in extinction of aversive memories. However, the life-long absence of CB1 could result in developmental defects leading to the phenotype observed. It, furthermore, precludes any temporal dissection of the involvement of the endogenous cannabinoid system in different stages of memory formation. Thus, we treated wild-type C57BL/6J mice with the CB1 antagonist SR141716A (ref. 13), either before conditioning, or before the first extinction trial. Systemic application of SR141716A 20 min before the first extinction trial impaired both short-term and long-term extinction of the freezing response as compared with both vehicle-treated controls and animals treated with SR141716A before conditioning (treatment × time interaction: $F_{10,160} = 2.72$, $P < 0.005$), with no difference between the two latter treatments and with a similar performance of all three groups in the beginning of the first extinction trial (Fig. 2a). These data largely confirm the phenotype of $CB1^{-/-}$ mice (Fig. 1a, b, g), indicating that endocannabinoids have only a negligible function in memory acquisition, consolidation and recall (indicated by the similar performance at the beginning of the first extinction trial), but selectively interfere with extinction of the freezing response to the tone. Mice treated with SR141716A before the first extinction trial showed an attenuated extinction of freezing not only during the first tone presentation (short-term extinction) but also in the absence of pharmacological treatment during the first 60 s of tone presentation at day 6 (long-term extinction). Spontaneous recovery of the behavioural performance from the end of the first (day 1) to the beginning of the second tone presentation session (day 6) was not different among the three groups ($F_{2,34} = 0.29$, $P = 0.744$; Supplementary Information). Together, these findings support the idea that CB1 might be particularly important for the extinction of acute responses to the tone (short-term extinction), which, in turn, relates to behavioural extinction over repeated tone presentations (long-term extinction), without affecting spontaneous recovery of the behavioural performance. Accordingly, the CB1 antagonist had to be present at the time of tone presentation (that is, during aversive memory recall) in order to interfere with memory extinction, as SR141716A failed to affect extinction if administered immediately at the end of the extinction trial (data not shown) or 10 min later (Fig. 2b).

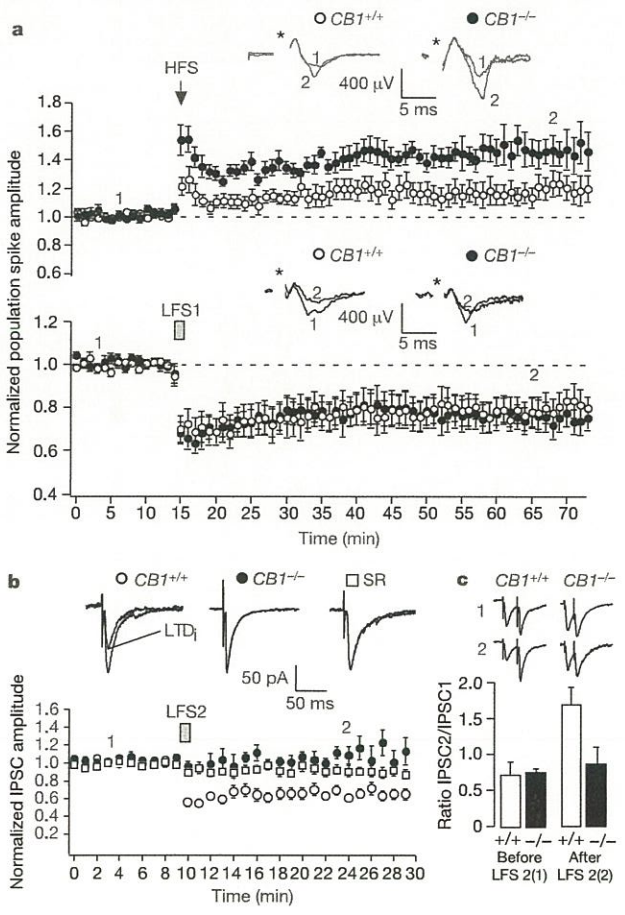

Figure 4 Endogenous cannabinoid system and synaptic plasticity in the basolateral amygdala. **a**, LTP (top) and LTD (bottom) in slices from $CB1^{+/+}$ and $CB1^{-/-}$ mice, induced by high-frequency stimulation (HFS) and low-frequency stimulation (LFS 1), respectively. Asterisks indicate stimulus artefacts. **b**, Long-term depression of IPSCs (LTD_i) requires CB1 activation. In principal neurons of slices of $CB1^{+/+}$ mice, low-frequency stimulation (LFS 2) induced a reduction of the amplitudes of isolated IPSCs. Slices of $CB1^{+/+}$ mice pre-incubated in SR141716A (SR) showed no LTD_i. LFS 2 had no effect in $CB1^{-/-}$ mice. **c**, LTD_i was accompanied by increased PPF, which was absent in $CB1^{-/-}$ mice. Insets show representative traces before and after HFS or LFS (1, 2, respectively). Means ± s.e.m. are shown.

If the endogenous cannabinoid system is activated during tone presentation, how exactly does it facilitate memory extinction? To answer this question, we performed a series of electrophysiological experiments in the BLAC of brain slices from $CB1^{-/-}$ and $CB1^{+/+}$ mice. Basic electrical properties were similar in $CB1^{-/-}$ and $CB1^{+/+}$ littermates, including input resistance and resting membrane potential (data not shown). High-frequency stimulation (HFS) in the lateral amygdala close to the external capsule induced long-term potentiation (LTP) in the basolateral amygdala of both genotypes (Fig. 4a). This effect was significantly more pronounced in $CB1^{-/-}$ than in $CB1^{+/+}$ mice (potentiation of population spike amplitude to 147 ± 11% in $CB1^{-/-}$ compared with 117 ± 8% in $CB1^{+/+}$ mice, $n = 9$, $P < 0.05$). However, we failed to affect basal synaptic transmission and LTP induction in wild-type slices superfused with SR141716A (5 μM; data not shown). This indicates that the enhanced LTP in $CB1^{-/-}$ mice might reflect long-term develop-

These observations, together with the pharmacokinetics of SR141716A (ref. 14), led us to assume that presentation of the tone during the extinction trial causes an instantaneous rise in endocannabinoid levels. To confirm this assumption, we measured in C57BL/6J mice levels of the two major endocannabinoids, anandamide (AEA) and 2-arachidonoylglycerol (2-AG), in brain punches of the medial prefrontal cortex (mPFC) and the basolateral amygdala complex (BLAC), both of which are thought to have central roles in extinction of aversive memories[9,15]. In those animals forming an association between tone and foot-shock, levels of AEA and 2-AG were significantly higher in the BLAC at the end of tone presentation of the extinction trial on day 1, as compared with animals with unpaired tone and foot-shock presentation on the previous day and with animals with paired tone and foot-shock presentation but no re-exposure to the tone (Fig. 3). There were no significant differences in levels of AEA and 2-AG in the mPFC, suggesting a specific involvement of endocannabinoids in extinction processes within the BLAC. Data of the two control groups indicate that both a successful tone–foot-shock association and re-exposure to the tone are required to trigger the acute increase of endocannabinoid levels.

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mental adaptations to life-long absence of CB1, and cannot be easily attributed to the lack of CB1 during LTP induction. Low-frequency stimulation with 900 pulses at 1 Hz (LFS 1) of the same pathway induced a persistent decrease in excitatory synaptic transmission (long-term depression, LTD) in both $CB1^{-/-}$ and $CB1^{+/+}$ mice with no difference between genotypes (depression of population spike amplitude to 75 ± 7% in $CB1^{-/-}$ compared with 80 ± 7% in $CB1^{+/+}$ mice, $n = 9$, $P > 0.05$; Fig. 4a).

As several recent studies indicate an involvement of CB1 in GABA-mediated synaptic transmission in hippocampus[16,17] and amygdala[6], we next looked for possible differences in this process within the basolateral amygdala of $CB1^{-/-}$ and $CB1^{+/+}$ mice. Low-frequency stimulation with 100 pulses at 1 Hz (LFS 2) of the lateral amygdala close to the external capsule induced a significant suppression of isolated $GABA_A$ receptor-mediated inhibitory post-synaptic currents (IPSCs) in principal neurons of the basolateral amygdala of $CB1^{+/+}$ mice. This suppression lasted for more than 20 min (hereafter called long-term depression of IPSCs, LTD_i, to 66.7 ± 5.4%, $n = 8$, $P < 0.05$; Fig. 4b). Importantly, LTD_i was blocked in $CB1^{+/+}$ mice by SR141716A (5 μM; Fig. 4b), showing an acute involvement of the endocannabinoid system in the development of LTD_i. The involvement of CB1 in LTD_i was confirmed in $CB1^{-/-}$ mice in which LTD_i was completely abolished (to 110.1 ± 13.8%, $n = 8$, $P < 0.01$ compared with $CB1^{+/+}$; Fig. 4b). Consistent with previous reports[16,17], suppression of GABA-mediated synaptic transmission also increased paired-pulse facilitation (PPF) in $CB1^{+/+}$ ($P < 0.05$) but not in $CB1^{-/-}$ mice (Fig. 4c), indicating a local CB1-dependent decrease in GABA release from axon terminals in $CB1^{+/+}$ slices.

Extinction of aversive memories is thought to be an active mnemonic process[2]. As a new memory, it shares several attributes with other steps of memory formation[9,10,18]; however, there is increasing evidence that some cellular pathways are specifically involved in extinction, but not in acquisition or consolidation of fear memories[15,19,20]. We demonstrated a specific involvement of CB1-mediated neurotransmission in extinction of aversive memories. In principle, the enhanced excitatory synaptic plasticity in $CB1^{-/-}$ mice (LTP; Fig. 4a) might explain the prolonged maintenance of aversive memories observed in these animals (Fig. 1a, b, g). However, an enhanced LTP is expected to coincide with an increased initial freezing response in the first extinction trial[21], which was not observed in $CB1^{-/-}$ mice. Accordingly, acute blockade of CB1 by a selective antagonist failed to affect LTP induction as well as acquisition and consolidation of the aversive memory. In contrast, the same approach revealed a significant involvement of CB1 in extinction (Fig. 2a). Tone-induced recall of the aversive memory was accompanied by an activation of the endocannabinoid system within the BLAC (Fig. 3), which possibly leads to a decrease of GABA-mediated transmission in a CB1-dependent manner (LTD_i; Fig. 4b, c).

The role of GABA-mediated transmission for extinction is, however, controversial[22,23]. Within the amygdala, CB1 immunoreactivity was detected in a distinct subset of GABA-containing interneurons of the BLAC[6] (one of the sites where aversive memories might be formed and stored[24]), but not in the central nucleus of the amygdala[6] (the principal output site of the amygdala[1]). Taking into consideration that principal neurons of the BLAC and neurons of the central nucleus of the amygdala might be inversely correlated in their activities[25,26], we propose that the CB1-mediated decrease of activity of local inhibitory networks within the BLAC leads to a disinhibition of principal neurons and finally to extinction of the freezing response. The selective and locally restricted inhibition of GABA-mediated transmission might not be easily reproduced by systemic administration of GABA-interfering drugs[22,23]. Thus, future studies will have to confine such treatments to the BLAC to validate that CB1-mediated inhibition of GABA-mediated transmission is indeed crucially involved in the extinction of aversive memories mediated by CB1. It remains to be shown whether CB1 is not only involved in extinction of aversive memories but also in adaptation to aversive situations in general and/or in extinction of memories, independently from their emotional value.

Overall, our findings suggest that the endogenous cannabinoid system could represent a therapeutic target for the treatment of diseases associated with inappropriate retention of aversive memories or inadequate responses to aversive situations, such as post-traumatic stress disorders[2], phobias, and certain forms of chronic pain[11]. □

Methods

Animals

Adult male C57BL/6JOlaHsd mice (6–8 weeks; Harlan–Winkelmann) and male $CB1^{-/-}$ and $CB1^{+/+}$ littermates (10–16 weeks; see Supplementary Information) were housed individually with an inverse 12/12 h light/dark cycle (lights off at 8:00) for at least 2 weeks before starting the experiments.

Behavioural studies

Experimental procedures were approved by the Committee on Animal Health and Care of local Government. Experiments were performed between 9:00 and 14:00. Animal's behaviour was analysed in a blind fashion with regards to genotype and drug treatment. Data were analysed by analysis of variance (ANOVA) followed by Fisher's least significant difference test for planned comparisons, Mann-Whitney *U*-test or unpaired Student's *t*-test. A *P*-value of <0.05 was considered statistically significant. Experimental procedures for pain threshold and unconditioned freezing, elevated plus maze and open field are described in Supplementary Information.

Fear conditioning

For conditioning, animals were placed into conditioning chambers (MED Associates). After 3 min, a 20-s tone (9 kHz, 80 dB) was presented that co-terminated with a 2-s electric foot-shock (0.7 mA). In pharmacological experiments animals received a 1-s shock to avoid ceiling effects in the freezing response due to the combination of foot-shock and injection stress. Animals were returned to their home cages 60 s after shock application. At the given time points after conditioning, animals were placed into transparent plexiglas cylinders that differed from the conditioning context, and a 60-s or 180-s tone was presented 3 min later (extinction trials). Animals were returned to their home cages after another 60 s. Mice were experimentally naive except for the stronger extinction protocol, where they had been tested on the elevated plus maze 5 days before. Freezing behaviour (defined as the absence of all movements except for respiration) was quantified from videotapes by trained observers that were blind to genotype and drug treatment, and data were normalized to the respective observation periods.

Pharmacological treatment

SR141716A (NIMH Chemical Synthesis and Drug Supply Program) was dissolved in vehicle solution (1 drop of Tween-80 in 3 ml 2.5% dimethylsulphoxide in saline). SR141716A (3 mg per kg body weight) and vehicle were injected subcutaneously at 20 ml per kg body weight under light isofluran anaesthesia.

Measurement of endocannabinoids

C57BL/6JOlaHsd mice were randomly assigned to three groups ($n = 20$ each). On the conditioning day, two groups were conditioned as described before (paired). The remaining group received the foot-shock first and a 20 s tone 3 min later (unpaired). On the next day, all animals were placed into the cylinders, but only one of the paired groups and the unpaired group were exposed to a 3-min tone. Immediately after the end of the tone (or equivalent time in cylinder), animals were killed, brains were quickly removed and snap-frozen in isopentane/dry ice. mPFC and BLAC were punched from the frozen brain using a cryocut and cylindric brain punchers (Fine Science Tools, internal diameter 2.0 mm and 0.8 mm, respectively). Length of punches was approximately 1.6 mm for mPFC (start: bregma +2.8 mm[27]) and 1.2 mm for BLAC (start: bregma −1.0 mm[27]). Brain tissue of mPFC and bilateral BLAC, respectively, of 5 mice was pooled to obtain a single data point. Tissues (10–15 mg per data point) were dounce-homogenized with chloroform/methanol/Tris-HCl 50 mM, pH 7.4 (1/1/1 by volume) containing 5 pmol of octa-deuterated (d_8)-anandamide and 50 pmol of d_8-2-arachidonoylglycerol (Cayman Chemicals) as internal standards. Lipid-containing organic phase was dried down, weighed and pre-purified by open-bed chromatography on silica gel, and analysed by liquid chromatography-atmospheric pressure chemical ionization-mass spectrometry (LC-APCI-MS) using a Shimadzu high-performance liquid chromatography (HPLC) apparatus (LC-10ADVP) coupled to a Shimadzu quadrupole mass spectrometer (LCMS-2010) via a Shimadzu APCI interface. Mass spectrometry analyses were carried out in the selected ion-monitoring (SIM) mode as described previously[28]. Temperature of the APCI source was 400 °C; HPLC column was a Phenomenex (5 μm, 150 × 4.5 mm) reverse phase column, eluted as described[28]. Anandamide (retention time of 14.5 min) and 2-AG (retention time of 17.0 min) quasi-molecular ions were quantified by isotope dilution with the above-mentioned deuterated standards[28] and their amounts in pmols normalized per mg of lipid extract. Data were statistically evaluated by ANOVA.

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Electrophysiology

Brain slices were prepared essentially as described[29]. IPSCs and population spikes were evoked by square pulse stimuli (0.066 Hz, 5–12 mA, 200 μs) delivered by means of bipolar tungsten electrodes positioned within the lateral amygdala close to the external capsule. Population spikes were recorded in the basolateral amygdala close to lateral amygdala using glass microelectrodes (2–3 MΩ) filled with artificial cerebrospinal fluid (ACSF)[29]. HFS (five trains at 100 Hz for 1 s, 10-s interstimulus interval) was applied to induce LTP, and LFS1 (900 pulses at 1 Hz) was applied to induce LTD. Whole-cell GABA-mediated currents were isolated by adding NBQX (0.005 mM) and D-(-)-2-amino-5-phosphopentanoic acid (AP5; 0.05 mM) to ACSF (bubbled with 95% O_2/5% CO_2; pH 7.3), and were recorded from visually identified somata of principal neurons of the basolateral amygdala[30] by glass electrodes (4.5–5 MΩ)[16] containing (in mM): Mg-ATP 2, $CsCH_3SO_3$ 100, CsCl 60, EGTA 0.2, HEPES 10, $MgCl_2$ 1, QX314 5 and Na_3GTP 0.3 (pH 7.3). Patch clamp experiments were performed at 24 ± 1 °C at a holding potential of −70 mV. LTD_1 was induced by 100 stimuli at 1 Hz (LFS 2). PPF was induced as described[30]. Data are expressed as means ± s.e.m. We tested significance using the Student's *t*-test.

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1. LeDoux, J. E. Emotion circuits in the brain. *Annu. Rev. Neurosci.* **23,** 155–184 (2000).
2. Davis, M., Falls, W. A. & Gewirtz, J. in *Contemporary Issues in Modeling Psychopathology* (eds Myslobodsky, M. S. & Weiner, I.) 113–141 (Kluwer Academic, Norwell, 2000).
3. Matsuda, L. A., Lolait, S. J., Brownstein, M. J., Young, A. C. & Bonner, T. I. Structure of a cannabinoid receptor and functional expression of the cloned cDNA. *Nature* **346,** 561–564 (1990).
4. Di Marzo, V., Melck, D., Bisogno, T. & De Petrocellis, L. Endocannabinoids: endogenous cannabinoid receptor ligands with neuromodulatory action. *Trends Neurosci.* **21,** 521–528 (1998).
5. Marsicano, G. & Lutz, B. Expression of the cannabinoid receptor CB1 in distinct neuronal subpopulations in the adult mouse forebrain. *Eur. J. Neurosci.* **11,** 4213–4225 (1999).
6. Katona, I. *et al.* Distribution of CB1 cannabinoid receptors in the amygdala and their role in the control of GABAergic transmission. *J. Neurosci.* **21,** 9506–9518 (2001).
7. Hampson, R. E. & Deadwyler, S. A. Role of cannabinoid receptors in memory storage. *Neurobiol. Dis.* **5,** 474–482 (1998).
8. Reibaud, M. *et al.* Enhancement of memory in cannabinoid CB1 receptor knock-out mice. *Eur. J. Pharmacol.* **379,** R1–R2 (1999).
9. Falls, W. A., Miserendino, M. J. & Davis, M. Extinction of fear-potentiated startle: blockade by infusion of an NMDA antagonist into the amygdala. *J. Neurosci.* **12,** 854–863 (1992).
10. Lu, K. T., Walker, D. L. & Davis, M. Mitogen-activated protein kinase cascade in the basolateral nucleus of amygdala is involved in extinction of fear-potentiated startle. *J. Neurosci.* **21,** RC162 (2001).
11. Pertwee, R. G. Cannabinoid receptors and pain. *Prog. Neurobiol.* **63,** 569–611 (2001).
12. Rodriguez de Fonseca, F., Del Arco, I., Martin-Calderon, J. L., Gorriti, M. A. & Navarro, M. Role of the endogenous cannabinoid system in the regulation of motor activity. *Neurobiol. Dis.* **5,** 483–501 (1998).
13. Rinaldi-Carmona, M. *et al.* SR141716A, a potent and selective antagonist of the brain cannabinoid receptor. *FEBS Lett.* **350,** 240–244 (1994).
14. Petitet, F., Jeantaud, B., Bertrand, P. & Imperato, A. Cannabinoid penetration into mouse brain as determined by *ex vivo* binding. *Eur. J. Pharmacol.* **374,** 417–421 (1999).
15. Morgan, M. A., Romanski, L. M. & LeDoux, J. E. Extinction of emotional learning: contribution of medial prefrontal cortex. *Neurosci. Lett.* **163,** 109–113 (1993).
16. Wilson, R. I. & Nicoll, R. A. Endogenous cannabinoids mediate retrograde signalling at hippocampal synapses. *Nature* **410,** 588–592 (2001).
17. Ohno-Shosaku, T., Maejima, T. & Kano, M. Endogenous cannabinoids mediate retrograde signals from depolarized postsynaptic neurons to presynaptic terminals. *Neuron* **29,** 729–738 (2001).
18. Santini, E., Muller, R. U. & Quirk, G. I. Consolidation of extinction learning involves transfer from NMDA-independent to NMDA-dependent memory. *J. Neurosci.* **21,** 9009–9017 (2001).
19. El Ghundi, M., O'Dowd, B. F. & George, S. R. Prolonged fear responses in mice lacking dopamine D1 receptor. *Brain Res.* **892,** 86–93 (2001).
20. Quirk, G. J., Russo, G. K., Barron, J. L. & Lebron, K. The role of ventromedial prefrontal cortex in the recovery of extinguished fear. *J. Neurosci.* **20,** 6225–6231 (2000).
21. Tang, Y. P. *et al.* Genetic enhancement of learning and memory in mice. *Nature* **401,** 63–69 (1999).
22. Harris, J. A. & Westbrook, R. F. Evidence that GABA transmission mediates context-specific extinction of learned fear. *Psychopharmacology (Berl.)* **140,** 105–115 (1998).
23. McGaugh, J. L., Castellano, C. & Brioni, J. Picrotoxin enhances latent extinction of conditioned fear. *Behav. Neurosci.* **104,** 264–267 (1990).
24. Medina, J. F., Repa, C. J., Mauk, M. D. & LeDoux, J. E. Parallels between cerebellum- and amygdala-dependent conditioning. *Nature Rev. Neurosci.* **3,** 122–131 (2002).
25. Collins, D. R. & Paré, D. Reciprocal changes in the firing probability of lateral and central medial amygdala neurons. *J. Neurosci.* **19,** 836–844 (1999).
26. Royer, S., Martina, M. & Paré, D. An inhibitory interface gates impulse traffic between the input and output stations of the amygdala. *J. Neurosci.* **19,** 10575–10583 (1999).
27. Franklin, K. B. J. & Paxinos, G. *The Mouse Brain* (Academic, San Diego, 1997).
28. Di Marzo, V. *et al.* Levels, metabolism, and pharmacological activity of anandamide in CB(1) cannabinoid receptor knockout mice: evidence for non-CB(1), non-CB(2) receptor-mediated actions of anandamide in mouse brain. *J. Neurochem.* **75,** 2434–2444 (2000).
29. Rammes, G. *et al.* Synaptic plasticity in the basolateral amygdala in transgenic mice expressing dominant-negative cAMP response element-binding protein (CREB) in forebrain. *Eur. J. Neurosci.* **12,** 2534–2546 (2000).
30. Rammes, G., Eder, M., Dodt, H.-U., Kochs, E. & Zieglgänsberger, W. Long-term depression in the basolateral amygdala of the mouse involves the activation of interneurons. *Neuroscience* **107,** 85–97 (2001).

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Competing interests statement

The authors declare that they have no competing financial interests.

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A transcription factor response element for gene expression during circadian night

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Mammalian circadian clocks consist of complex integrated feedback loops[1–10] that cannot be elucidated without comprehensive measurement of system dynamics and determination of network structures[11]. To dissect such a complicated system, we took a systems-biological approach based on genomic, molecular and cell biological techniques. We profiled suprachiasmatic nuclei and liver genome-wide expression patterns under light/dark cycles and constant darkness. We determined transcription start sites of human orthologues for newly identified cycling genes and then performed bioinformatical searches for relationships between time-of-day specific expression and transcription factor response elements around transcription start sites. Here we demonstrate the role of the Rev-ErbA/ROR response element in gene expression during circadian night, which is in phase with *Bmal1* and in antiphase to *Per2* oscillations. This role was verified using an *in vitro* validation system, in which cultured fibroblasts transiently transfected with clock-controlled reporter vectors exhibited robust circadian bioluminescence[12].

To perform comprehensive measurement of mammalian circadian gene expression, we profiled genome-wide expression patterns of central (suprachiasmatic nuclei, SCN) and peripheral (liver) clocks every four hours during light/dark cycles (LD) or constant darkness (DD) over two days. We extracted total RNA from 50 pooled SCNs and four pooled livers at each time point, prepared biotinylated complementary RNA and used an Affymetrix mouse high-density oligonucleotide probe array (GeneChip) to determine SCN and liver gene expression.

The data obtained were analysed through two statistical cosine filters, one for LD and the other for DD time courses (see

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Behavioral/Systems/Cognitive

Cannabinoid CB1 Receptor Mediates Fear Extinction via Habituation-Like Processes

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The interplay between fear expression and fear extinction provides an important prerequisite for adequate coping with aversive encounters. Current models propose that extinction of conditioned fear is mediated by associative safety learning. Here, we demonstrate that the cannabinoid CB1 receptor, which is crucially involved in fear extinction, is dispensable for associative safety learning. In fact, our results indicate that CB1 mediates fear extinction primarily via habituation-like processes. CB1 null-mutant mice were severely impaired not only in extinction of the fear response to a tone after fear conditioning but also in habituation of the fear response to a tone after sensitization with an inescapable footshock. Surprisingly, long-term habituation was generally affected even in situations with proper short-term adaptation, suggesting the existence of two separated CB1-dependent effector systems for short- and long-term fear adaptation. Our findings underscore the importance of habituation as a determinant of fear extinction in mice and characterize the cannabinoid CB1 receptor as an essential molecular correlate of this process.

Key words: **sensitization; fear conditioning; anxiety; memory; endocannabinoids; stress**

Introduction

In laboratory animals, a discrete stimulus (e.g., a tone) can elicit fear reactions (e.g., freezing) in multiple ways: for example, a tone may trigger an innate fear response in naive animals once its intensity exceeds a certain threshold (Ohman and Mineka, 2001; Lamprea et al., 2002). This threshold critically depends on the life history of the animals, because previous aversive encounters (e.g., a footshock) are able to sensitize animals to tones (Kamprath and Wotjak, 2004). Sensitization is a nonassociative learning process characterized by the general increase in responsiveness to potentially harmful stimuli after an aversive/stressful experience. Accordingly, inescapable footshocks result in long-lasting alterations in behavioral and endocrine parameters (Van Dijken et al., 1992a,b, 1993). On the other hand, if a tone was explicitly paired with a punishment, as done in fear conditioning paradigms, reexposure to the tone activates the memory of the tone–punishment association and thus causes a conditioned freezing response (LeDoux, 2000; Maren and Quirk, 2004). In a typical fear-conditioning task, however, associative learning and sensitization (caused by application of an inescapable footshock during fear conditioning) may occur in parallel, with the consequence that freezing responses of conditioned mice to the tone are determined by both associative and nonassociative memory components (Kamprath and Wotjak, 2004).

In an auditory fear-conditioning task, not only fear acquisition but also fear extinction can be achieved by associative and/or nonassociative learning processes. On the one hand, animals might form an association between the tone and the nonappearance of the predicted punishment (safety learning) that suppresses expression of the memory of the tone–shock association in a process called extinction (for review, see Myers and Davis, 2002). On the other hand, repeated nonreinforced tone presentations may decrease the responsiveness to the tone in the stimulus–response pathways because of habituation-like processes (Thompson and Spencer, 1966; Groves and Thompson, 1970; McSweeney and Swindell, 2002; Kamprath and Wotjak, 2004). In the recent years, molecular correlates of extinction were identified (for review, see Myers and Davis, 2002). Among them, the cannabinoid CB1 receptor (CB1) (for review, see Di Marzo et al., 2004) plays a special role, because its implication in extinction seems to be restricted to aversive test conditions: whereas genetic ablation or pharmacological blockade of CB1 impairs the extinction of fear memories (Marsicano et al., 2002; Suzuki et al., 2004; Chhatwal et al., 2005) and spatial memories acquired under stressful conditions (Varvel and Lichtman, 2002; Varvel et al., 2005), CB1 does not seem to be important for memory extinction in operant conditioning tasks involving positive reinforcement (Holter et al., 2005). One explanation might be that CB1 is not involved in associative extinction learning but rather plays a role in nonassociative learning processes (e.g., habituation), which contribute to the decrease in the fear response (Kamprath and

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Wotjak, 2004). Here, we tested this hypothesis by examining the role of CB1 in extinction and habituation of acquired fear responses. Our results point to an involvement of endocannabinoids in the modulation of habituation-like processes during fear extinction, whereas CB1 seems to be dispensable for associative safety learning.

Materials and Methods

Animals

All experiments were approved by the Committee on Animal Health and Care of the State of Bavaria (Regierung von Oberbayern) and performed in strict compliance with the European Union recommendations for the care and use of laboratory animals (86/609/CEE).

A total of 118 male cannabinoid CB1 receptor null-mutant mice ($CB1^{-/-}$), 135 male wild-type littermate controls ($CB1^{+/+}$), and 34 male C57BL/6NCrl mice (Charles River, Sulzfeld, Germany) were included in the experiments. $CB1^{-/-}$ and $CB1^{+/+}$ derived from heterozygous breeding pairs [backcrossed to C57BL/6NCrl to F5/F6 generation (Marsicano et al., 2002)] that were kept in the vivaria of the Forschungszentrum für Umwelt und Gesundheit (Neuherberg, Germany) and the Max Planck Institute of Psychiatry in Munich, Germany. Mice were genotyped after weaning and regenotyped at the end of the experiments as described previously (Marsicano et al., 2002).

All animals were separated at the age of 6–14 weeks and kept singly in standard macrolon type II cages with sawdust bedding (Altromin Faser Einstreu; Altromin, Lage-Lippe, Germany), water and food *ad libitum*, at 22 ± 2°C room temperature and 55 ± 5% humidity, under an inverse 12 h light/dark cycle (lights off at 8:00 A.M.) for at least 14 d before starting the experiment.

Experiments

All experiments were performed during the activity phase (dark phase) of the animals between 9:30 A.M. and 5:00 P.M. Experiments were designed to minimize the number of animals tested. Animals of a given experiment derived from the same batch and were tested simultaneously. Experiments were performed over the course of 3 years.

Conditioning, sensitization, backward conditioning, and unconditioned freezing. Setups and procedures were essentially the same as described previously (Kamprath and Wotjak, 2004). Briefly, two different contexts were used (conditioning chamber and test context) that differed considerably in material, shape, surface texture, bedding, and odor of the cleaning solutions. Mice received the footshocks via metal grid floors in the conditioning chamber (MED Associates, St. Albans, VT). Tones were generated by audio stimulus generators (MED Associates). The animals' behavior was videotaped.

For conditioning, mice were placed in the conditioning context. Three minutes later, a tone (80 dB, 9 kHz sine wave, 10 ms rising and falling time) was presented to the animals for 20 s that coterminated with a 2 s scrambled electric footshock of 0.7 mA. Mice were returned to their home cages 60 s later. For sensitization, mice were treated similarly to the conditioning procedure, except for the fact that only the 2 s footshock (0.7 mA) was presented, but not the tone. For backward conditioning, tone and footshock were presented in reverse order compared with the conventional conditioning procedure (i.e., mice perceived a 2 s footshock of 0.7 mA 198 s after insertion into the conditioning chamber that was immediately followed by a 20 s tone).

To measure the freezing response to the tone without confounding influences of contextual memory, sensitized and conditioned mice were placed into a neutral environment (test context), and the house light was switched on. Three minutes later, a 3 min tone was presented (9 kHz; 80 dB if not stated otherwise). Mice were returned to their home cages 60 s after the end of tone presentation. For measuring the innate fear response to the tone, naive mice underwent the same test procedure, but with a tone intensity of 95 dB without previous sensitization/conditioning procedures.

Recording of auditory-evoked potentials. $CB1^{-/-}$ ($n = 12$) and $CB1^{+/+}$ ($n = 12$) were chronically equipped with a bipolar recording electrode aimed at the CA1 region of the dorsal hippocampus essentially as described previously (Tang et al., 2003). A recovery period of 14 d was followed by a test period, in which the optimal tone intensity for auditory-evoked potential recordings was defined for each animal and tone frequency (6 or 12 kHz). Furthermore, the stability of responses was assessed. Animals not showing stable auditory-evoked potentials were excluded from the experiment. Test recordings were followed by the experimental period, which consisted of 2 d of baseline recording, the conditioning day without recording, and 2 d of post-conditioning recording (compare Fig. 2*A* for the temporal schedule). At each recording day, mice were slightly anesthetized with isoflurane to connect them to the recording device (Tang et al., 2003) and placed into the test context. After a recovery period of 30 min, auditory-evoked potentials were triggered by series of 90 50 ms tones of either 6 or 12 kHz presented at one tone per second. The two tone series were separated by a 3 min interval. Tones of 6 kHz always preceded the tones of 12 kHz. Three days before conditioning (day −3), intensity–response curves were established for each animal and each tone frequency. Afterward, the intensity of the tones was adjusted individually to cause a 40–50% maximal excitatory field potential in the subsequent baseline recordings and kept constant throughout the experiment. Basal responses were recorded at days −2 and −1 before conditioning by presenting a single series of 6 and 12 kHz tones. If the basal responses were stable, mice were conditioned the next day (day 0). For each mouse, one of the two tone frequencies was chosen for the conditioned stimulus (CS) in a counterbalanced manner per genotype. The CS+ (paired tone) consisted of a series of 20 50 ms tones of the same characteristics as used for the baseline recordings. The CS+ coterminated with a 1 s footshock of 0.7 mA. The conditioning procedure was repeated two more times. We have made an effort to minimize the number of tone–shock pairings as well as the intensity of the shock. In our experience (J. Tang and C. T. Wotjak, unpublished observations), the protocol used here is the weakest procedure that consistently caused a potentiation of auditory-evoked potentials in wild-type mice. Most other experiments aimed at measuring learning-induced changes in auditory-evoked potentials used 5–10 tone–shock pairings (Rogan et al., 1997; Tang et al., 2001, 2003). For post-conditioning recordings, mice were transferred back to the electrophysiology laboratory, and the baseline procedure was repeated in the test context.

For data analysis, the 90 auditory-evoked potentials evoked by the 50 ms tones were averaged per tone series and recording day. Averaged auditory-evoked potentials were analyzed off-line by measuring peak latencies, amplitudes, and slopes of the most negative components as described previously (Tang et al., 2003). Data were normalized to the averaged preconditioning values (baseline, 100%) separately per animal and tone frequency.

After completion of the experiment, mice were deeply anesthetized with pentobarbital (100 mg/kg), and an anodal current (200 μA, 4 s) was passed through the tungsten wire to identify the electrode placement. Approximately 15 min later, animals were killed by an overdose of isoflurane and their brains were removed. Electrode placement was assessed in 20 μm cryosections stained with cresyl violet. Animals with a misplaced recording electrode were excluded from analysis.

Drugs. SR141716A [Rimonabant, 3 mg/kg; kindly provided by the National Institute of Mental Health (Bethesda, MD) Chemical Synthesis and Drug Supply Program] was dissolved in vehicle solution (2.5% DMSO and 1 drop of Tween 80 per 3 ml of saline) and injected subcutaneously at 20 ml/kg body weight 30 min before the behavioral test. Injections were given under light isoflurane anesthesia.

Analyses

Behavioral analysis. The behavior of the mice was videotaped and scored off-line by trained observers that were blind to the animals' treatment as described previously (Kamprath and Wotjak, 2004). Freezing was defined as the absence of all movements, except for those related to respiration.

Statistical analysis. Data were summarized to distinct intervals, as indicated in the text or the figure legends, and normalized to the respective time. If not stated otherwise, data were analyzed by two-way (genotype/drug, interval) ANOVA for repeated measures (interval) separately per test day (analysis of the freezing data in 20 s intervals) or by two-way ANOVA (genotype/drug, day) for repeated measures (day; analysis of the

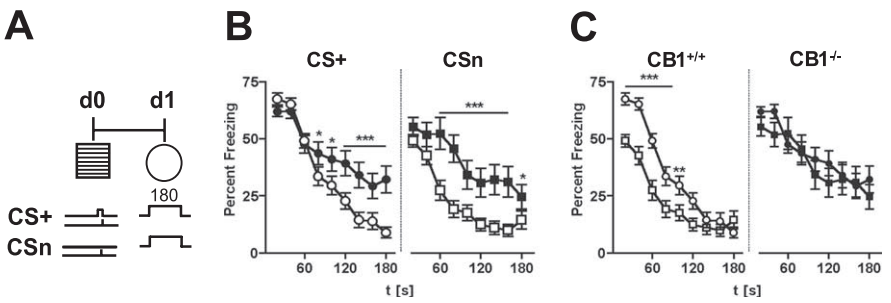

Figure 1. $CB1^{-/-}$ mice are impaired in within-session extinction and adaptation, but not in acquisition, of conditioned and sensitized fear. ***A***, $CB1^{-/-}$ and $CB1^{+/+}$ littermate controls were either conditioned with a single tone–shock pairing or sensitized with a footshock only in the conditioning chamber. All animals were exposed to a 180 s tone in a neutral environment 24 h after the conditioning (CS+) and the sensitization (CSn) procedure, respectively. d0, Day 0; d1, day 1. ***B***, Both conditioned $CB1^{-/-}$ (●, $n = 36$) and sensitized $CB1^{-/-}$ (■, $n = 20$) showed a sustained freezing response over the course of the 180 s tone presentation compared with the respective $CB1^{+/+}$ (○, $n = 37$; □, $n = 26$). ***C***, As revealed by comparison of the freezing responses with the tone after conditioning (CS+; ●, ○) and sensitization procedures (CSn; ■, □) separately per genotype, conditioned $CB1^{+/+}$ showed a stronger freezing response than sensitized $CB1^{+/+}$, whereas conditioned and sensitized $CB1^{-/-}$ froze at the same level. Data were normalized to the 20 s observation intervals (mean ± SEM). $*p < 0.05$, $**p < 0.01$, $***p < 0.001$ versus $CB1^{+/+}$ (***B***) or sensitized $CB1^{+/+}$ (***C***). CS+, Tone after conditioning procedure; CSn, tone after sensitization procedure.

freezing data averaged over the entire 3 min observation period). *Post hoc* comparisons were performed by the Newman–Keuls test, if appropriate. Statistical significance was accepted if $p < 0.05$. For the sake of clarity and brevity, only relevant results of the statistical analyses are reported. Statistical analyses were performed by specialized software [GraphPad Prism 3.0 (GraphPad, San Diego, CA), Statistica 5.0 (StatSoft, Tulsa, OK), and SPSS version 11.0 (SPSS, Chicago, IL)].

Results

We refer to procedures with tone–shock pairings as conditioning procedures, with reverse order of shock and tone presentation as backward conditioning ("safety learning") and with shock presentation only as sensitization procedures. The term "sensitization" describes the general increase in responsiveness to potentially harmful stimuli after perception of a punishment, on the basis of nonassociative learning processes. Accordingly, we call mice that underwent sensitization procedures sensitized mice and mice that underwent conditioning procedures conditioned mice. The freezing response of naive (i.e., nonshocked) mice to a tone is called unconditioned freezing or innate fear response. Fear adaptation generally describes the development of the fear response to a tone. More specifically, the decrease in freezing over the course of tone presentation is called within-session extinction after conditioning and within-session adaptation after sensitization procedures. Accordingly, the decrease in freezing from the first to a second tone presentation is called long-term extinction and long-term adaptation/habituation, respectively.

$CB1^{-/-}$ mice are impaired in within-session extinction and adaptation, but not in acquisition, of conditioned and sensitized fear

CB1 plays a crucial role in extinction of the freezing response after fear-conditioning procedures (Marsicano et al., 2002). We wondered whether CB1 is involved in processing of associative or nonassociative memory components that determine extinction of the freezing response of conditioned mice (Kamprath and Wotjak, 2004). Because sensitization of a behavioral response is thought to be a nonassociative learning process that is diminished by counteracting nonassociative adaptation processes (Kamprath and Wotjak, 2004), we compared the tone-induced freezing responses of mice with genetic ablation of CB1 [$CB1^{-/-}$ (cf. Marsicano et al., 2002)] with that of their wild-type littermate controls ($CB1^{+/+}$) after conditioning and sensitization procedures.

For conditioning, $CB1^{-/-}$ and $CB1^{+/+}$ received a 0.7 mA footshock in the conditioning chamber in association with a 20 s, 80 dB tone. On the next day, mice were re-exposed to the same tone in a novel environment (test context) for 3 min (Fig. 1*A*). $CB1^{-/-}$ showed a prolonged freezing reaction to the tone compared with $CB1^{+/+}$ (genotype: $F_{(1,71)} = 4.0$, $p = 0.049$; genotype-by-interval interaction: $F_{(8,568)} = 9.5$, $p < 0.0001$) (Fig. 1*B*), thus confirming our previous observation of impaired within-session extinction in $CB1^{-/-}$ (Marsicano et al., 2002; Cannich et al., 2004). It is of note that both genotypes showed similar freezing in the very beginning of the first nonreinforced tone presentation after the conditioning procedure pointing to a normal acquisition of fear conditioning in $CB1^{-/-}$ (Marsicano et al., 2002).

For sensitization, $CB1^{-/-}$ and $CB1^{+/+}$ received a 0.7 mA footshock in the conditioning chamber without any tone presentation. Mice were subsequently exposed to a 3 min tone in a novel environment (test context) on the next day, similarly to conditioned mice (Fig. 1*A*). Again, $CB1^{-/-}$ mice showed a prolonged and stronger freezing reaction to the tone compared with $CB1^{+/+}$ (genotype: $F_{(1,44)} = 9.7$, $p = 0.003$; genotype-by-interval interaction: $F_{(8,352)} = 3.5$, $p < 0.001$) (Fig. 1*B*). Importantly, both $CB1^{-/-}$ and $CB1^{+/+}$ showed the same levels of freezing at the beginning of tone presentation, indicating that CB1 is not involved in the sensitization process per se. Taking into consideration that the freezing response to a tone after inescapable footshocks is primarily determined by sensitization and nonassociative adaptation processes, the sustained freezing response of $CB1^{-/-}$ over the course of tone presentation suggests that CB1 deficiency delays fear adaptation in a nonassociative manner.

To estimate the contribution of associative memory components to the freezing response of the animals, we compared the freezing response of conditioned and sensitized mice separately per genotype. As expected, conditioned $CB1^{+/+}$ showed significantly more freezing to the tone than sensitized $CB1^{+/+}$ (CS: $F_{(1,61)} = 8.8$, $p = 0.004$; CS-by-interval interaction: $F_{(8,488)} = 8.2$, $p < 0.0001$) (Fig. 1*C*), indicating that fear conditioning comprises the parallel processing of a stimulus–punishment association and nonassociative sensitization with the consequence that associative and nonassociative memory components together determine the freezing response after conditioning procedures (Kamprath and Wotjak, 2004). In contrast, $CB1^{-/-}$ showed a similar freezing response to a tone, no matter whether they had received a shock only or whether the tone had been paired with a shock before (CS: $F_{(1,54)} = 0.3$, $p = 0.582$; CS-by-interval interaction: $F_{(8,432)} = 1.6$, $p = 0.117$) (Fig. 1*C*). At first sight, these data imply that $CB1^{-/-}$ were not able to associate tone and footshock during the conditioning procedure, with the consequence that their freezing response was solely determined by nonassociative memory components. However, an intact tone–shock association in $CB1^{-/-}$ cannot be entirely ruled out by behavioral data alone, because an impaired adaptation of nonassociative memory com-

ponents might simply have masked differences in freezing between conditioned and sensitized CB1 $^{-/-}$. Therefore, we decided to investigate whether CB1 $^{-/-}$ are able to form an associative memory at all by electrophysiological means. To this end, we recorded auditory-evoked potentials in the CA1 region of the dorsal hippocampus of freely behaving CB1 $^{-/-}$ and CB1 $^{+/+}$. Evidence exists that conditioning-related changes in auditory-evoked potentials recorded from the lateral amygdala and the CA1 region are exclusively determined by the associative history of the CS (Goosens et al., 2003; Tang et al., 2003). Because both brain structures show a high synchronicity in memory-related changes in neuronal activity (Seidenbecher et al., 2003; Tang et al., 2003), we targeted the more easily accessible CA1 region instead of the lateral amygdala. Moreover, we confirmed that recall of auditory cued fear memories activates the endocannabinoid system not only within the amygdala (Marsicano et al., 2002) but also within the dorsal hippocampus (supplemental Fig. 1, available at www.jneurosci.org as supplemental material).

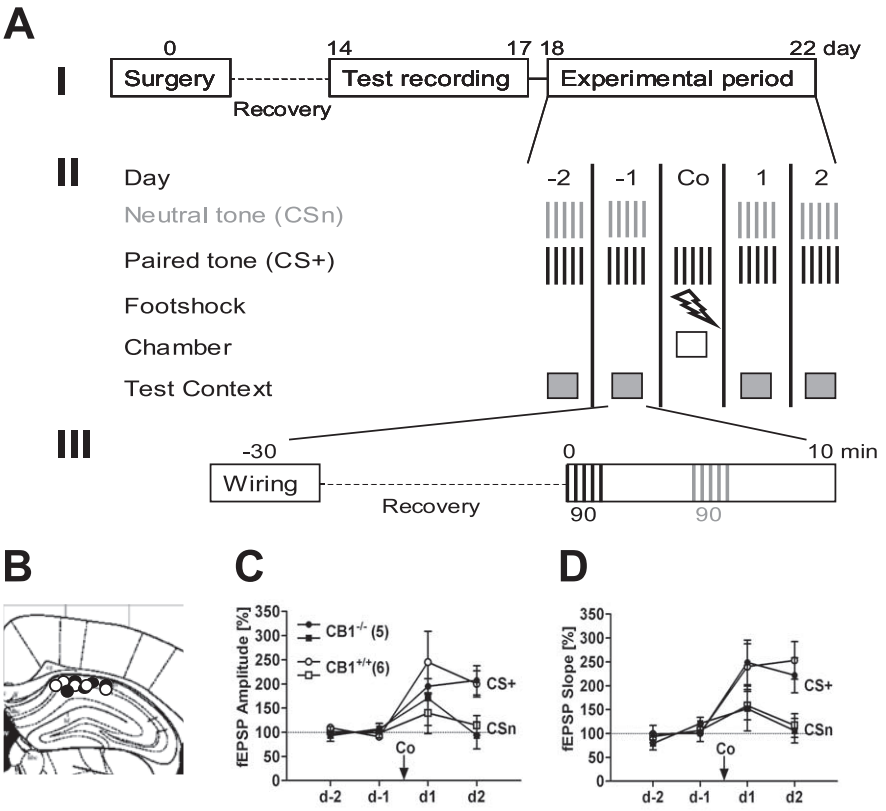

Figure 2. CB1 deficiency does not affect the formation of associative fear memories. ***A***, Experimental procedure of the discriminatory fear-conditioning task. ***I***, General timetable of the experiments. CB1 $^{-/-}$ ($n = 5$) and CB1 $^{+/+}$ ($n = 6$) were chronically equipped with a recording electrode aimed at the CA1 region of the dorsal hippocampus. After a recovery period of 14 d, the optimal tone intensity for auditory-evoked potential recordings (~40% of maximal response) was defined per animal and tone frequency (6 and 12 kHz), followed by measurement of the stability of the responses (test recording). ***II***, Schematic representation of the procedures used in the experimental period. Conditioning was performed after 2 d of baseline recordings. At the conditioning day, a series of 20 50 ms tones of either 6 or 12 kHz coterminated with an electric footshock (flash) in the conditioning chamber. The conditioning procedure was repeated two times. Tone series of the frequency used for the conditioning procedures were termed paired tones, and those of the other frequency, which was not presented at the conditioning day, were termed neutral tones. Tone frequencies for the conditioning procedure were counterbalanced among the animals of a given genotype. During the two preconditioning and the two postconditioning days, paired and neutral tones were presented in the neutral test context. ***III***, Time schedule for a recording day. Animals were slightly anesthetized with isoflurane to connect the electrode assembly to the recording hardware. After a 27 min recovery period, the video recorder was switched on. Three minutes later, two 90 tone series, one of 6 kHz and the other of 12 kHz, were presented at a 3 min interval. ***B***, Schematic brain section according to Franklin and Paxinos (1997) showing the correct placement of the electrodes within the CA1 region of the dorsal hippocampus in CB1 $^{-/-}$ (●) and CB1 $^{+/+}$ (○) ***C***, ***D***, Both amplitude (***C***) and slope (***D***) of the most-negative-going component of the auditory-evoked potentials were potentiated for the paired tone (●, ○) but not for the neutral tone (■, □) after conditioning. Data (mean ± SEM) were normalized individually to the averaged preconditioning responses (dotted line, 100%). ■ and ●, CB1 $^{-/-}$; □ and ○, CB1 $^{+/+}$. Co, Conditioning day; CS+, paired tones; CSn, neutral tones; fEPSP, field EPSP.

Potentiation of auditory-evoked potentials after auditory fear conditioning points to normal memory of the tone–shock association in CB1 $^{-/-}$ mice

Mice were chronically equipped with a recording electrode aimed at the CA1 region of the dorsal hippocampus and tested in a discriminatory fear-conditioning task (Fig. 2*A*). At first, both 6 and 12 kHz tones were presented to the animals at 2 consecutive days to record a stable baseline. For conditioning, only one of these frequencies was used (i.e., series of either 6 or 12 kHz tones were paired with a footshock). At the 2 d after conditioning, series of both 6 and 12 kHz tones were presented to the mice to assess frequency-specific potentiation of auditory-evoked potentials.

Of the 24 animals equipped with electrodes, 11 showed stable responses to the tone series with correct placement of the electrode in the CA1 region of the dorsal hippocampus (Fig. 2*B*). During test recordings, CB1 $^{-/-}$ ($n = 5$) and CB1 $^{+/+}$ ($n = 6$) showed a similar sensitivity to 6 and 12 kHz tones of increasing intensity (data not shown). Furthermore, there were no genotype differences in basic characteristics of the auditory-evoked potentials assessed at days −2 and −1 before the conditioning procedure (Table 1). Fear conditioning led to a significant potentiation of amplitude [CS: $F_{(1,9)} = 13.6$, $p = 0.005$; time: $F_{(3,27)} = 6.9$, $p = 0.001$; CS-by-time interaction: $F_{(3,27)} = 5.7$, $p = 0.004$; three-way ANOVA (genotype, CS, time) for repeated measures (CS, time)] (Fig. 2*C*) and slope (CS: $F_{(1,9)} = 11.8$, $p = 0.007$; time: $F_{(3,27)} = 14.4$, $p < 0.0001$; CS-by-time interaction: $F_{(3,27)} = 8.1$, $p = 0.001$) (Fig. 2*D*) of the most-negative-going component of the field EPSPs evoked by the paired tones, with no significant differences between CB1 $^{-/-}$ and CB1 $^{+/+}$ in either parameter (genotype: $F_{(1,9)} < 0.06$, $p > 0.810$; CS by genotype: $F_{(1,9)} < 0.4$, $p > 0.560$; CS by time by genotype: $F_{(1,9)1,9} < 1.3$, $p > 0.300$).

These data show that genetic ablation of CB1 does not alter the potentiation of auditory-evoked potentials induced by the formation of a tone–shock association, indicating that both CB1 $^{+/+}$ and CB1 $^{-/-}$ were able to discriminate between paired and neutral tones and therefore to form an associative memory component during fear conditioning. There is still the option that CB1 $^{-/-}$ might be able to form a tone–shock association after three tone–shock pairings (as used here) but not after a single tone–shock pairing, as used in the behavioral experiments. However, the similar initial freezing response of CB1 $^{-/-}$ and CB1 $^{+/+}$ at day 1 (Fig. 1*B*) as well as our previous study showing that

Table 1. Basic characteristics of auditory-evoked potentials evoked by tone series of two different frequencies within the CA1 region of the dorsal hippocampus in CB1$^{-/-}$ ($n = 5$) and CB1$^{+/+}$ ($n = 6$) during the baseline period day −2 and day −1 (compare Fig. 2)

	6 kHz			12 kHz		
Genotype	Latency (ms)	Amplitude (μV)	Slope (μV/ms)	Latency (ms)	Amplitude (μV)	Slope (μV/ms)
CB1$^{-/-}$	28.9 ± 2.2	195.6 ± 44.3	−24.6 ± 6.0	28.1 ± 1.8	172.3 ± 41.3	−21.2 ± 3.9
CB1$^{+/+}$	27.3 ± 3.1	113.4 ± 25.0	−21.3 ± 4.5	27.3 ± 2.6	144.7 ± 25.3	−26.3 ± 5.5

None of these parameters differed significantly between the two genotypes and the two tone frequencies (genotype: $F_{(1,10)} < 1.9, p > 0.202$; frequency: $F_{(1,10)} < 0.5, p > 0.510$; genotype by frequency: $F_{(1,10)} < 1.6, p > 0.240$).

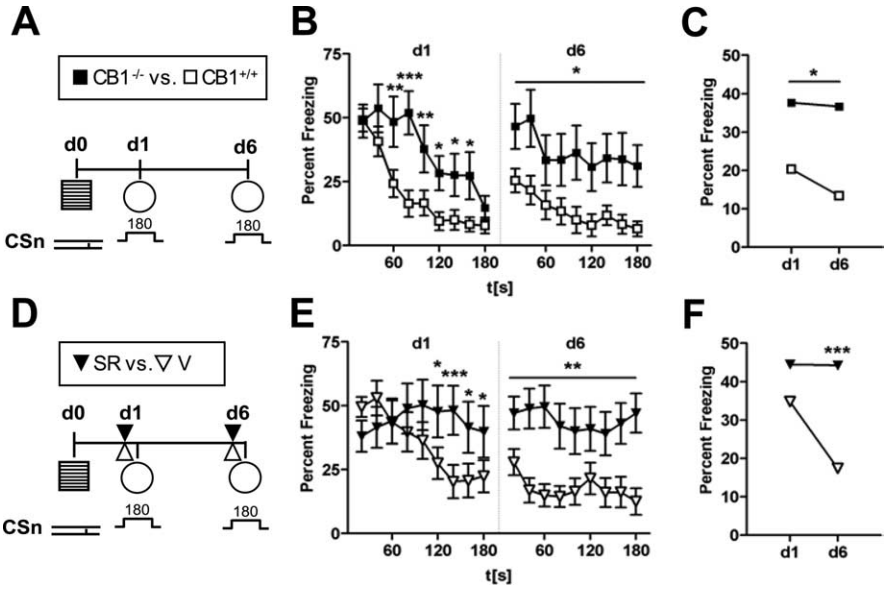

Figure 3. CB1 deficiency impairs both within-session and long-term adaptation of sensitized fear. ***A***, ***D***, Mice with genetic ablation of CB1 (***A***; CB1$^{-/-}$, $n = 10$; CB1$^{+/+}$, $n = 15$; included in Fig. 1*B*) and inbred mice with acute pharmacological blockade of CB1 before tone presentation [***D***; 3 mg/kg SR141716A (SR), $n = 17$; vehicle (V), $n = 17$] were exposed to a 180 s tone not only 1 d but also 6 d after sensitization. ***B***, ***E***, Genetic ablation (***B***) and pharmacological inactivation (***E***) of CB1 caused similar impairments in the decrease in sensitized fear during the first tone presentation. These impairments became even more pronounced during the second tone presentations (mean ± SEM). ***C***, ***F***, Both genetic ablation (***C***) and acute pharmacological blockade (***F***) of CB1 impaired the long-term decrease in the freezing response from the first to the second tone presentation (mean). Data were normalized either to 20 s observation intervals (***B***, ***D***) or to the entire 180 s observation period (***C***, ***F***). *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$. CSn, Tone after sensitization procedure; ■, CB1$^{-/-}$; □, CB1$^{+/+}$; ▼, C57BL/6N mice treated with 3 mg/kg SR141716A; ▽, C57BL/6N mice treated with vehicle; d0, day 0; d1, day 1; d6, day 6.

pharmacological blockade of CB1 before tone re-exposure, but not before conditioning, leads to a sustained freezing response to the tone (Marsicano et al., 2002) strongly argue against impairments in memory acquisition because of CB1 deficiency. Consequently, the similarities in the freezing response of conditioned and sensitized CB1$^{-/-}$ (Fig. 1*C*) cannot be explained by impairments in the formation of associative memories (Varvel and Lichtman, 2002; Cannich et al., 2004; Suzuki et al., 2004; Holter et al., 2005; Varvel et al., 2005), but rather by alterations in the processing of nonassociative memory components after fear conditioning and sensitization procedures.

To further characterize the role of CB1 in fear extinction, from now on we mainly focused on sensitization experiments, because nonassociative memories are directly accessible in such tasks and not confounded by the behavioral expression of associative memory components. Importantly, the nonassociative nature of the sensitization procedures could be confirmed in a control experiment, which demonstrates not only that the increased freezing response to the tone after sensitization persists for ~1 month but also that it is independent from contextual memory/context generalization (supplemental Fig. 2, available at www.jneurosci.org as supplemental material). To substantiate this conclusion, we additionally analyzed freezing responses to the novel context before tone presentation and observed either no or no consistent involvement of CB1 that would support the idea that alterations in context generalization or interference learning could explain the sustained freezing response of CB1$^{-/-}$ to the tone (supplemental Fig. 3, available at www.jneurosci.org as supplemental material).

CB1 controls both within-session and long-term fear adaptation after sensitization

In previous studies, we showed that CB1$^{-/-}$ are impaired in both within-session extinction and long-term extinction after fear conditioning (Marsicano et al., 2002; Cannich et al., 2004). Now we investigated the role of CB1 in the decrease in the freezing response after repeated tone presentations after sensitization procedures (Fig. 3*A*). CB1$^{-/-}$ showed a prolonged and stronger freezing reaction to the tone than CB1$^{+/+}$ not only during the first tone presentation (genotype: $F_{(1,23)} = 7.0$, $p = 0.014$; genotype by interval: $F_{(8,184)} = 2.9$, $p = 0.004$) but also during the second tone presentation 5 d later (genotype: $F_{(1,22)} = 7.8$; $p = 0.010$) (Fig. 3*B*). The same was the case in wild-type C57BL/6N mice with pharmacological blockade of CB1 with SR141716A before tone presentation (Fig. 3*D*), both at day 1 (drug by interval: $F_{(8,256)} = 5.4$, $p < 0.0001$) and at day 6 (drug: $F_{(1,32)} = 9.8$; $p = 0.003$) (Fig. 3*E*). Accordingly, analysis of the development of the total freezing response from day 1 to day 6 revealed significant effects of genotype ($F_{(1,22)} = 7.3$; $p = 0.012$) (Fig. 3*C*) and drug ($F_{(1,32)} = 4.7$; $p = 0.036$) as well a significant drug-by-day interaction ($F_{(1,32)} = 4.7$; $p = 0.037$) (Fig. 3*F*). Whereas CB1$^{+/+}$ showed a decrease in freezing from day 1 to day 6 ($t_{(14)} = 2.3$; $p = 0.039$; paired *t* test), CB1$^{-/-}$ failed to do so ($t_{(9)} = 0.24$; $p = 0.815$) (Fig. 3*C*). Also, vehicle-treated ($t_{(16)} = 4.5$; $p < 0.0001$) but not antagonist-treated ($t_{(16)} = 0.03$; $p = 0.971$) C57BL/6N mice (Fig. 3*F*) showed a significant decrease in freezing from day 1 to day 6. Together, these data demonstrate that the phenotype of CB1$^{-/-}$ cannot be ascribed to developmental effects of the mutation or altered fear sensitization but to an acute involvement of CB1 in within-session and in long-term fear adaptation.

CB1$^{-/-}$ mice show normal safety learning

In general, the impairment of CB1$^{-/-}$ in extinction of the fear response could be explained by an involvement of CB1 either in inhibitory associative (i.e., safety) learning [tone–no shock association (Myers and Davis, 2002)] or in habituation-like processes

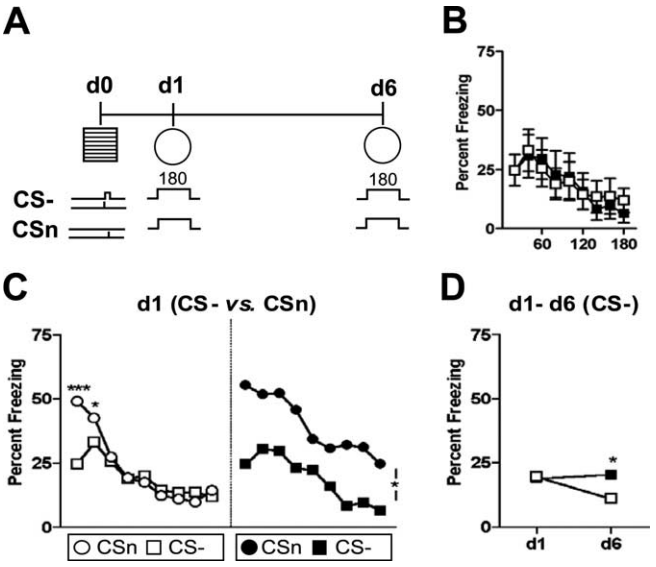

Figure 4. CB1 deficiency does not affect safety learning in a backward conditioning task. ***A***, Both CB1$^{-/-}$ and CB1$^{+/+}$ underwent a backward conditioning procedure in the conditioning chamber, during which application of the footshock preceded the presentation of the 20 s tone. Mice were re-exposed to the tone for 180 s in the test context the next day and 6 d later. ***B***, CB1$^{-/-}$ (■, $n = 10$) and CB1$^{+/+}$ (□, $n = 12$) showed the same freezing response to the tone at day 1 after the backward conditioning procedure (mean ± SEM). ***C***, Both CB1$^{-/-}$ and CB1$^{+/+}$ showed a significantly smaller freezing response to the tone after the backward conditioning procedure compared with the respective sensitized mice (●, ○; compare Fig. 1*C*). ***D***, CB1$^{+/+}$, but not CB1$^{-/-}$, showed a decrease in the freezing responses to the tones from day 1 to day 6 (mean). Data were normalized either to 20 s observation intervals (***B***, ***C***) or to the entire 180 s observation period (***D***). *$p < 0.05$; ***$p < 0.001$. ■ and ●, CB$^{-/-}$; □ and ○, CB1$^{+/+}$. CS−, tone after backward conditioning procedure; CSn, tone after sensitization procedure; d0, day 0; d1, day 1; d6, day 6.

(McSweeney and Swindell, 2002; Kamprath and Wotjak, 2004). The first option seems to be unlikely, because CB1 is dispensable for the formation of associative memories (i.e., tone–shock associations) (Figs. 1, 2) and extinction in an appetitive conditioning task (Holter et al., 2005). Nevertheless, because acquisition and extinction of fear behavior could rely on different molecular mechanisms (Suzuki et al., 2004), CB1 could be specifically implicated in extinction of aversive memories through the formation of "tone–no shock" associations. To address this possibility, we tested the tone–no shock association (i.e., safety learning) of CB1$^{-/-}$ in a backward conditioning task (Moscovitch and LoLordo, 1968; Mackintosh, 1974), in which the shock preceded the tone presentation (Fig. 4*A*). After this procedure, there were no significant differences between CB1$^{-/-}$ and CB1$^{+/+}$ in the freezing response to the tone on the next day (Fig. 4*B* and data not shown). However, both CB1$^{+/+}$ and CB1$^{-/-}$ froze at substantially lower levels than the respective sensitized mice (compare Fig. 1*B*), indicating that not only CB1$^{+/+}$ (protocol by interval: $F_{(8,288)} = 4.5$, $p < 0.0001$) (Fig. 4*C*) but also CB1$^{-/-}$ (protocol: $F_{(1,28)} = 5.6$, $p = 0.025$) (Fig. 4*C*) were able to form an inhibitory association between the tone and the shock that partially suppressed expression of sensitized fear.

Importantly, although the two genotypes froze at comparable levels at day 1, they differed in the development of the freezing response from day 1 to day 6 (genotype by day: $F_{(1,20)} = 6.4$, $p = 0.020$) (Fig. 4*D*) with CB1$^{+/+}$ ($t_{(11)} = 3.1$; $p = 0.001$; paired *t* test) but not CB1$^{-/-}$ ($t_{(9)} = 0.5$; $p = 0.606$) decreasing their freezing response on repeated tone presentation. Because safety learning seems to be intact in CB1$^{-/-}$, these differences point to an involvement of CB1 in long-term habituation.

CB1$^{-/-}$ mice are impaired in long-term habituation to a tone

To test whether CB1 receptors are generally involved in habituation to a tone, we exposed naive (nonshocked) CB1$^{+/+}$ and CB1$^{-/-}$ mice to a loud 3 min tone of 95 dB at days 1 and 6 (Fig. 5*A*). At day 1, CB1$^{-/-}$ and CB1$^{+/+}$ showed a similar freezing response to this loud tone (genotype: $F_{(1,26)} = 0.0$, $p = 0.952$; interval: $F_{(8,208)} = 31.0$, $p < 0.0001$; genotype-by-interval interaction: $F_{(8,208)} = 0.4$, $p = 0.919$) (Fig. 5*B*), indicating that CB1 does not affect perception of and behavioral response to a loud tone. At day 6, however, CB1$^{-/-}$ consistently froze at higher levels than CB1$^{+/+}$ (genotype: $F_{(1,26)} = 5.3$, $p = 0.029$) (Fig. 5*B*), with no difference in baseline freezing (supplemental Fig. 4, available at www.jneurosci.org as supplemental material). Comparison of the development of the total freezing response from day 1 to day 6 revealed a significant genotype-by-day interaction ($F_{(1,26)} = 5.8$; $p = 0.022$), reflecting the inability of CB1$^{-/-}$ ($t_{(12)} = 0.3$; $p = 0.803$; paired *t* test) but not CB1$^{+/+}$ ($t_{(14)} = 6.0$; $p < 0.0001$) to decrease their freezing response from day 1 to day 6 (Fig. 5*C*). These data indicate that CB1 plays an important role in long-term habituation to an aversive tone in mice without previous shock experience, even in situations when CB1 is not acutely involved in within-session adaptation during the first tone presentation.

Impairments in the decrease in freezing to a tone in sensitized CB1$^{-/-}$ mice relate to impaired habituation

To confirm that CB1-mediated habituation processes are responsible for the decrease in freezing after repeated tone presentation in sensitized mice, we studied the consequences of pre-exposure to a 3 min tone of 80 dB on the freezing response to the same tone presented 1 and 6 d after sensitization (Fig. 6*A*). If long-term habituation processes indeed account for adaptation of acquired fear responses, as assessed during a second tone presentation, it should not matter whether the first tone presentation (i.e., the induction of long-term habituation to that stimulus) occurred before or after the sensitization procedure (Kamprath and Wotjak, 2004). Indeed, on day 1 after the sensitization procedure, CB1$^{+/+}$ with pre-exposure to the tone froze significantly less than CB1$^{+/+}$ without tone pre-exposure (protocol: $F_{(1,31)} = 11.1$, $p = 0.002$; protocol by interval: $F_{(8,248)}$ 2.8, $p = 0.006$) (Fig. 6*B*). Moreover, CB1$^{+/+}$ showed similarly low freezing levels during the second tone presentation, regardless of whether the first tone presentation occurred before or after the sensitization procedure (Fig. 6*D*). CB1$^{-/-}$, in contrast, showed a strong freezing response at day 1 (protocol: $F_{(1,25)} = 0.3$, $p = 0.541$; protocol by interval: $F_{(8,200)} = 1.3$, $p = 0.228$) (Fig. 6*B*), regardless of whether the tone was previously presented or not. Noteworthy, tone pre-exposure at day 5 (Fig. 6*A*) elicited only a negligible freezing response compared with mice without tone presentation (CB1$^{+/+}$: 3.0 ± 0.7% vs 0.1 ± 0.0%; CB1$^{-/-}$: 3.7 ± 1.6% vs 0.5 ± 0.2%) with no differences between the two genotypes (statistics not shown), indicating that expression of fear is not necessary for induction of CB1-dependent long-term habituation to the tone.

At day 6, CB1$^{-/-}$ showed a generally increased freezing response compared with CB1$^{+/+}$ (genotype: $F_{(1,56)} = 10.6$, $p = 0.002$) (Fig. 6*C*), independently of the protocol [genotype by protocol: $F_{(1,56)} = 0.0$, $p = 0.902$; genotype by protocol by interval: $F_{(8,448)} = 0.8$, $p = 0.602$; three-way ANOVA (genotype, protocol, interval) for repeated measures (interval)]. As illustrated in Figure 6*E*, CB1$^{-/-}$ were not at all able to adapt to the tone, despite repeated tone presentations [genotype: $F_{(1,56)} = 5.0$, $p = 0.029$; genotype by day: $F_{(1,56)} = 15.8$, $p < 0.001$; genotype by protocol

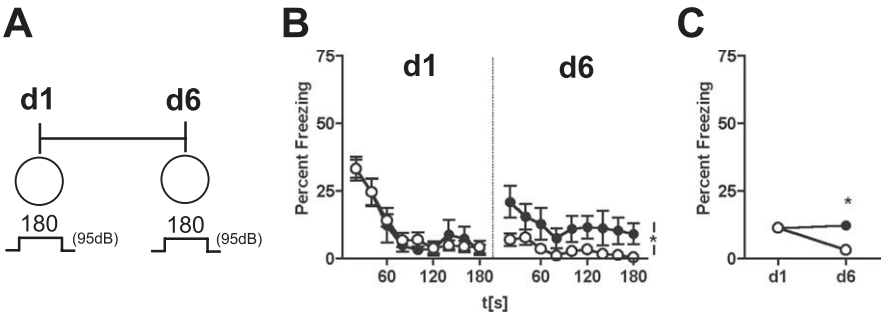

Figure 5. CB1 deficiency impairs long-term habituation to a loud tone. ***A***, Naive CB1 $^{-/-}$ ($n = 13$) and CB1 $^{+/+}$ ($n = 15$) were exposed to a loud tone of 95 dB at days 1 and 6. ***B***, CB1 $^{-/-}$ and CB1 $^{+/+}$ showed a similar short-term habituation to the first tone presentation but differed in their freezing response to the second tone presentation (mean ± SEM). ***C***, CB1 $^{+/+}$, but not CB1 $^{-/-}$, showed a decrease in the freezing responses to the tones from day 1 to day 6 (mean). Data were normalized either to 20 s observation intervals (***B***) or to the entire 180 s observation period (***C***). *$p < 0.05$. ●, CB1 $^{-/-}$; ○, CB1 $^{+/+}$; d1, day 1; d6, day 6.

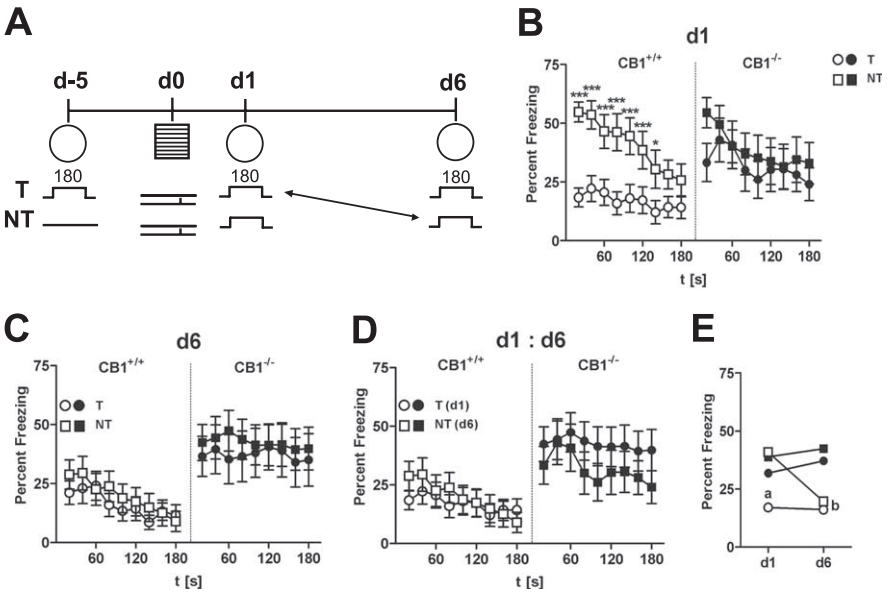

Figure 6. Impaired long-term adaptation in CB1-deficient mice after sensitization results from impaired long-term habituation. ***A***, CB1 $^{-/-}$ and CB1 $^{+/+}$ were randomly assigned to one of two groups. Mice of the first group [tone (T); CB1 $^{-/-}$, $n = 12$; CB1 $^{+/+}$, $n = 17$] were exposed to a 180 s tone in the test context 5 d before the sensitization procedure. Mice of the second group [no tone (NT); CB1 $^{-/-}$, $n = 15$; CB1 $^{+/+}$, $n = 16$] were also placed into the test context 5 d before the sensitization procedure, but without tone presentation. All mice were exposed to 180 s tones at days 1 and 6 after sensitization with a single 0.7 mA footshock. ***B***, Freezing response to the tone at day 1. ***C***, Freezing responses to the tone at day 6. ***D***, Freezing response to the second tone presentation of mice with (T, day 1) and without (NT, day 6) tone presentation before the sensitization procedure. ***E***, Development of the freezing responses to the tones from days 1–6. Data were normalized either to 20 s observation intervals (mean ± SEM; ***B–D***) or to the entire 180 s observation period (mean; ***E***). *$p < 0.05$; ***$p < 0.001$; [a]$p < 0.001$ versus all other groups; [b]$p < 0.0005$ versus the two CB1 $^{-/-}$ groups. ■, CB1 $^{-/-}$ without tone pre-exposure; ●, CB1 $^{-/-}$ with tone pre-exposure; □, CB1 $^{+/+}$ without tone pre-exposure; ○, CB1 $^{+/+}$ with tone pre-exposure; d-5, 5 d before the sensitization procedure; d0, day 0; d1, day 1; d6, day 6.

by day: $F_{(1,56)} = 5.7$, $p = 0.021$; three-way ANOVA (genotype, protocol, day) for repeated measures (day)]. *Post hoc* analyses revealed that, at day 1, only CB1 $^{+/+}$ with pre-exposure to the tone froze significantly less than all of the other groups ($p < 0.001$). At day 6, both CB1 $^{+/+}$ groups (with and without pre-exposure to the tone) froze at similar levels and considerably less than CB1 $^{-/-}$ ($p < 0.001$). In accordance with our previous observations in naive mice (compare Fig. 5*C*), in mice after sensitization (Fig. 3, compare *C*, *F*), and in mice after backward conditioning procedures (compare Fig. 4*D*), CB1 $^{+/+}$ without pre-exposure to the tone showed a significant decrease in freezing from day 1 to day 6 ($t_{(15)} = 32.6$; $p < 0.0001$; paired t test), whereas the two CB1 $^{-/-}$ groups failed to do so ($t < 1.2$; $p > 0.297$).

Together, these data demonstrate that in wild-type mice, the decrease in freezing on repeated tone presentation is independent of whether the first tone presentation occurred before or after the sensitization procedure. In CB1-deficient mice, in contrast, pre-exposure to the tone failed to significantly affect the freezing response to a second tone presentation. The most parsimonious interpretation of these observations is that CB1 plays a central role in long-term habituation to the tone and that CB1-dependent habituation primarily accounts for the decrease in acquired fear after sensitization procedures.

Discussion

Extinction of aversive memories involves at least two different processes: learning about the association between a stimulus and the nonappearance of a punishment [here called safety learning (cf. Myers and Davis, 2002)] and habituation to a repeatedly presented stimulus (cf. McSweeney and Swindell, 2002; Kamprath and Wotjak, 2004). Recent work has shown that different molecular mechanisms relate to different aspects of extinction processing. NMDA receptors, for instance, seem to mediate predominantly associative memory components of extinction (Falls et al., 1992; Santini et al., 2001; Walker et al., 2002). The same is the case for L-type voltage-gated calcium channels (Cain et al., 2002), which are also involved in latent inhibition but not in reduced contingency effects (Cain et al., 2005). Calcineurin, in contrast, mediates extinction by circuit depotentiation (i.e., weakening of the original signaling) (Lin et al., 2003).

Although extinction of conditioned fear critically depends on the endocannabinoid system of the brain (Marsicano et al., 2002; Cannich et al., 2004; Suzuki et al., 2004; Chhatwal et al., 2005), the mechanism by which CB1 mediates fear extinction remained speculative. This study points to a specific involvement of the endocannabinoid system in the habituation component of fear extinction. However, besides habituation, there are still alternative explanations for the data of the present study. For example, the experiment using tone pre-exposure (Fig. 6) strongly resembles latent inhibition procedures, in which pre-exposure to the to-be CS (e.g., a tone) before conditioning results in a decreased conditioned response (e.g., freezing) after conditioning (for review, see Lubow, 1973). When we applied tone pre-exposure before sensitization, we observed a decreased freezing response to the tone after sensitization in CB1 $^{+/+}$ but not in CB1 $^{-/-}$. Thus, CB1 $^{-/-}$ are possibly impaired in latent inhibition, which belongs, like extinction, to interference paradigms. However, we did not use a true latent inhibition

paradigm because we used sensitization instead of conditioning: Mice received tone pre-exposure in the test context; 5 d later they were sensitized in a different context without tone presentation and re-exposed to the tone in the test context. To obtain interference in terms of latent inhibition, the animals should generalize between the different contexts (Bouton, 1993).

Context generalization is known to be a general problem of shock-sensitization paradigms, which relates to the fact that the procedure of shock sensitization is essentially the same as the contextual fear-conditioning procedure. The important difference between these paradigms relates to the test stimuli used for memory retrieval: in contextual fear conditioning, the response to the conditioning context is tested, and in sensitization, the response to an unrelated ("nonassociative") stimulus is tested in a different context. Because shock sensitization of the startle response, which was originally attributed to nonassociative processes (Davis, 1989), was shown to relate to context generalization (Richardson, 2000), the nonassociative nature of fear responses after sensitization is arguable. Thus, freezing responses to the tone after sensitization could be attributed to the fact that the entire experimental procedure, including experimental handling, signals the aversive experience, thus leading to interference learning and context generalization. However, after a contextual fear-conditioning/sensitization procedure, both $CB1^{-/-}$ and $CB1^{+/+}$ displayed less conditioned freezing to the "shock context" than to an unconditioned tone in a different context [supplemental Fig. 2 (available at www.jneurosci.org as supplemental material) vs Fig. 3], indicating that the animals used in our experiments are inferior in contextual learning. On repeated nonreinforced exposures to the shock context, $CB1^{-/-}$ initially developed a more pronounced freezing response, which was later extinguished (supplemental Fig. 2, available at www.jneurosci.org as supplemental material). Nevertheless, $CB1^{-/-}$ still showed considerably more freezing than $CB1^{+/+}$ to a subsequently presented tone. These results indicate that behavioral adaptation of contextual fear and of unconditioned fear to the tone rely on different mechanisms both involving CB1.

In general, the context is known to play a crucial role in different learning paradigms, for instance in extinction and latent inhibition but also in habituation (Marlin and Miller, 1981; for review, see Bouton, 1993). To further investigate the role of the context in sensitized fear, we additionally analyzed the fear responses to the test context before tone presentation (supplemental Fig. 3, available at www.jneurosci.org as supplemental material). It became evident that $CB1^{-/-}$ froze even less in the test context than $CB1^{+/+}$. Moreover, freezing responses to the tone were more pronounced than freezing responses to the test context, thus rendering it unlikely that they result from context generalization. However, application of the CB1 antagonist SR141716A to wild-type mice resulted in slightly increasing fear responses to the context, which was not observed in $CB1^{-/-}$ before the first tone exposure (supplemental Fig. 3, available at www.jneurosci.org as supplemental material) and might therefore relate to unspecific effects of SR141716A. Baseline freezing before the second tone exposure was generally increased compared with baseline freezing before the first tone exposure (supplemental Fig. 3, available at www.jneurosci.org as supplemental material). This could be explained by a secondary learning process associating the test context with the aversive tone exposure. Additional experiments are necessary to verify this hypothesis. However, in summary, our data indicate that the role of CB1 in adaptation of sensitized fear to the tone cannot be ascribed to context generalization and thus to differences in extinction of contextual memory. Nevertheless, CB1 seems to be involved, in some aspects, in contextual learning (Suzuki et al., 2004; Pamplona and Takahashi, 2006). The underlying mechanisms remain to be investigated, but they seem to be independent from CB1-mediated habituation of the fear response to the tone.

In some of our experiments (e.g., unconditioned freezing to a loud tone, backward conditioning, tone pre-exposure), CB1 was shown to mediate long-term habituation without affecting within-session fear adaptation during the first tone presentation. It is likely that the sensitization procedure acutely activates certain neurotransmitter/modulator systems (e.g., corticotrophin-releasing hormone) (Koob et al., 1993; Walker et al., 2003), which in turn increase the general responsiveness to potentially dangerous stimuli. During the first tone presentation, endocannabinoids may counteract this potentiation as retrograde messengers that reduce neurotransmitter release from presynaptic terminals (Schlicker and Kathmann, 2001; Alger, 2002; Wilson and Nicoll, 2002; Freund et al., 2003) once the averseness of the situation exceeds a certain intensity. This downregulation of neuronal activation might explicitly involve a reduction in glutamatergic transmission (Marsicano et al., 2003;Azad et al., 2003). For long-term habituation, in contrast, processing of the tone might be accompanied by an activation of the endocannabinoid system within an innate stimulus–response pathway (e.g., Mongeau et al., 2003), even if the tones are not sufficient per se for inducing a significant freezing response. In this scenario, activation of CB1 would lead to changes in neuronal excitability in the stimulus–response pathway [e.g., by changing the activity status of kinases and phosphatases (Cannich et al., 2004) that have been implicated in long-term retention of extinction of conditioned fear (Lu et al., 2001; Lin et al., 2003)]. The differences in the involvement of CB1 in short-term adaptation and long-term habituation might also relate to CB1 activation on different neuronal populations (Marsicano and Lutz, 1999; Marsicano et al., 2003). It is conceivable that physicochemical characteristics of CB1 (e.g., binding affinity to endocannabinoids) or the spatial proximity of CB1 to the release sites of endocannabinoids differ between GABAergic interneurons and glutamatergic neurons. In addition, CB1 might be coupled to different intracellular pathways, in particular, because GABAergic interneurons do not express the phosphatase calcineurin (Sik et al., 1998).

In general, the involvement of CB1 in fear extinction strikingly resembles the role of the endocannabinoid system in adaptation of stress responses (for review, see Hill and Gorzalka, 2005; Viveros et al., 2005). This has potential implications for the interaction of the two processes (for review, see Korte, 2001), in particular because tone presentations after fear conditioning or sensitization procedures can be regarded as psychological stressors. Nevertheless, it seems to be unlikely that a sustained activation of the hypothalamic–pituitary–adrenocortical axis in CB1-deficient mice after stressor exposure (Cota et al., 2003; Di et al., 2003) plays a significant role in within-session extinction of the fear response, because unrestrained release of corticosterone attributable to CB1 deficiency would be expected to facilitate rather than to attenuate fear extinction (Yang et al., 2006). Long-term fear extinction, in contrast, might well share common mechanisms with adaptation to homotypic stressors. In this context, Patel et al. (2005) showed that the endocannabinoid system mediates habituation to repeated restraint stress. They observed an increase in the level of 2-arachidonoyl glycerol in brain punches from the amygdala complex from the first to the fifth restraint stress episode. Furthermore, pharmacological blockade of CB1 during the fifth restraint episode resulted in a reversal of the

habituation-like reduction in Fos expression in the infralimbic and prelimbic cortices (Patel et al., 2005). Interestingly, these brain regions are known to play a pivotal role in extinction of conditioned fear (Milad and Quirk, 2002; Quirk et al., 2003; Pare et al., 2004) and therefore may be involved in the endocannabinoid-mediated long-term habituation described in the present study as well.

In summary, our study provides a new conceptual framework for understanding the role of CB1 in short- and long-term adaptation to aversive situations and emphasizes the importance of CB1-mediated habituation in extinction of acquired fear. Our protocol of prolonged stimulus presentations resembles the principle of exposure therapy, which is successfully used for the treatment of phobias and posttraumatic stress disorder (Wolpe, 1958; Bartling et al., 1980; Ost et al., 1991; Foa and Riggs, 1993). Our findings imply that the success of such therapies is, at least partially, based on successful habituation, thus suggesting the endocannabinoid system as both a vulnerability factor and potential therapeutic target of anxiety disorders.

References

Alger BE (2002) Retrograde signaling in the regulation of synaptic transmission: focus on endocannabinoids. Prog Neurobiol 68:247–286.

Azad SC, Eder M, Marsicano G, Lutz B, Zieglgansberger W, Rammes G (2003) Activation of the cannabinoid receptor type 1 decreases glutamatergic and GABAergic synaptic transmission in the lateral amygdala of the mouse. Learn Mem 10:116–128.

Bartling G, Fiegenbaum W, Krause R (1980) Reizüberflutung. Theorie und Praxis. Stuttgart, Germany: Kohlhammer.

Bouton ME (1993) Context, time, and memory retrieval in the interference paradigms of Pavlovian learning. Psychol Bull 114:80–99.

Cain CK, Blouin AM, Barad M (2002) L-type voltage-gated calcium channels are required for extinction, but not for acquisition or expression, of conditional fear in mice. J Neurosci 22:9113–9121.

Cain CK, Godsil BP, Jami S, Barad M (2005) The L-type calcium channel blocker nifedipine impairs extinction, but not reduced contingency effects, in mice. Learn Mem 12:277–284.

Cannich A, Wotjak CT, Kamprath K, Hermann H, Lutz B, Marsicano G (2004) CB1 cannabinoid receptors modulate kinase and phosphatase activity during extinction of conditioned fear in mice. Learn Mem 11:625–632.

Chhatwal JP, Davis M, Maguschak KA, Ressler KJ (2005) Enhancing cannabinoid neurotransmission augments the extinction of conditioned fear. Neuropsychopharmacology 30:516–524.

Cota D, Marsicano G, Tschop M, Grubler Y, Flachskamm C, Schubert M, Auer D, Yassouridis A, Thone-Reineke C, Ortmann S, Tomassoni F, Cervino C, Nisoli E, Linthorst AC, Pasquali R, Lutz B, Stalla GK, Pagotto U (2003) The endogenous cannabinoid system affects energy balance via central orexigenic drive and peripheral lipogenesis. J Clin Invest 112:423–431.

Davis M (1989) Sensitization of the acoustic startle reflex by footshock. Behav Neurosci 103:495–503.

Di S, Malcher-Lopes R, Halmos KC, Tasker JG (2003) Nongenomic glucocorticoid inhibition via endocannabinoid release in the hypothalamus: a fast feedback mechanism. J Neurosci 23:4850–4857.

Di Marzo V, Bifulco M, De Petrocellis L (2004) The endocannabinoid system and its therapeutic exploitation. Nat Rev Drug Discov 3:771–784.

Falls WA, Miserendino MJ, Davis M (1992) Extinction of fear-potentiated startle: blockade by infusion of an NMDA antagonist into the amygdala. J Neurosci 12:854–863.

Foa EB, Riggs DS (1993) Post-traumatic stress disorder in rape victims. In: American Psychiatric Press review of psychiatry (Oldham J, Riba MB, Tasman A, eds), pp 273–303. Washington, DC: American Psychiatric.

Franklin KN, Paxinos G (1997) The mouse brain in stereotactic coordinates. London: Academic.

Freund TF, Katona I, Piomelli D (2003) Role of endogenous cannabinoids in synaptic signaling. Physiol Rev 83:1017–1066.

Goosens KA, Hobin JA, Maren S (2003) Auditory-evoked spike firing in the lateral amygdala and Pavlovian fear conditioning: mnemonic code or fear bias? Neuron 40:1013–1022.

Groves PM, Thompson RF (1970) Habituation: a dual-process theory. Psychol Rev 77:419–450.

Hill MN, Gorzalka BB (2005) Is there a role for the endocannabinoid system in the etiology and treatment of melancholic depression? Behav Pharmacol 16:333–352.

Holter SM, Kallnik M, Wurst W, Marsicano G, Lutz B, Wotjak CT (2005) Cannabinoid CB1 receptor is dispensable for memory extinction in an appetitively-motivated learning task. Eur J Pharmacol 510:69–74.

Kamprath K, Wotjak CT (2004) Nonassociative learning processes determine expression and extinction of conditioned fear in mice. Learn Mem 11:770–786.

Koob GF, Heinrichs SC, Pich EM, Menzaghi F, Baldwin H, Miczek K, Britton KT (1993) The role of corticotropin-releasing factor in behavioural responses to stress. Ciba Found Symp 172:277–289.

Korte SM (2001) Corticosteroids in relation to fear, anxiety and psychopathology. Neurosci Biobehav Rev 25:117–142.

Lamprea MR, Cardenas FP, Vianna DM, Castilho VM, Cruz-Morales SE, Brandao ML (2002) The distribution of fos immunoreactivity in rat brain following freezing and escape responses elicited by electrical stimulation of the inferior colliculus. Brain Res 950:186–194.

LeDoux JE (2000) Emotion circuits in the brain. Annu Rev Neurosci 23:155–184.

Lin CH, Yeh SH, Leu TH, Chang WC, Wang ST, Gean PW (2003) Identification of calcineurin as a key signal in the extinction of fear memory. J Neurosci 23:1574–1579.

Lu KT, Walker DL, Davis M (2001) Mitogen-activated protein kinase cascade in the basolateral nucleus of amygdala is involved in extinction of fear-potentiated startle. J Neurosci 21:RC162(1–5).

Lubow RE (1973) Latent inhibition. Psychol Bull 79:398–407.

Mackintosh NJ (1974) The psychology of animal learning. London: Academic.

Maren S, Quirk GJ (2004) Neuronal signalling of fear memory. Nat Rev Neurosci 5:844–852.

Marlin NA, Miller RR (1981) Associations to contextual stimuli as a determinant of long-term habituation. J Exp Psychol Anim Behav Process 7:313–333.

Marsicano G, Lutz B (1999) Expression of the cannabinoid receptor CB1 in distinct neuronal subpopulations in the adult mouse forebrain. Eur J Neurosci 11:4213–4225.

Marsicano G, Wotjak CT, Azad SC, Bisogno T, Rammes G, Cascio MG, Hermann H, Tang J, Hofmann C, Zieglgansberger W, Di M, V, Lutz B (2002) The endogenous cannabinoid system controls extinction of aversive memories. Nature 418:530–534.

Marsicano G, Goodenough S, Monory K, Hermann H, Eder M, Cannich A, Azad SC, Cascio MG, Gutierrez SO, van der SM, Lopez-Rodriguez ML, Casanova E, Schutz G, Zieglgansberger W, Di M, V, Behl C, Lutz B (2003) CB1 cannabinoid receptors and on-demand defense against excitotoxicity. Science 302:84–88.

McSweeney FK, Swindell S (2002) Common processes may contribute to extinction and habituation. J Gen Psychol 129:364–400.

Milad MR, Quirk GJ (2002) Neurons in medial prefrontal cortex signal memory for fear extinction. Nature 420:70–74.

Mongeau R, Miller GA, Chiang E, Anderson DJ (2003) Neural correlates of competing fear behaviors evoked by an innately aversive stimulus. J Neurosci 23:3855–3868.

Moscovitch A, LoLordo VM (1968) Role of safety in the Pavlovian backward fear conditioning procedure. J Comp Physiol Psychol 66:673–678.

Myers KM, Davis M (2002) Behavioral and neural analysis of extinction. Neuron 36:567–584.

Ohman A, Mineka S (2001) Fears, phobias, and preparedness: toward an evolved module of fear and fear learning. Psychol Rev 108:483–522.

Ost LG, Salkovskis PM, Hellstrom K (1991) One-session therapist-directed exposure vs self-exposure in the treatment of spider phobia. Behav Ther 22:407–422.

Pamplona FA, Takahashi RN (2006) WIN 55212–2 impairs contextual fear conditioning through the activation of CB1 cannabinoid receptors. Neurosci Lett 397:88–92.

Pare D, Quirk GJ, LeDoux JE (2004) New vistas on amygdala networks in conditioned fear. J Neurophysiol 92:1–9.

Patel S, Roelke CT, Rademacher DJ, Hillard CJ (2005) Inhibition of restraint stress-induced neural and behavioural activation by endogenous cannabinoid signalling. Eur J Neurosci 21:1057–1069.

Quirk GJ, Likhtik E, Pelletier JG, Pare D (2003) Stimulation of medial prefrontal cortex decreases the responsiveness of central amygdala output neurons. J Neurosci 23:8800–8807.

Richardson R (2000) Shock sensitization of startle: learned or unlearned fear? Behav Brain Res 110:109–117.

Rogan MT, Staubli UV, LeDoux JE (1997) Fear conditioning induces associative long-term potentiation in the amygdala. Nature 390:604–607.

Santini E, Muller RU, Quirk GJ (2001) Consolidation of extinction learning involves transfer from NMDA-independent to NMDA-dependent memory. J Neurosci 21:9009–9017.

Schlicker E, Kathmann M (2001) Modulation of transmitter release via presynaptic cannabinoid receptors. Trends Pharmacol Sci 22:565–572.

Seidenbecher T, Laxmi TR, Stork O, Pape HC (2003) Amygdalar and hippocampal theta rhythm synchronization during fear memory retrieval. Science 301:846–850.

Sik A, Hajos N, Gulacsi A, Mody I, Freund TF (1998) The absence of a major Ca^{2+} signaling pathway in GABAergic neurons of the hippocampus. Proc Natl Acad Sci USA 95:3245–3250.

Suzuki A, Josselyn SA, Frankland PW, Masushige S, Silva AJ, Kida S (2004) Memory reconsolidation and extinction have distinct temporal and biochemical signatures. J Neurosci 24:4787–4795.

Tang J, Wotjak CT, Wagner S, Williams G, Schachner M, Dityatev A (2001) Potentiated amygdaloid auditory-evoked potentials and freezing behavior after fear conditioning in mice. Brain Res 919:232–241.

Tang J, Wagner S, Schachner M, Dityatev A, Wotjak CT (2003) Potentiation of amygdaloid and hippocampal auditory-evoked potentials in a discriminatory fear-conditioning task in mice as a function of tone pattern and context. Eur J Neurosci 18:639–650.

Thompson RF, Spencer WA (1966) Habituation: a model phenomenon for the study of neuronal substrates of behavior. Psychol Rev 73:16–43.

Van Dijken HH, Van der Heyden JA, Mos J, Tilders FJ (1992a) Inescapable footshocks induce progressive and long-lasting behavioural changes in male rats. Physiol Behav 51:787–794.

Van Dijken HH, Mos J, Van der Heyden JA, Tilders FJ (1992b) Characterization of stress-induced long-term behavioural changes in rats: evidence in favor of anxiety. Physiol Behav 52:945–951.

Van Dijken HH, de Goeij DC, Sutanto W, Mos J, de Kloet ER, Tilders FJ (1993) Short inescapable stress produces long-lasting changes in the brain-pituitary-adrenal axis of adult male rats. Neuroendocrinology 58:57–64.

Varvel SA, Lichtman AH (2002) Evaluation of CB1 receptor knockout mice in the Morris water maze. J Pharmacol Exp Ther 301:915–924.

Varvel SA, Anum EA, Lichtman AH (2005) Disruption of CB(1) receptor signaling impairs extinction of spatial memory in mice. Psychopharmacology (Berl) 179:863–872.

Viveros MP, Marco EM, File SE (2005) Endocannabinoid system and stress and anxiety responses. Pharmacol Biochem Behav 81:331–342.

Walker DL, Ressler KJ, Lu KT, Davis M (2002) Facilitation of conditioned fear extinction by systemic administration or intra-amygdala infusions of D-cycloserine as assessed with fear-potentiated startle in rats. J Neurosci 22:2343–2351.

Walker DL, Toufexis DJ, Davis M (2003) Role of the bed nucleus of the stria terminalis versus the amygdala in fear, stress, and anxiety. Eur J Pharmacol 463:199–216.

Wilson RI, Nicoll RA (2002) Endocannabinoid signaling in the brain. Science 296:678–682.

Wolpe J (1958) Psychotherapy by reciprocal inhibition. Stanford, CA: Stanford UP.

Yang YL, Chao PK, Lu KT (2006) Systemic and intra-amygdala administration of glucocorticoid agonist and antagonist modulate extinction of conditioned fear. Neuropsychopharmacology 31:912–924.

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Preclinical Research

Enhancing Cannabinoid Neurotransmission Augments the Extinction of Conditioned Fear

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Abstract

The endogenous cannabinoid (eCB) system represents a major therapeutic target for the treatment of a variety of anxiety-related disorders. A recent study has demonstrated that pharmacologic or genetic disruption of CB1-receptor-mediated neurotransmission decreases the extinction of conditioned fear in mice. Here, we examined whether CB1 blockade would similarly disrupt extinction in rats, using fear-potentiated startle as a measure of conditioned fear. We also examined whether pharmacologic enhancement of CB1 activation would lead to enhancements in extinction. Our results indicate that systemic administration of the CB1 antagonist rimonabant (SR141716A) prior to extinction training led to significant, dose-dependent decreases in extinction. While the administration of the CB1 agonist WIN 55,212-2 did not appear to affect extinction, administration of AM404, an inhibitor of eCB breakdown and reuptake, led to dose-dependent enhancements in extinction. In addition to showing decreased fear 1 and 24 h after extinction training, AM404-treated animals showed decreased shock-induced reinstatement of fear. Control experiments demonstrated that the effects of AM404 could not be attributed to alterations in the expression of conditioned fear, locomotion, shock reactivity, or baseline startle, as these parameters seemed unchanged by AM404. Furthermore, coadministration of rimonabant with AM404 blocked this enhancement of extinction, suggesting that AM404 was acting to increase CB1 receptor activation during extinction training. These results demonstrate that the eCB system can be modulated to enhance emotional learning, and suggest that eCB modulators may be therapeutically useful as adjuncts for exposure-based psychotherapies such as those used to treat Post-Traumatic Stress Disorder and other anxiety disorders.

Keywords: amygdala, fear, extinction, PTSD, cannabinoid, phobia

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Review

The spontaneously hypertensive-rat as an animal model of ADHD: evidence for impulsive and non-impulsive subpopulations

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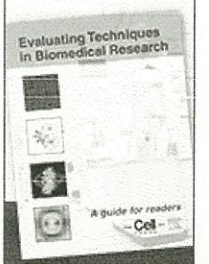

Abstract

Attention-deficit hyperactivity disorder (ADHD) is a neuropsychiatric syndrome, affecting human infants and adolescents. Two main behavioural features are reported: (1) impaired attention and (2) an impulsive–hyperactive behavioural trait. The latter has been studied in a series of experiments, using the spontaneously hypertensive-rat (SHR) strain (which is regarded as a validated animal model for ADHD) in operant tasks. Food-restricted SHRs and their Wistar–Kyoto (WKY) controls were tested during adolescence (i.e. post-natal days 30–45), in operant chambers provided with two nose-poking holes. Nose-poking in one hole (H1) resulted in the immediate delivery of a small amount of food, whereas nose-poking in the other hole (H5) delivered a larger amount of food after a delay, which was increased progressively each day (0–100 s). As expected, all animals showed a shift in preference from the large (H5) to the immediate (H1) reinforcer as the delay length increased. Impulsivity can be measured by the steepness of this preference–delay curve. The two strains differed in home-cage circadian activity, SHRs being more active than WKYs at several time-points. During the test for impulsivity, inter-individual differences were completely absent in the WKY strain, whereas a huge inter-individual variability was evident for SHRs. On the basis of the median value of average hole-preference, we found an 'impulsive' SHR subgroup, with a very quick shift towards the H1 hole, and a flat-slope ('non-impulsive') SHR subgroup, with little or no shift. The impulsive subpopulation also presented reduced noradrenaline levels in both cingulated and medial–frontal cortex, as well as reduced serotonin turnover in the latter. Also, cannabinoid CB1 receptor density resulted significantly lower in the prefrontal cortex of impulsive SHRs, when compared to both the non-impulsive subgroup and control WKYs. Interestingly, acute administration of a cannabinoid agonist (WIN 55,212, 2 mg/kg s.c.) normalized the impulsive behavioural profile, without any effect on WKY rats. Thus, two distinct subpopulations, differing

for impulsive behaviour and specific neurochemical parameters, were evidenced within adolescent SHRs. These results support the notion that a reduced cortical density of cannabinoid CB1 receptors is associated with enhanced impulsivity. This behavioural trait can be positively modulated by administration of a cannabinoid agonist. Present results confirm and extend previous literature, indicating that adolescent SHRs represent a suitable animal model for the preclinical investigation of the early-onset ADHD syndrome.

Author Keywords: Impulsivity; Spontaneously hypertensive-rat; Attention-deficit hyperactivity disorder; Cannabinoid; CB1 receptor; Rat

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BEYOND THC: THE COMPLEMENTARY THERAPEUTIC ROLE OF MINOR PHYTOCANNABINOIDS, TERPENOIDS AND FLAVONOIDS

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Introduction: Since its formal discovery and synthesis in 1964, THC has been the pre-eminent focus of research into both therapeutic and adverse effects of cannabis. In recent times, however, increasing interest has developed in complementary effects of minor phytocannabinoids (CBD, CBG, CBC, THCV and other propyl series components), cannabis terpenoids and flavonoids (see McPartland-Russo, *Journal of Cannabis Therapeutics* 2001; 1(3-4):103-132 for earlier review). The current presentation will focus on newer data for these agents and future targets for research.

Methods: The PubMed/NLM database was reviewed for numerous pertinent keywords. The author's extensive library of books and older articles were also examined for additional material. Particular focus was placed on research of the last several years, and that done on agents not addressed in previous publications, especially the cannabis terpenoids, such as nerolidol, caryophyllene oxide, and phytol.

Results: CBD displays myriad activities as an anti-anxiety, antioxidant, analgesic, cytotoxic agent, etc. (Russo-Guy, *Medical Hypotheses* 2006; 66(2): 234-46), with recent evidence that it also may prevent delayed vomiting in chemotherapy, is anti-inflammatory by increasing adenosine signaling via inhibition of its uptake, limits endothelial, retinal and islet cell damage in diabetes, and β-amyloid damage in Alzheimer disease. CBC and CBG have shown promise in animal models of anxiety and depression, while the latter has also demonstrated cytotoxic properties in various cancer cell lines, and prominent antihypertensive activity. THCV shows great promise as an anorectic agent and anticonvulsant. Cannabis terpenoids show promise as modulators of THC function to reduce memory impairment, boost analgesia via complementary mechanisms, etc. Phytol produces sedative effects, and counters teratogenesis produced by retinoids. Nerolidol may increase skin penetration of topical medicines, and produce antiparasitic effects in leishmaniasis and malaria. Caryophyllene oxide is a potent antifungal agent and antibiotic against *Proteus mirabilis,* a common urinary tract pathogen. Data will be presented to support the premise that even tiny concentrations of these agents may be sufficient to produce important pathophysiological benefits Experiments are planned to assess cannabinoid-terpenoid interactions in the CNS pertinent to anxiety, depression and other processes.

Conclusion: Minor phytocannabinoids, cannabis terpenoids and flavonoids produce an entourage effect that may offer important contributions to enhance benefits of and reduce adverse events attributable to THC.

EFFECTS OF NON-PSYCHOACTIVE CANNABINOIDS ON PRECLINICAL AND CLINICAL PSYCHIATRIC/PSYCHOLOGICAL DISORDERS: A REVIEW AND RECOMMEDATIONS FOR FURTHER RESEARCH

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Introduction:
Recent evidence has revealed the potential therapeutic effects of constituents of the *cannabis* plant other than Δ^9-tetrahydrocannabinol (THC), namely cannabidiol (CBD), cannabichrome (CBC) and cannibigerol (CBG). Each of these it at various stages of research progress.

Results:
CBD: There are both animal model and human research data that indicate that CBD has anxiolytic and anti-schizophrenic properites. There are substantial animal data that support the view that CBD has anxyioltic properties, namely on the conflict test, the Vogel test, the condioned emotional response test, and the elevated plus maze test. In human research, in healthy vounteers, CBD reduces feelings of anxiety, psychotic symptoms, and feelings of cannabis intoxication. CBD also reduces anxiety induced by a public speaking test. Finally, CBD reduces anxiety during a regional cerebral blod flow test (rCBF) test and increases activity on the rCBF scan in the left parahippocampal gyrus. In regard to other anxiety disorders, there is some data that CBD might reduce symptoms in bipolar disorder. In regard to Schizophrenia, there is one small of N=1 patients that indicates CDB may reduce symptoms and futhermore a double-blind study revealed that CBD reduced symotms of acute psychosis but this study clearly needs replication and extension.

CBC: There is evidence that CBC has antidepressant properties in animal models of depression. In addition, it blocks the anxiogenic effect of THC administered in a high dose in animals.

CBG: There is also evidence that CBG has antidepressant properties in an animal model of depression.

Conclusions:
Taken together, these data suggest that much more research should be conducted on these *cannabis* constituents.

Research problems with these *cannabis* compounds include bioavailability, route and type of administration, as well as the the formulation, pure compounds vs. extracts. Significant collaboration should occur to advance this potential therapeutic work.

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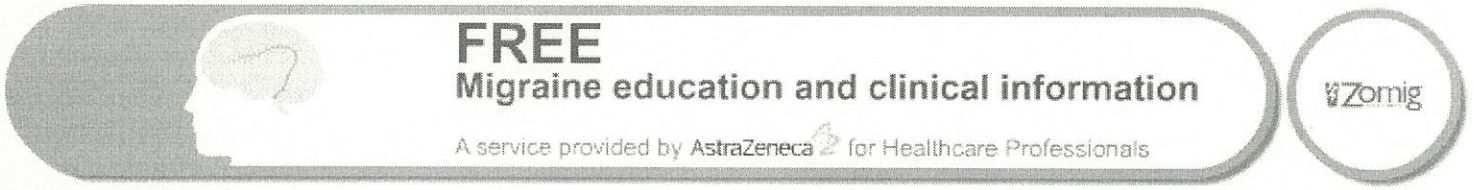

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Brain inflammation is a sign of autism

Medical Studies/Trials
Published: Monday, 15-Nov-2004

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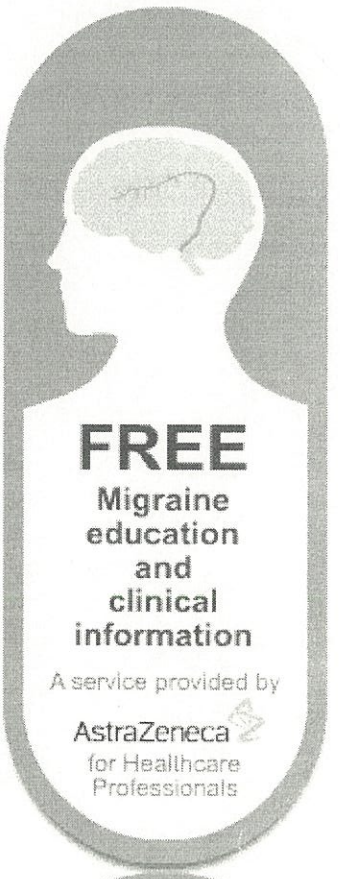
A new study by scientists at Johns Hopkins shows that brain inflammation is a sign of autism. The findings are reported in the online edition of Annals of Neurology, the scientific journal of the American Neurological Association.

Strong evidence that certain immune system components that promote inflammation are consistently activated in people with autism was unearthed.

"These findings reinforce the theory that immune activation in the brain is involved in autism, although it is not yet clear whether it is destructive or beneficial, or both, to the developing brain," said senior author Carlos A. Pardo-Villamizar, M.D., at the Johns Hopkins University School of Medicine in Baltimore, Maryland.

Autism is a brain disorder that begins in early childhood and persists throughout adulthood; affects three crucial areas of development: communication, social interaction, and creative or imaginative play. It is estimated to afflict between 2 and 5 of every 1000 children and is four times more likely to strike boys than girls. Children with autism have difficulties in social interaction and communication and may show repetitive behaviors and have unusual attachments to objects or routines.

Autism has a strong genetic component, and in some families, autism tends to be more



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prevalent. In identical twins with autism both are usually affected. However, the number of children with autism appears to be increasing more than expected for a genetic disorder. This suggests to scientists that genetic abnormalities require the influence of other factors to cause the disorder. Birth complications, toxins, diet, and viruses and other pathogens have been suggested, though there is no strong evidence for any of these.

In recent years, there have been scientific hints of immune system irregularities in children with autism, but not all studies have confirmed this. Pardo and his colleagues sought a more definitive answer by looking not at the immune system overall, but at immune components inside the relatively sealed environment of the nervous system.

Led by first author Diana L. Vargas, MD, a post-doctoral fellow working in Pardo's laboratory, the researchers examined brain tissue from 11 people with autism, aged 5 to 44 years, who had died of accidents or injuries.

Compared with normal control brains, the brains of the people with autism featured immune system activation and inflammation in the brain.

"This ongoing inflammatory process was present in different areas of the brain and produced by cells known as microglia and astroglia," said Pardo.

When the researchers measured brain levels of immune system proteins called cytokines and chemokines, they found abnormal patterns consistent with inflammation.

"The pattern of cellular and protein findings indicate that they are part of the 'innate' immune system in the brain, and do not appear to be caused by immune abnormalities from outside the brain," said Pardo.

The findings in the brain tissue were corroborated by studies of cerebrospinal fluid obtained from six children with autism (ages 5 to 12 years), in which cytokines that promote inflammation were found to be elevated.

It is conceivable that signs of inflammation in the cerebrospinal fluid could one day be used to diagnose autism, or even that doctors could treat inflammation to prevent or combat autism, however this is still speculative, according to Andrew W. Zimmerman, a pediatric neurologist at the Kennedy-Krieger Institute in Baltimore and co-author of the paper. For one thing, it is possible that the inflammation represents the brain's efforts to combat some other process damaging to brain cells.

"These findings open new possibilities for

understanding the dynamic changes that occur in the brain of autistic patients during childhood and adulthood. Although they may lend themselves to development of new medical treatments for autism, much more research would be needed to establish the validity of this approach," said Pardo.

Among the next steps in this line of research, Pardo and colleagues are studying how the genetic background of patients and families may influence the development of immunological reactions in the brain that confer susceptibility to autism.

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Cannabinoid CB2 receptors are expressed by perivascular microglial cells in the human brain: an immunohistochemical study.

Núñez E, Benito C, Pazos MR, Barbachano A, Fajardo O, González S, Tolón RM, Romero J.

Laboratorio de Apoyo a la Investigación, Fundación Hospital Alcorcón, 28922, Alcorcón, Madrid, Spain.

Two types of cannabinoid receptors have been characterized so far, CB1 and CB2. While CB1 receptors are present both in the CNS and in the periphery, CB2 receptors showed an almost exclusive distribution within the immune system. We now report that CB2 receptors are present in a specific microglial cell type of the human cerebellum. Thus, we have performed immunohistochemical analysis of tissue sections of white matter areas of the human cerebellum and detected the presence of CB2 receptors in perivascular microglial cells. These findings match with the well-known immunomodulatory role of CB2 receptors and open new perspectives on the possible role that these receptors may play in pathophysiological events.

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Reduced endocannabinoid immune modulation by a common cannabinoid 2 (CB2) receptor gene polymorphism: possible risk for autoimmune disorders.

Sipe JC, Arbour N, Gerber A, Beutler E.

Department of Molecular and Experimental Medicine, The Scripps Research Institute (MEM-215), La Jolla, CA 92037, USA. jcsipe@scripps.edu

Immune system responsiveness results from numerous factors, including endogenous cannabinoid signaling in immunocytes termed the "immunocannabinoid" system. This system can be an important signaling pathway for immune modulation. To assess the immunomodulating role of the cannabinoid 2 (CB2) receptor, we sought polymorphisms in the human gene, identified a common dinucleotide polymorphism, and investigated its effect on endocannabinoid-induced inhibition of T lymphocyte proliferation. The CB2 cDNA 188-189 GG/GG polymorphism predicts the substitution of glutamine at amino acid position 63 by arginine. T lymphocytes from CB2 188-189 GG/GG homozygotes had approximately twofold reduction of endocannabinoid-induced inhibition of proliferation compared with cells from CB2 188-189 AA/AA homozygotes. In GG/GG subjects, the reduced endocannabinoid inhibitory response was highly significant for N-arachidonylglycine and nearly significant for 2-arachidonylglycerol, and a specific CB2 receptor antagonist partially blocked these effects. Also, patients with autoimmune diseases had an increased prevalence of the homozygous GG/GG genotype. Collectively, these results demonstrate reduced endogenous fatty acid amide immunomodulatory responses in individuals with the CB2 188-189 GG/GG genotype and suggest that this CB2 gene variation may be a risk factor for autoimmunity. The results also support the proposition that the CB2 receptor may represent a novel pharmacological target for selective agonists designed to suppress autoreactive immune responses while avoiding CB1 receptor-mediated cannabinoid adverse effects.

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Cannabinoid receptors in microglia of the central nervous system: immune functional relevance

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Department of Microbiology and Immunology, Virginia Commonwealth University, School of Medicine, Richmond, Virginia

Abstract: Microglia, resident macrophages of the brain, function as immune effector and accessory cells. Paradoxically, they not only play a role in host defense and tissue repair but also have been implicated in a variety of neuropathological processes. Microglia, in addition to exhibiting phenotypic markers for macrophages, express CB_1 and CB_2 cannabinoid receptors. Recent studies suggest the existence of a third, yet-to-be cloned, non-CB_1, non-CB_2 cannabinoid receptor. These receptors appear to be functionally relevant within defined windows of microglial activation state and have been implicated as linked to cannabinoid modulation of chemokine and cytokine expression. The recognition that microglia express cannabinoid receptors and that their activation results in modulation of select cellular activities suggests that they may be amenable to therapeutic manipulation for ablating untoward inflammatory responses in the central nervous system. *J. Leukoc. Biol.* 78: 1192–1197; 2005.

Key Words: *brain immune modulation · cytokines · marijuana · nitric oxide*

Marijuana is a complex plant material, which can elicit a variety of pharmacological and immunological effects. Its major psychoactive component is Δ-9-tetrahydrocannabinol (THC), a compound that has been reported to account for the majority of effects on the immune system [1, 2]. Several modes of action have been proposed as accounting for the effects of THC on immune cells. At high concentrations, such as those exceeding micromolar levels, THC and other cannabinoids may have direct effects on membranes, as they are highly lipophilic [3]. However, stereospecificity and structural requirements for biological activity indicate that cannabinoids also act through specific receptors. To date, two unique cannabinoid receptors have been identified. The CB_1 is located primarily in the brain and is responsible for most, if not all, of the centrally mediated effects of cannabinoids [4, 5]. The CB_2 is present primarily in cells of the immune system but has been detected in adult human uterine tissue and embryonic organs and adult rat retina [6, 7]. Both receptors are $G_{i/o}$ protein-coupled, as evidenced by inhibition of adenylyl cyclase [8], inhibition of N-type calcium channels [9], and increased binding of nonhydrolyzable guanylyl-5′-O-(γ-thio)-triphosphate in the presence of cannabinoids [10]. The CB_1 differs from the CB_2, however, in that it also modulates Q-type calcium channels [11]. Recent studies suggest the existence of a third receptor, a non-CB_1/non-CB_2 receptor [12–14].

Major targets of marijuana and exogenous cannabinoids in the immune system are cells of macrophage lineage. Ultrastructural abnormalities have been observed in alveolar macrophages of humans who have been heavy users of marijuana [15] and in peritoneal macrophages of mice exposed in vitro to various concentrations of pure THC [16]. In addition, various functional defects of alveolar or peritoneal macrophages obtained from humans, rats, or mice following in vivo or in vitro exposure to marijuana or THC have been observed [17]. Microglia constitute a resident population of macrophages in the brain, the spinal cord, and retina and are morphologically, phenotypically, and functionally related to cells of macrophage lineage [18–21]. The function of quiescent microglia in normal brain is not well understood, but in pathological conditions, these cells play an active role as immunoeffector/accessory cells. Microglia migrate and proliferate during and after injury and inflammation [22–25]. Once activated, they produce various cytokines including interleukin-1 (IL-1), IL-6, and tumor necrosis factor-α (TNF-α) and express major histocompatibility complex classes I and II antigens and the complement receptor, CR3. Microglia are also phagocytic and can process antigens and exert cytolytic functions. Paradoxically, these cells not only play a role in host defense and tissue repair in the central nervous system [26, 27] but also have been implicated in nervous system disorders such as multiple sclerosis [28], Alzheimer's disease [29], Parkinson's disease [30], and AIDS dementia [31–33].

Microglia, as macrophage-like cells, undergo a process of maturation, differentiation, and activation, which is characterized by differential gene expression and correlative acquisition of specified functions [22–25, 34, 35]. This pattern of differential expression also applies to cannabinoid receptors (**Fig. 1**). Using an in vitro model of multistep activation, in which

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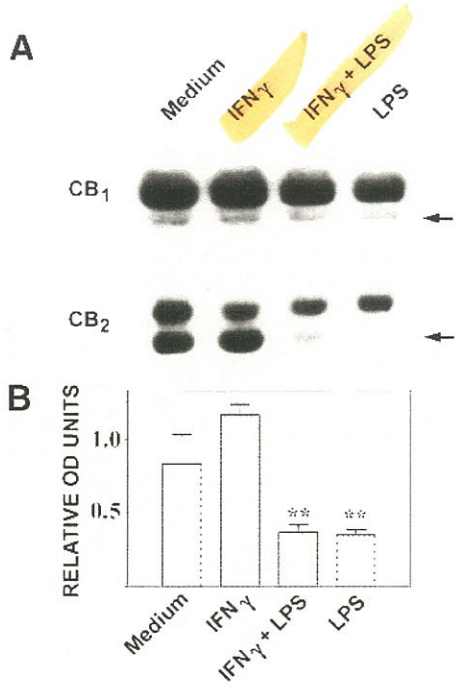

Fig. 1. Differential levels of CB_2 mRNA are detected in neonatal rat brain cerebral cortex microglia in relation to cell activation state. (A) Southern blot of mutagenic reverse transcriptase-polymerase chain reaction (MRT-PCR) products from total nucleic acid of microglia maintained in medium ("responsive") or treated (6 h) with 100 U/ml rat interferon-γ (IFN-γ; "primed"), 100 U/ml rat IFN-γ plus 100 ng/ml lipopolysaccharide (LPS; multisignal-activated), or 1 μg/ml LPS ("fully activated"). MRT-PCR was performed as described [36]. RT primers were used, which introduced a single base mismatch into the cDNA of CB_1 or CB_2 to generate a unique *Msp*I or *Hind*III restriction site, respectively. The upper band of the doublet is amplified genomic DNA (gDNA). The lower band of the doublet (arrow) represents an amplified cDNA product from mRNA. (Upper panel) CB_1 mRNA was detected at low levels for all treatment groups. (Lower panel) High levels of CB_2 mRNA were detected for microglia maintained in medium or IFN-γ (100 U/ml), and low levels were detected for cells treated with IFN-γ plus LPS or with LPS alone. (B) Graphic representation of relative levels of CB_2 mRNA depicted by MRT-PCR. The graph represents a single experiment performed in triplicate. The ordinate designated as Relative OD Units represents densitometric analysis of cDNA-amplified product based on area X pixel density and is represented relative to the corresponding densitometry obtained for the gDNA-amplified product. A significant decrease in levels of CB_2 mRNA was recorded for cells treated with LPS or with LPS plus IFN-γ as compared with those for untreated cells. Error bars are ± SD, n = 3, **, $P < 0.01$. OD, Optical density.

microglia are driven sequentially from a "resting" state to responsive, primed, and fully activated states, the CB_1 was found at constitutive low levels at all states of cell activation. In contrast, the CB_2 was found to be expressed inducibly and at maximal levels when microglia were in responsive and primed states. Collectively, the observations that expression of CB_1 is constitutive, and that of the CB_2 is inducible, that the two receptors are present at disparate levels, and that they exhibit distinctive compartmentalization [36, 37] suggest that the two receptors have discriminative, functional relevance in microglia.

We have demonstrated that cannabinoids inhibit the production of inducible nitric oxide (iNO) by microglia, which are fully activated, in a mode that is mediated, at least in part, by the CB_1 [36]. **Table 1** lists select ligands, which have been used to study cannabinoid receptor-associated functions. Pretreatment of microglia with the CB_1/CB_2 high-affinity binding enantiomer CP55940 (K_i=0.9 nM) resulted in inhibition of iNO (**Fig. 2**) elicited in response to bacterial LPS used in combination with IFN-γ. A less-inhibitory effect was exerted by the lower affinity-binding, paired enantiomer CP56667 (K_i=62 nM). The differential effect exerted by CP55940 versus CP56667 is consistent with a role of a cannabinoid receptor in the inhibition of iNO production, as selective binding affinity of paired cannabinoid stereoisomers has been shown to correlate with bioactivity in vivo and in vitro [5], and differential dose-related effects of one enantiomer versus its enantiomeric pair are implicative of a functional linkage to a receptor. The receptor, which was found as linked to the cannabinoid-mediated inhibition of iNO production, was CB_1-based through the application of antagonist experiments. Treatment of microglia with the CB_1-selective antagonist SR141716A prior to exposure to the agonist CP55940 blocked the CP55940-mediated inhibition of iNO production.

Cannabinoid-mediated alteration of microglial activities, as linked to the CB_2, appears to be limited to that window of cell activation encompassing the responsive and primed states, for which the CB_2 is expressed at high levels. Critical activities of responsive and primed microglia include chemotaxis and antigen processing/presentation, respectively. We have demonstrated that the partial agonist THC and the full agonist CP55940 inhibit the processing of select antigens by murine peritoneal macrophages and that this effect is mediated, at least in part, by the CB_2 [47, 48]. These observations suggest that a similar effect may occur for microglia. There is, however, accumulating evidence that the CB_2 is expressed in vivo by microglia in the context of a variety of inflammatory states [49]. We have shown that *Acanthamoeba culbertsoni* (**Fig. 3A**), an opportunistic, human pathogen, which is the causative agent of granulomatous amebic encephalitis (GAE) [50], can induce increased levels of CB_2 in microglia in vitro (Fig. 3B). Comparable results were obtained when total RNA from brain of mice inoculated with *A. culbertsoni* was assessed for CB_2 mRNA (Fig. 3C). Histopathological analysis of brain sections revealed focal brain lesions containing amebae circumscribed by cells exhibiting morphological features typical of microglia (Fig. 3D). Collectively, these results, although not definitive, are consistent with microglia as the source of inducible expression of CB_2 in vivo and suggest a potential target for ablating inflammation associated with GAE.

Recent studies suggest that a third, yet-to-be cloned receptor, a non-CB_1, non-CB_2 receptor [12–14], can also play a role in cannabinoid mediation of microglial activities. We have shown that the potent cannabinoid receptor agonist levonantradol (K_i=1.06 nM) inhibits the inducible expression of mRNAs for the proinflammatory cytokines IL-1α and TNF-α in a mode that is not blocked by the CB_1 antagonist SR141716A or the CB_2 antagonist SR144528 (**Fig. 4**). Similarly, the partial agonist THC (K_i=42 nM) and the agonist CP55940 (K_i=0.9 nM) inhibited the induction of

TABLE 1. Properties of Select Cannabinoid Receptor Ligands

Ligand[a]	Description	Receptor target	Action	Comments	References
THC	Partial agonist	CB_1/CB_2	Activates CB_1/CB_2	Has lower intrinsic activity than full agonist and produces lower maximum effect; CB_1 Ki = 40.7 nM; CB_2 Ki = 36.4 nM	[38]
CP55940	Agonist	CB_1/CB_2	Activates CB_1/CB_2	CB_1/CB_2 Ki = 0.9 nM	[5, 39–41]
CP56667	Stereoisomer of CP55940	–	–	Pharmacologically less active than CP55940; CB_1/CB_2 Ki = 62 nM	[5, 36]
HU210	Agonist	CB_1/CB_2	Activates CB_1/CB_2	CB_1Ki = 0.0606 nM; CB_2 Ki = 0.524 nM	[11, 42, 43]
HU211	Stereoisomer of HU210	–	–	Pharmacologically less active than HU210; CB_1 Ki > 10 μM	[44]
Levonantradol	Agonist	CB_1/CB_2	Activates CB_1/CB_2	CB_1/CB_2 Ki = 1.06 nM	[45]
Dextronantradol	Stereoisomer of levonantradol	–	–	Pharmacologically less active than levonantradol; CB_1/CB_2 Ki = 3100 nM	[45]
SR141716A	Antagonist	CB_1	Blocks agonist activation of CB_1	CB_1 Ki = 5.6 nM; CB_2 Ki = 1000 nM	[39]
SR144528	Antagonist	CB_2	Blocks agonist activation of CB_2	CB_1 Ki = 437 nM; CB_2 Ki = 0.60 nM	[46]

[a] Ligand abbreviations: CP55940: (1R,3R,4R)-3-[2-hydroxy-4-(1,1-dimethylheptyl)phenyl]-4-(3-hydroxypropyl)cyclohexan-1-ol; CP56667: (1S,3S,4S)-3-[2-hydroxy-4-(1,1-dimethylheptyl)phenyl]-4-(3-hydroxypropyl)cyclohexan-1-ol; HU210: (6aR,10aR)-3-(1,1′-dimethylheptyl)-6a,7,10,10a-tetrahydro-1-hydroxy-6,6-dimethyl-6H-dibenzo[b,d]pyran-9-methanol; HU211: 3-(1,1-dimethylheptyl)-6aS,7,10,10aS-tetrahydro-1-hydroxy-6,6-dimethyl-6H-dibenzo[b,d]pyran-9-methanol; SR141716A: N-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide hydrochloride; SR144528: N[(1S)-endo-1,3,5-trimethylbicyclo[2.2.1]heptan-2-yl]-5-(4-chloro-3-methylphenyl)-1-(4-methylbenzyl)pyrazole-3-carboxamide. Ki: molar association constant.

cytokine mRNAs for IL-1α, IL-1β, IL-6, and TNF-α in a mode that was not blocked by the CB_1 or the CB_2 antagonist (data not shown). Furthermore, enantiomeric selectivity for the CB_1/CB_2 high-affinity ligands, as compared with the paired, lower affinity counterparts, was not observed [51]. The "less bioactive" enantiomers CP56667 and HU211 exhibited inhibitory activity comparable with that of the potent CB_1/CB_2 agonists CP55940 and HU210, respectively. A similar outcome was obtained when the stereoisomers levonantradol (K_i=1.06 nM) and dextranantradol (K_i=3100 nM) were used. Collectively, the observations that gene expression for proinflammatory cytokines is associated with microglia that are fully activated and express low levels of CB_2, that stereoselective paired cannabinoids exert comparable inhibitory effects on the induction of proinflammatory cytokine mRNAs, and that the CB_1 and CB_2 selective antagonists SR141716A and SR144528 do not block the inhibition of cytokine gene expression by the agonists CP55940 and levonantradol indicate that cannabinoid-mediated modulation of proinflammatory cytokine gene expression is not linked to the CB_1 or the CB_2. Whether these results are indicative of the presence of a non-CB_1, non-CB_2 receptor in microglia, which is functionally relevant when these cells are in a state of full activation, awaits biochemical and molecular analysis.

In summary, microglia are macrophage-like cells that undergo a multistep process to full activation during inflammation. This multistep process is associated with differential gene expression and the acquisition of correlative functional activities. The differential expression of genes in relation to cell activation also applies to cannabinoid receptors. The CB_1 is expressed constitutively and at low levels throughout multistep activation, indicating a potential for this receptor to be functionally relevant for a broad spectrum of cannabinoid-mediated effects. However, as it is expressed at low levels, it may exhibit less "sensitivity" to the action of nonselective CB_1/CB_2 agonists as compared with the CB_2. In contrast, the CB_2 is expressed inducibly and is present at high levels as compared with the CB_1 when microglia are in responsive and primed states of activation. A signature, functional activity attributed to microglia, when in a responsive state, is chemotaxis, and these observations are consistent with reports of cannabinoid effects on cell migration, which is linked to the CB_2 [52]. Recent pharmacological data suggest the existence of a third cannabinoid receptor, a non-CB_1, non-CB_2 receptor, which may play a role in cannabinoid-mediated inhibition of proin-

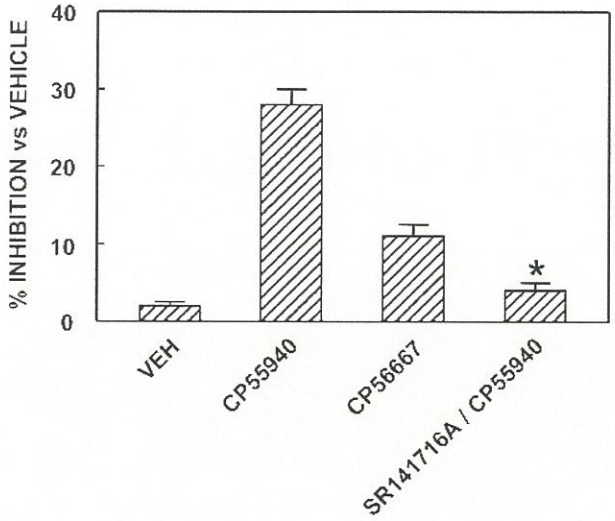

Fig. 2. CP55940-mediated inhibition of iNO release by microglia is blocked by the CB_1 receptor-selective antagonist SR141716A. Microglia were pretreated (1 h) with 5×10^{-7} M SR141716A prior to exposure (8 h) to 5×10^{-6} M CP55940 or CP56667 and LPS plus IFN-γ activation (24 h). Culture supernatants were assayed for nitrite using the Griess reagent. The CP55940 inhibition was stereoselective, as the paired enantiomer CP56667 was less bioactive. Results (mean±SEM of triplicate wells) are expressed as percent inhibition versus vehicle control (*, $P<0.01$, vs. SR141716A). Nitrite accumulation in LPS plus IFN-γ-treated vehicle control cultures was 29.3 ± 3.5 ($\mu M/10^6$ cells). VEH, Vehicle (0.01% ethanol).

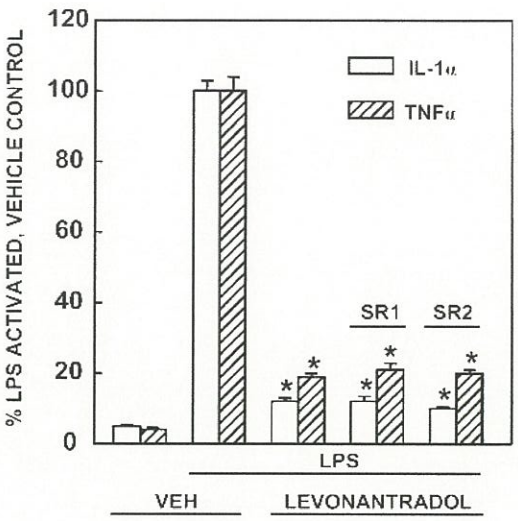

Fig. 4. The CB_1 and CB_2 receptor-specific antagonists do not block the inhibitory effects of levonantradol ($K_i = 1.06$ nM) on cytokine mRNA expression. Microglia were treated (4 h) with levonantradol or pretreated (1 h) with 1×10^{-6} M SR141716A or SR144528 prior to exposure (3 h) to 1×10^{-6} M levonantradol. Cells then were treated (6 h) with LPS (100 ng/ml), and levels of cytokine mRNA were determined using the RiboQuant multiprobe RPA (PharMingen). The ordinate designates that the data are presented as the percent LPS-treated (100 ng/ml) vehicle control. Results are expressed as the mean percent cytokine mRNA expression of triplicate cultures versus that for the LPS-treated vehicle mean ± SEM (*, $P<0.01$; two-tailed Student's t-test). SR1: CB_1-selective antagonist SR141716A; SR2: CB_2-selective antagonist SR144528; VEH: 0.01% ethanol.

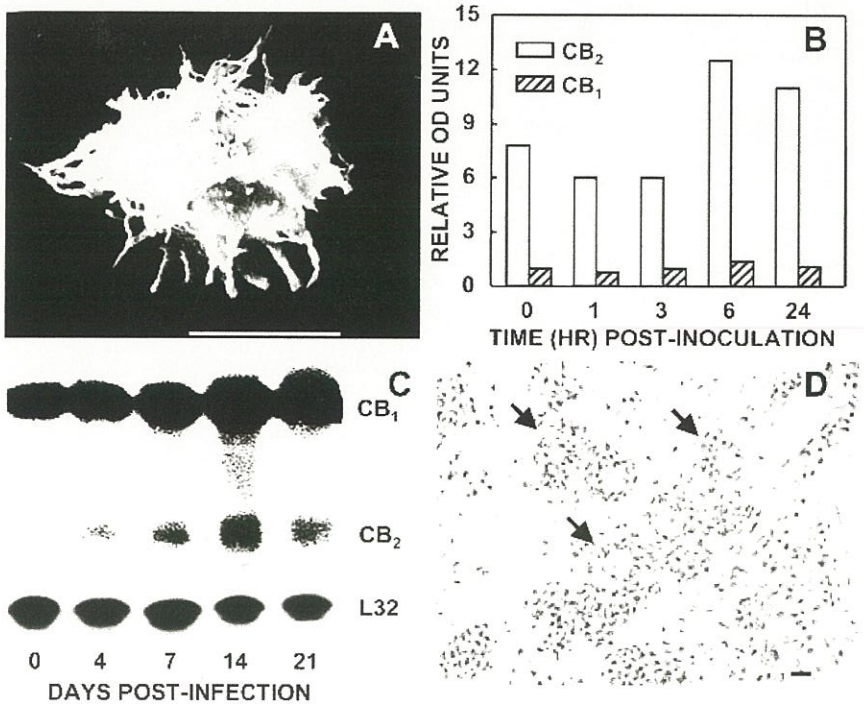

Fig. 3. Augmentation of CB_2 mRNA levels in response to *A. culbertsoni*. (A) Scanning electron micrograph of an *A. culbertsoni* trophozoite. (B) Graphic representation of relative levels of cannabinoid receptor mRNA. Microglia were incubated with *A. culbertsoni* at a microglia:ameba ratio of 10:1. Levels of mRNA for the CB_1 receptor remained unaffected, but those for the CB_2 receptor exhibited a time-dependent augmentation as determined by RNase protection assay (RPA; PharMingen, San Diego, CA). The ordinate designated as Relative OD Units represents densitometric analysis based on area X pixel density relative to that of constitutively expressed L32 ribosomal (L32) mRNA product for each sample. The graph represents a representative single experiment. (C) RPA detection of mRNA for CB_1 and CB_2 receptors from whole brain homogenates of mice infected intranasally with *A. culbertsoni* (1×10^5 50% lethal dose). As expected, an excess of mRNA for the CB_1 was obtained from whole brain homogenates. An apparent increase in levels of CB_1 mRNA was noted for homogenates of brain obtained at 14 and 21 days. mRNA levels for the CB_2 receptor exhibited a time-related increase following infection with amebae. Infection was confirmed by isolation of amebae in culture from mouse brains. (D) Hematoxylin and eosin-stained murine brain section demonstrating multiple foci of amebae surrounded by cells (arrows), which morphologically resemble microglia. (A, D) Original bars, 10 μm.

flammatory cytokine production, an activity that may be associated with microglia when fully activated.

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REFERENCES

1. Nahas, G., Latour, C. (1992) The human toxicity of marijuana. *Med. J. Aust.* **156,** 495–497.
2. Turner, C. E., Elsohly, M. A., Boeren, E. G. (1980) Constituents of *Cannabis sativa* L. XVII. A review of the natural constituents. *J. Nat. Prod.* **43,** 169–234.
3. Wing, D. R., Leuschner, J. T. A., Brent, G. A., Harvey, D. J., Paton, W. D. M. (1985) Quantitation of in vivo membrane-associated Δ-1-tetrahydrocannabinol and its effect on membrane fluidity. In *Marihuana '84: Proceedings of the Oxford Symposium on* Cannabis (D. J. Harvey, ed.), Oxford, UK, IRL.
4. Matsuda, L. A., Lolait, S. J., Brownstein, M. J., Young, A. C., Bonner, T. I. (1990) Structure of a cannabinoid receptor and functional expression of the cloned cDNA. *Nature* **346,** 561–564.
5. Compton, D. R., Rice, K. C., De Costa, B. R., Razdan, R. K., Melvin, L. S., Johnson, M. R., Martin, B. R. (1993) Cannabinoid structure-activity relationships: correlation of receptor binding in vivo activities. *J. Pharmacol. Exp. Ther.* **265,** 218–226.
6. Munro, S., Thomas, K. L., Abu-Shaar, M. (1993) Molecular characterization of a peripheral receptor for cannabinoids. *Nature* **365,** 61–65.
7. Galiegue, S., Mary, S., Marchand, J., Dussossoy, D., Carriere, D., Carayon, P., Bouaboula, M., Shire, D., Le Fur, G., Casellas, P. (1995) Expression of central and peripheral cannabinoid receptors in human immune tissues and leukocyte subpopulations. *Eur. J. Biochem.* **232,** 54–61.
8. Howlett, A. C., Qualy, J. M., Khachatrian, L. L. (1986) Involvement of Gi in the inhibition of adenylate cyclase by cannabimimetic drugs. *Mol. Pharmacol.* **29,** 307–313.
9. Mackie, K., Hille, B. (1992) Cannabinoids inhibit N-type calcium channels in neurobalstoma-glioma cells. *Proc. Natl. Acad. Sci. USA* **89,** 3825–3829.
10. Sim, L. J., Hampson, R. E., Deadwyler, S. A., Childers, S. R. (1996) Effects of chronic treatment with Δ^9-tetrahydrocannabinol on cannabinoid-stimulated [^{35}]GTPγS autoradiography in rat brain. *J. Neurosci.* **16,** 8057–8066.
11. Felder, C. C., Joyce, K. E., Briley, E. M., Mansouri, J., Mackie, K., Blond, O., Lai, Y., Ma, A. L., Mitchell, R. L. (1995) Comparison of the pharmacology and signal transduction of the human cannabinoid CB_1 and CB_2 receptors. *Mol. Pharmacol.* **48,** 443–450.
12. Breivogel, C. S., Griffin, G., Di Marzo, V., Martin, B. R. (2001) Evidence for a new G protein-coupled cannabinoid receptor in mouse brain. *Mol. Pharmacol.* **60,** 155–163.
13. Di Marzo, V., Breivogel, C. S., Tao, Q., Bridgen, D. T., Razdan, R. K., Zimmer, A. M., Zimmer, A., Martin, B. R. (2000) Levels, metabolism, and pharmacological activity of anandamide in CB(1) cannabinoid receptor knockout mice: evidence for non-CB(1), non-CB(2) receptor-mediated actions of anandamide in mouse brain. *J. Neurochem.* **75,** 2434–2444.
14. Jarai, Z., Wagner, J. A., Varga, K., Lake, K. D., Compton, D. R., Martin, B. R., Zimmer, A. M., Bonner, T. I., Buckley, N. E., Mezey, E., Razdan, R. K., Zimmer, A., Kunos, G. (1999) Cannabinoid induced mesenteric vasodilation through an endothelial site distinct from CB1 or CB2 receptors. *Proc. Natl. Acad. Sci. USA* **96,** 14136–14141.
15. Mann, P. E. G., Cohen, A. B., Finley, T. N., Ladman, A. J. (1971) Alveolar macrophages. Structural and functional differences between non-smokers and smokers of marijuana in vitro. *Lab. Invest.* **25,** 111–120.
16. Raz, A., Goldman, R. (1976) Effect of hashish compounds on mouse peritoneal macrophages. *Lab. Invest.* **34,** 69–76.
17. Cabral, G. A., Dove Pettit, D. A. (1998) Drugs and immunity: cannabinoids and their role in decreased resistance to infectious disease. *J. Neuroimmunol.* **83,** 116–123.
18. Aloisi, F., Ria, F., Penna, G., Adorini, L. (1998) Microglia are more efficient than astrocytes in antigen processing and in Th1 but not Th2 cell activation. *J. Immunol.* **160,** 4671–4680.
19. Gehrmann, J., Matsumoto, Y., Kreutzberg, G. W. (1995) Microglia: intrinsic immuneffector cell of the brain. *Brain Res. Brain Res. Rev.* **20,** 269–287.
20. Ling, E. A., Wong, W. C. (1993) The origin and nature of ramified and amoeboid microglia: a historical review and current concepts. *Glia* **7,** 9–18.
21. Stoll, G., Jander, S. (1999) The role of microglia and macrophages in the pathophysiology of the CNS. *Prog. Neurobiol.* **58,** 233–247.
22. Benveniste, E. N. (1997) Role of macrophages/microglia in multiple sclerosis and experimental allergic encephalomyelitis. *J. Mol. Med.* **75,** 165–173.
23. Kreutzberg, G. W. (1995) Microglia, the first line of defense in the brain pathologies. *Arzneimittelforschung* **45,** 357–360.
24. Kreutzberg, G. W. (1996) Microglia: a sensor for pathological events in the CNS. *Trends Neurosci.* **19,** 312–318.
25. Leong, S., Ling, E. (1992) Ameboid and ramified microglia: their interrelationship and response to brain injury. *Glia* **6,** 39–47.
26. Perry, S. W. (1990) Organic mental disorders caused by HIV: update on early diagnosis and treatment. *Am. J. Psychiatry* **147,** 696–710.
27. Streit, W. J., Graeber, M. B., Kreutzberg, G. W. (1988) Functional plasticity of microglia: a review. *Glia* **1,** 301–307.
28. Matsumoto, Y., Ohmori, K., Fujiwara, M. (1992) Microglial and astroglial reactions to inflammatory lesions of experimental autoimmune encephalomyelitis in the rat central nervous system. *J. Neuroimmunol.* **37,** 23–33.
29. Rogers, J., Luber-Nardo, J., Styren, S. D., Civin, W. H. (1988) Expression of immune system-associated antigens by cells of the central nervous system: relationship to the pathology of Alzheimer's disease. *Neurobiol. Aging* **9,** 339–349.
30. McGeer, P. L., Itagaki, S., Boyes, B. E., McGeer, E. G. (1988) Reactive microglia are positive for HLA-DR in the substantia nigra of Parkinson's and Alzheimer's disease brains. *Neurology* **38,** 1285–1291.
31. Dickson, D. W., Mattiace, L. A., Kure, K., Hutchins, K., Lyman, W. D., Brosnan, C. F. (1991) Microglia in human disease, with an emphasis on acquired immune deficiency syndrome. *Lab. Invest.* **64,** 135–156.
32. Merrill, J. E., Chen, I. S. Y. (1991) HIV-1, macrophages, glial cells, and cytokines in AIDS nervous system disease. *FASEB J.* **5,** 2391–2397.
33. Spencer, D. C., Price, R. W. (1992) Human immunodeficiency virus and the central immune system. *Annu. Rev. Microbiol.* **46,** 655–693.
34. Giulian, D., Baker, T. J., Shih, L. N., Lachman, L. B. (1986) IL-1 of the central nervous system is produced by ameboid microglia. *J. Exp. Med.* **164,** 594–604.
35. Reid, D. M., Perry, V., Andersson, P., Gordon, S. (1993) Mitosis and apoptosis of microglia in vivo induced by an anti-CR3 antibody which crosses the blood-brain barrier. *Neuroscience* **56,** 529–533.
36. Waksman, Y., Olson, J. M., Carlisle, S. J., Cabral, G. A. (1999) The central cannabinoid receptor (CB1) mediates inhibition of nitric oxide production by rat microglial cells. *J. Pharmacol. Exp. Ther.* **288,** 1357–1366.
37. Carayon, P., Marchand, J., Dussossoy, D., Derocq, J. M., Jbilo, O., Bord, A., Bouaboula, M., Galiegue, S., Mondiere, P., Penarier, G., Fur, G. L., Defrance, T., Casellas, P. (1998) Modulation and functional involvement of CB2 peripheral cannabinoid receptors during B-cell differentiation. *Blood* **92,** 3605–3615.
38. Showalter, V. M., Compton, D. R., Martin, B. R., Abood, M. E. (1996) Evaluation of binding in a transfected cell line expressing a peripheral cannabinoid receptor (CB2); identification of cannabinoid receptor subtype selective agonists. *J. Pharmacol. Exp. Ther.* **278,** 989–999.
39. Rinaldi-Carmona, M., Barth, F., Heaulme, M., Shire, D., Calandra, B., Congy, C., Martinez, S., Maruani, J., Neliat, G., Caput, D., Ferrara, P., Soubrie, P., Breliere, J. C., Le Fur, G. (1994) SR141716A, a potent and selective antagonist of the brain cannabinoid receptor. *FEBS Lett.* **350,** 240–244.
40. Wiley, J. L., Barrett, R. L., Lowe, J., Balster, R. L., Martin, B. R. (1995) Discriminative stimulus effects of CP 55,940 and structurally dissimilar cannabinoids in rats. *Neuropharmacology* **34,** 669–676.
41. Thomas, B. F., Gilliam, A. F., Burch, D. F., Roche, M. J., Seltzman, H. H. (1998) Comparative receptor binding analyses of cannabinoid agonists and antagonists. *J. Pharmcol. Exp. Ther.* **285,** 285–292.
42. Mechoulam, R., Feigenbaum, J. J., Lander, N., Segal, M., Jarbe, T. U., Hiltunen, A. J., Consroe, P. (1988) Enantiomeric cannabinoids: stereospecificity of psychotropic activity. *Experientia* **44,** 762–764.
43. Howlett, A. C., Champion, T. M., Wilken, G. H., Mechoulam, R. (1990) Stereochemical effects of 11-OH-Δ^8-tetrahydrocannabinol-dimethylheptyl

to inhibit adenylate cyclase and bind to the cannabinoid receptor. *Neuropharmacology* **29,** 161–165.
44. Pertwee, R. G. (1999) Pharmacology of cannabinoid receptor ligands. *Curr. Med. Chem.* **6,** 635–664.
45. Howlett, A. C., Fleming, R. M. (1984) Cannabinoid inhibition of adenylate cyclase. Pharmacology of the response in neuroblastoma cell membranes. *Mol. Pharmacol.* **26,** 532–538.
46. Rinaldi-Carmona, M., Barth, F., Millan, J., Derocq, J-M., Casellas, P., Congy, C., Oustric, D., Sarran, M., Bouaboula, M., Calandra, B., Portier, M., Shire, D., Breliere, J. C., Le Fur, G. L. (1998) SR 144528, the first potent and selective antagonist of the CB2 cannabinoid receptor. *J. Pharmcol. Exp. Ther.* **284,** 644–650.
47. McCoy, K. L., Gainey, D., Cabral, G. A. (1995) Δ-9-Tetrahydrocannabinol modulates antigen processing by macrophages. *J. Pharmacol. Exp. Ther.* **273,** 1216–1223.
48. McCoy, K. L., Matveyeva, M., Carlisle, S. J., Cabral, G. A. (1999) Cannabinoid inhibition of the intact lysozyme by macrophages: evidence for CB2 receptor participation. *J. Pharmacol. Exp. Ther.* **289,** 1620–1625.
49. Benito, C., Nunez, E., Tolon, R. M., Carrier, E. J., Rabano, A., Hillard, C. J., Romero, J. (2003) Cannabinoid CB2 receptors and fatty acid amide hydrolase are selectively overexpressed in neuritic plaque-associated glia in Alzheimer's disease brains. *J. Neurosci.* **23,** 11136–11141.
50. Marciano-Cabral, F., Cabral, G. A. (2003) *Acanthamoeba* spp. as agents of disease in humans. *Clin. Microbiol. Rev.* **16,** 273–307.
51. Puffenbarger, R. A., Boothe, A. C., Cabral, G. A. (2000) Cannabinoids inhibit LPS-inducible cytokine mRNA expression in rat microglial cells. *Glia* **29,** 58–69.
52. Walter, L., Franklin, A., Witting, A., Wade, C., Xie, Y., Kunos, G., Mackie, K., Stella, N. (2003) Nonpsychotropic cannabinoid receptors regulate cell migration. *J. Neurosci.* **23,** 1398–1405.

Chronic Microglial Activation and Excitotoxicity Secondary to Excessive Immune Stimulation: Possible Factors in Gulf War Syndrome and Autism

Russell L. Blaylock, M.D.

ABSTRACT

There is considerable and growing evidence that chronic microglial activation plays a major role in numerous neurological conditions including Alzheimer's dementia, Parkinson's disease, ALS, strokes, and inflammatory brain diseases. The release of toxic elements from activated microglia, such as cytokines and excitotoxins, is known to produce neurodegeneration. Peripheral immune stimulation has been shown to activate CNS microglia, and when excessive can lead to neurodegeneration and cognitive defects commonly associated with both the Gulf War Syndrome (GWS) and autism. This paper summarizes the mechanism linking these two disorders with excessive immune stimulation secondary to overvaccination.

Clinical Features of Gulf War Syndrome and Autism

Following the first Gulf War, tens of thousands of American and British veterans began to suffer from numerous complaints, which have become known as the Gulf War Syndrome (GWS). These included recurrent fevers, cognitive difficulties, neuropsychiatric problems, polyarthralgias, chronic fatigue syndrome, and numerous allergies where none existed before.

In addition, an increase in birth defects has been documented in the offspring of Gulf veterans, including tricuspid valve insufficiency, aortic valve stenosis, renal agenesis or hypoplasia, and hypospadias.[1]

One recent study documented reduced cerebrovascular flow and impaired ability to handle complex cognitive tasks in a substantial number of these veterans.[2] Another study found that cognitive symptoms correlated closely with immune dysfunction in Gulf War veterans, but not with nonveteran chronic fatigue controls.[3] The Gulf War veteran was exposed to as many as 17 inoculations over a very short period.

In many ways, behavioral problems associated with this syndrome resemble astheno-emotional disorder, which is characterized by fatigue with prolonged mental activity, stress intolerance, decreased concentration and short term memory, headache with mental processing, irritability, and sensitivity to sounds or lights.[4] The anatomic substrate of this syndrome–ascribed to the hippocampus, limbic connections, temporal lobes, and parts of the frontal lobes–overlaps those most damaged by neuroinflammation and attendant excitotoxicity.

In the instance of autism, the significant and unexplained increase beginning in the 1980s paralleled the introduction of a host of new vaccines,[5] although vaccine authorities do not accept a causal connection. The neurologic dysfunction seen in autism and the autism spectrum disorders, like that of the GWS, involves the limbic connections. Because of the rapid development of the child's brain and the immaturity of various biochemical systems, symptoms are seen in the autistic child that would not be expected in the adult, especially echolalia, inappropriate laughing and giggling, and various language-related symptoms. As with GWS, significant immune dysfunction is seen in cases of autism.

The Effect of Immune Stimulation on the Central Nervous System

There is growing evidence that overstimulation of systemic immunity can produce deleterious effects on nervous system function, including neurodegeneration. While most are aware that autoimmunity can occur when nervous system components are involved in immune reactions–for example, with postvaccinal encephalitis and subacute sclerosing panencephalitis–few are aware of chronic neurodegeneration without autoimmunity. Recent evidence indicates that a different type of reaction can occur that may have relevance not only to autism spectrum disorders and GWS, but also to neurotrauma, ischemia, and various neurodegenerative diseases.

McGeer and co-workers have recently defined an immune process not involving autoimmunity, but rather a nonspecific immune destruction of neurons, neurites, and synaptic connections,[6] referred to as autotoxicity. In this process, either systemic immune factors (cytokines) or local immune factors, such as ß-amyloid or viral components, can activate the brain's immune system via activation of astrocytes and microglia. In both instances brain levels of complement, cytokines, reactive oxygen and nitrogen species, cellular immune components, proteases, adhesion molecules, excitotoxins, arachidonic acid, and other chemokines are released. These toxic compounds cause bystander injury to surrounding normal neural elements.

The Role of the Microglia

The central nervous system's immune system is controlled by the microglia, a diffuse set of normally resting cells that when activated can assume ameboid activity and secrete numerous cytokines, chemokines, eicosanoids, proteases, complement,

and at least two excitotoxins. Under most acute conditions and during neurodevelopment, the immune cytokines can act as neurotrophic substances, protecting and promoting neurite growth. With intense activation and when chronically activated, these cytokines and other secretory components of the microglia can be very destructive.

High concentrations of interleukin-1ß (IL-1ß) injected into the brain can cause local inflammation and degeneration of neurons,[7] and even small concentrations in the presence of ß-amyloid, ischemia, trauma, or excitotoxins, can trigger destructive reactions.[8]

The key role of the microglia is demonstrated by the fact that mixed cultures of neurons and microglia will produce intense destruction of the neurons when lipopolysaccharide is added. This does not occur in the absence of microglia.

Activation of microglia has been shown to be an early event, both in experimental models and in human cases of Parkinson's and Alzheimer's diseases.[9,10] In fact, behavioral changes and clinical effects occur before the appearance of amyloid plaques and neurofibrillary tangles in the case of Alzheimer's disease. That these inflammatory processes can be aggravated by systemic activation of microglia has been recently shown in trials of experimental amyloid-beta (Abeta) vaccines used in Alzheimer's patient volunteers in whom several cases of encephalitis were seen.[11]

Microglial activation as a central destructive process has been demonstrated in a growing list of conditions, including multiple sclerosis (MS), Alzheimer's dementia, Parkinson's disease, amyotrophic lateral sclerosis (ALS), Huntington's disease, supranuclear palsy, macular degeneration, glaucoma, trauma, strokes, viral encephalopathy, human immunodeficiency virus (HIV)-associated dementia, and prion disorders.[12] It is also known that IL-1ß can greatly aggravate experimental allergic encephalitis (EAE) when injected into the spinal cord,[13] and that blocking tumor necrosis factor-alpha (TNF-alpha) and IL-1ß can significantly reduce the destruction of EAE.

Many events can activate the microglial immune cytokines, including introduction of heavy metals, aluminum, oxidized LDL, amyloid, viruses, mycoplasma, bacteria, and glutamate, and that the entry point on the microglial membrane is the mitogen-activated protein kinase (MAPK) and its stress protein enzyme (SAPK).[14]

Once microglia are activated, eicosanoid production is also elevated, primarily through the cyclooxygenase-1 (COX-1) enzyme.[15] At the same time there is increased secretion of arachidonic acid and production of prostaglandin E2, a powerful inflammatory cytokine. Release of these destructive elements in the zone of attack spreads to surrounding areas, causing what is called bystander damage, the extent of which depends on the intensity of the microglial activation and how long it persists. In most instances, activation of microglia terminates rapidly, thereby minimizing bystander damage. With chronic microglial activation, bystander damage can be extensive. This is what appears to occur in autism disorders and GWS.

Elevations of brain IL-1ß have been shown to interfere with long-term potentiation (LTP), a critical process in memory development.[16] IL-2 and IL-6 have also been implicated in altering memory and learning.[17] This is especially so in stressful situations, such as would be seen in a theater of war. Other studies have shown reduced hippocampal neurogenesis with chronic elevations of IL-6 and tumor necrosis factor-alpha (TNF-alpha).[18] This would be of special concern in infants and young children.

Microglial activation is known to occur in all of the viral encephalopathies and even spongioform encephalopathies.[19,20] This is particularly important when considering that the persistence of measles virus in the brain is known to occur in 20 percent of the cases of exposure to the virus. This is much higher than is clinically evident. It is not even necessary that the virus enter the neurons, as with HIV-1 dementia. In this instance a viral protein fragment (gp41) is sufficient to activate brain microglia, leading to dementia. *Herpes simplex* virus type 1 (HSV-1) has also been shown to produce brain degeneration by the same mechanism.

The Role of Excitotoxicity

Damage to neuronal mechanisms is not limited to direct cytokine effects. The excitotoxicity initiated by microglial activation is even more important. In cases of HIV-1 dementia, it is known that brain quinolinic acid, an excitotoxin secreted by activated microglia, can increase more than 300-fold.[21] In addition, glutamate is released in concentrations that are known to destroy neurons and/or synaptic connections.

Because glutamate can also activate microglia and enhance cytokine-induced neurodegeneration,[22] a vicious cycle is created in which immune cytokines can stimulate release of glutamate, and glutamate in turn enhances cytokine production and release. Moreover, cytokines inhibit glutamate transporters, which play a critical role in removal of excess extracellular glutamate. Intimately linked to excitotoxicity is the generation of destructive free radicals, especially the reactive nitrogen species such as peroxynitrite and nitrosoperoxycarbonate.[23] Much of the injury to dendrites, synapses and neurons, by both cytokines and excitotoxicity, is caused by free radicals.

The reactive nitrogen species are responsible for nitration of DNA residues, tyrosine, tryptophan, amines, and metalloproteins, thereby producing widespread disruption of numerous biochemical processes. In particular, peroxynitrite plays a major role in suppressing mitochondrial energy production. This not only interferes with brain function, but also greatly enhances excitotoxicity. In fact, it has been shown that when neuron energy supplies are low, even physiologically normal concentrations of excitatory amino acids can become excitotoxic.[24]

Excitotoxicity is also greatly enhanced by low magnesium levels, which have been reported in most cases of autism and are frequent in cases of stress, as would be seen in a Gulf War veteran. In fact, by removing magnesium from a cerebral tissue culture, excitotoxic sensitivity is increased 100-fold, just as with energy

depletion. In addition, low magnesium levels have been shown to enhance release of inflammatory cytokines.[25]

Injecting excitotoxins into the brain has been shown to increase expression of IL-1ß messenger RNA and its protein (IL-1ß) and that when both excitotoxins and IL-1ß are infused into the striatum of the brain, significant destruction occurs some distance away in the cortex as well as at the site of injection.[26] This finding demonstrates the widespread nature of the damage caused when combining immune stimulation and excitotoxicity, which is the effect of chronic microglial activation.

Another particularly destructive product is 4-hydroxynonenal (4-HNE), an aldehydic lipid peroxidation product found in abundant supply in cases of neurodegeneration. Interestingly, peroxylnitrite and 4-HNE are found in the same neurons. This lipid peroxidation product has been shown to be especially destructive of synaptic connections and mitochondrial enzymes and can significantly inhibit glutamate transport proteins. It is neutralized only by alpha-lipoic acid, glutathione, and certain flavonoids.

Elevations in brain glutamate and aspartate impair memory retention and damage hypothalamic neurons in both infant mice and adults.[27] This can be explained by the fact that the hippocampus and amygdala contain numerous glutamate-type receptors, which are thought to play a central role in learning and memory via LTP. In addition, their limbic connections, frontal and cingulate, play a major role in emotional elaboration. Moreover, microinjections of excitotoxins into the ventral subiculum of the hippocampus increase locomotor activity, strongly resembling that seen in some autistic children and attention deficit hyperactivity disorder (ADHD).[28] Hyperresponsiveness to stressful stimuli can be produced by excitotoxic damage to the ventral hippocampus at a very early age, something that would be expected in cases of autistic hyperimmune responses.

The consequence of damage to synapses, dendrites, and cell bodies would be different in the developing brain, especially during the period of the brain growth spurt from the last trimester of pregnancy to age two years. It has been shown that excitotoxicity cannot only disrupt neural elements and function but can alter brain pathway development, resulting in a "mis-wired" brain.[29]

In the adult, one would expect to see impairment of attention/memory, depression, and social withdrawal with similar injuries to the limbic system.

Neurologic and Behavioral Effects of Cytokines

Some of the best information we have concerning cytokine effects on brain function comes from their use in treating hepatitis and cancer patients. The effects of these substances are divided into acute and chronic. The acute effects resemble influenza and persist for one to three weeks. Chronic effects can occur with all dosages, routes of administration, and schedules. Higher doses are more likely to produce more profound and persistent effects.

A growing number of human studies have shown dramatic alterations in learning, memory, attention, and affective state, as well as perceptual and motor functions following cytokine treatments.[30] ALS patients treated with interferon have demonstrated significant impairment of verbal memory and calculation ability.[31] In another study involving 44 cancer patients receiving IL-2, it was found that 71 percent receiving the high dose developed mild-to-severe behavioral changes, including cognitive dysfunction.[32] All these behavioral symptoms resolved within three days of treatment cessation.

Patients receiving more than 1 million units of interferon–alpha show some constitutional symptoms. Chronic symptoms are seen with all doses, but are more likely at doses greater than 18 to 20 million units.[33] Renault et al. divided the behavioral symptoms into three categories: organic personality syndrome, organic affective syndrome, and delirium.[34]

One set of symptoms seems to be similar to those of autism spectrum patients, including uncontrollable overreaction to minor frustration, marked irritability, and a short temper. Gulf War veterans commonly display organic affective symptoms such as depression, feelings of hopelessness, and uncontrollable crying spells. Clouded consciousness, disorientation, irritability, and mood alterations similar to that seen in both autism patients and Gulf War veterans were also seen. In addition, patients receiving the interferon-alpha developed periods of severe agitation, became abusive, and often withdrew from others–other findings they have in common with the autistic child.

Psychomotor retardation was seen in 47 to 80 percent of patients treated with interferon-alpha, along with social withdrawal.[35] Cognitive changes also occurred, with a shortening of attention span, impaired short-termed memory, and mental fog. Other reports describe patients who exhibit periods of silence, and without warning stare vacantly even in mid-sentence.[36]

Most cognitive changes have been reported to be reversible, but some reports describe persistent cognitive problems lasting up to two years following cessation of therapy. In rare instances, patients will become fully demented.[37] This is understandable when we recall that interferon can activate excitotoxicity and produce severe free-radical injury to synapses and neurons. Interferon-gamma is reported to increase superoxide levels. Slowing of thought processes, confusion, and even Parkinsonian symptoms have been reported in patients using interferon-gamma.[38]

IL-2, used to treat infectious disease and cancer, has been shown to result in mental status changes, agitation, combativeness, hallucinations, difficulty concentrating, and delusions. IL-1 has been associated with ideational delusions, seizures, agitation, and somnolence, while TNF-alpha can cause transient amnesia, hallucinations, and even aphasia.

As we have seen, both IL-1ß and TNF-alpha can block glutamate reuptake, resulting in high levels of toxic extracellular glutamate.[39] Cognitive defects are also common following IL-1ß based on inhibition of LTP, which is essential for memory acquisition.

The Role of Vaccines

As stated above, peripheral immune stimulation readily activates the brain's immune system. In most instances this is short-lived, and neuron damage is minimal. Chronic activation of microglia, however, can lead to substantial disruption of neuronal function and even neurodegeneration.

Two basic processes seem to be responsible for the chronic stimulation of brain immunity: repeated, closely spaced inoculation without allowing brain recovery, and inoculation with live viruses or contaminant organisms that persist in the brain. Gulf War veterans were given some 17 inoculations very close together. Children are often given as many as five to seven inoculations during one visit to the pediatrician's office, several as combined vaccines, such as measles-mumps-rubella (MMR).

Of particular concern is the use of live organisms and contaminant organisms. Garth Nicolson and co-workers have demonstrated polymerase chain reaction (PCR) evidence of mycoplasma species in the blood samples of Gulf War veterans suffering from ALS, the incidence of which was found to be increased by 200 percent in this population.[40] Nicolson et al. found that 83 percent of veterans with ALS had positive tests, whereas positives were rarely seen in controls. It is hypothesized that the vaccines were contaminated primarily with *Mycoplasma fermentens*. Numerous activated microglia are found in the spinal cord of affected veterans. The involvement of live *M. fermentens* could also explain the appearance of similar illnesses in other household members.

Excitotoxins contribute to the damage in central nervous system infections. Cerebrospinal fluid glutamate levels rise in bacterial meningitis, and levels are directly correlated with prognosis. Extracellular glutamate levels are elevated in all cases of viral encephalopathies, including that of the acquired immunodeficiency syndrome (AIDS). Glutamate and aspartate levels in the plasma were also found to be elevated in 11 of 14 autistic children.[41] There is also evidence that viruses can enhance the toxicity of glutamate.[42]

Injection of the immune adjuvant lipopolysaccharide (LPS) closely resembles the vaccination process. In one study, it was shown for the first time that peripherally administered LPS decreased learning in mice.[43] The dose used did not produce observable injury to the neurons, but significantly impaired the animals' completing the Morris water maze and spontaneous alternation Y-maze, which tests spatial learning requiring a functional hippocampus. Associative learning was affected most. Memory retention was spared. LPS injection, by elevating IL-1 levels, has been shown to alter hippocampal norepinephrine and serotonin levels, as well as increasing glutamate levels.[44] Elevated serotonin levels have been described in autism.[45]

Long-term persistent immune activation and low-grade brain inflammation have been described in three children who recovered from *Herpex simplex* encephalitis before age two.[46] The children all demonstrated abundant activated microglia at brain biopsy and continued to deteriorate after viral treatment, indicating continued microgial activation. Viral fragments, without active infection, can produce this phenomenon.

Not all persistent viral infections are associated with obvious inflammatory responses. Using a hamster neurotrophic strain of measles, it was found that a noninflammatory encephalopathy could occur with destruction of the CA1 and CA3 segments of the hippocampus.[47] This could more closely resemble the situation in autistic child and some cases of GWS, since obvious clinical and laboratory signs of inflammation would be absent. Neurodegeneration caused by this neurotrophic measles virus was blocked using the NMDA receptor antagonist, MK-801, indicating an excitotoxic mechanism. (The NMDA receptor is the postsynaptic receptor for L-glutamate that can be activated by the drug N-methyl-D-aspartate.)

The smallpox vaccine is associated with postvaccinal encephalitis at a rate of 1 in 110,000 vaccinations. This includes only obvious cases of encephalitis; more chronic, subtle cases involving ill-defined neurological symptoms remote from the vaccination would be overlooked. Most vaccine follow-up studies do not extend beyond two weeks. It is obvious from the above studies that this follow-up period is far too short. More persistent neurotropic viruses now being discovered appear to be related to chronic neurodegeneration. These include HSV-1, coronavirus, measles virus, and human herpes viruses 6 and 7 (HHV-6 and HHV-7). A postvaccinal encephalopathy has been described in children under age two years following the smallpox vaccine.[48] Most of these occur as chronic conditions.

A Multifactorial Problem

Only part of the population exposed to these pathogenic factors have symptoms, and not everyone is affected in the same way. There is a critical interplay among many factors, including mercury exposure, exposure to other toxins (pesticides, chemical warfare agents, and insect repellants), other infectious agents, nutrition, antioxidant system status, and immune function.

Deficiencies in certain nutrients appear to be particularly common in the autistic child, including magnesium, pyridoxine (vitamin B6), and docosahexaenoic acid (DHA), an essential omega-3 fatty acid. Such deficiencies can enhance excitotoxicity. Vitamin B6 has been shown to lower blood and brain tissue glutamate levels. Magnesium acts at the NMDA receptor to down-regulate calcium entry into the neuron. DHA plays a particularly important role in cellular membrane function, especially mitochondrial membranes, and reduces excitotoxicity. Recent studies have also shown that it plays an important role in synaptic membranes as well.

Immune Dysfunction in GWS, Autism, and Other Behavioral Disorders

There is growing evidence that immunologic dysfunction plays a major role in both autism and GWS. Shifting the immune system from a Th1 profile to a Th2 pro-autoimmune profile has been described in the Gulf War veteran and in autistic children.[49,50] There is also compelling evidence that GWS is related to exposure to the vaccine adjuvant squalene.[51] In the autistic child, many observers have noted an increase in ear infections and upper respiratory infections.

There is growing evidence of autoimmunity in autism and other possibly related conditions, including ADHD. Warren and coworkers have described a common link in autism, ADHD, and dyslexia with the finding of an increased frequency of the C4B null gene (which does not produce the complement protein C4B).[52] Others have reported an increase in antibodies to basic myelin protein and neuronal axonal filaments in the brain.[52] Elevations of TNF-alpha have also been described in autism.[54]

This set of immune deficiencies would lead to direct cytokine activation of microglia by way of autoimmune-induced cytokine stimulation of microglial receptors. The immune deficiency caused by low levels of C4B complement, which is important in eliminating viruses, mycoplasma, and fungi would increase the likelihood of viral and mycoplasmal persistence in the brain. In both instances chronic activation of microglia would occur. Many cases of autism and GWS do not involve autoimmunity, but rather bystander injury.

Conclusions

The evidence suggests that overstimulation of the systemic immune system, as by repeated inoculations spaced close together, can result in chronic activation of brain microglia, the nervous system's immune mechanism.

There is abundant experimental and clinical evidence that elevations in cytokines can result in disruptions of brain function, both by generation of numerous free-radical types, and by release of the excitotoxins glutamate and quinolinic acid from activated microglia. Once activated, a complex interplay of oxygen and nitrogen stress, excitotoxicity, and immune cytokines alters synaptic connections, dendrite maintenance, and neuron function, leading to a myriad of symptoms like those seen in GWS and autism.

Both syndromes manifest an impaired peripheral immune system, a possible consequence of excessive vaccination itself, neurotoxic vaccine additives (aluminum and mercury), and immune-suppressive viruses such as the measles virus. This should serve as a caution to those who would add even more vaccines to a schedule already too crowded, as well as an indication to reassess the current schedule.

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REFERENCES

1. Araneta MR, Schlangen KM, Edmonds LD, et al. Prevalence of birth defects among infants of Gulf War veterans in Arkansas, Arizona, California, Georgia, Hawaii, and Iowa, 1989-1993. Birth Defects Research Part A. *Clin Mol Teratol* 2003;67:246-260.
2. Bugegin L, Mitzel HC, Miller CS, Gelineau JF, Tolstykh GP. Cognitive performance and cerebrohemodynamics associated with Persian Gulf Syndrome. *Toxicol Ind Health* 2001;17 (4):128-137.
3. Brimacombe M, Zhang Q, Lange G, Natelson BH. Immunological variables mediate cognitive dysfunction in Gulf War veterans but not civilians with chronic fatigue syndrome. *Neuroimmunomodulation* 2002;10(2):93-100.
4. Ronnback L, Hansson E. Does the astroglial network perform qualitative modifications of neuronal messages? In: Hansson E, Olsson T, Ronnback L, eds. *On Astrocytes and Glutamate Neurotransmission: New Waves in Brain Information Processing*. New York, N.Y.: Chapman & Hall; 1997:155-187.
5. Rimland B. The autism epidemic, vaccinations and mercury. *J Nutr Environ Med* 2000;10:261-266.
6. McGeer PL, McGeer EG. Local neuroinflammation and the progression of Alzheimer's disease. *J Neurovirol* 2002;8:529-538.
7. Wright JL, Merchant RE. Histopathological effects of intracerebral injections of human recombinant tumor necrosis factor-alpha in the rat. *Acta Neuropathol* (Berl) 1992;85:93-100.
8. Lawrence CB, Allan SM, Rothwell NJ. Interleukin-1ß and the interleukin receptor antagonist act in the stratum to modify excitotoxic brain damage in the rat. *Eur J Neurosci* 1998;10:1188-1195.
9. Mackenzie IA. Activated microglia in dementia with Lewy bodies. *Neurology* 2000;55:132-134.
10. Zhu X, Rottkamp CA, Hartzler A, et al. Activation of MKK6, an upstream activator of p38, in Alzheimer's disease. *J Neurochem* 2001;79:311-318.
11. Nicoll JA, Wilkinson D, Holmes C, et al. Neuropathology of human Alzheimer's disease after immunization with amyloid-beta peptide: a case report. *Nat Med* 2003;9:448-452.
12. Wood PL. Role of microglia in chronic neurodegenerative diseases. In: Wood PL, ed. *Neuroinflammation: Mechanisms and Management*. Totowa, N.J.: Humana Press; 2003:3-27.
13. Simmons RD, Willenborg DO. Direct injection of cytokines into the spinal cord causes autoimmune encephalomyelitis-like inflammation. *J Neurol Sci* 1990;100:37-42.
14. Jope RS, Zhang L, Song L. Peroxynitrite moderates the activation of p38 and extracellular regulated kinases in PC12 cells. *Arch Biochem Biophys* 2000;376:365-370.
15. Rothwell NJ, Luheshi GN. Interleukin 1 in the brain: biology, pathology, therapeutic target. *Trends Neurosci* 2000;23:618-625.
16. Murray CA, Lynch MA. Evidence that increased hippocampal expression of the cytokine interleukin-1ß is a common trigger for age- and stress-induced impairments in long-term potentiation. *J Neuroscience* 1998;18:2974-2981.
17. Vallieres L, Campbell IL, Gage FH, Sawchenko PE. Reduced hippocampal neurogenesis in adult transgenic mice with chronic astrocytic production of interleukin-6. *J Neurosci* 2002;22:486-492.
18. Neumann H, Schweigreiter R, Yamashita T, et al. Tumor necrosis factor inhibits neurite outgrowth and branching of hippocampal neurons by a Rho-dependent mechanism. *J Neurosci* 2002;22:854-862.

[19] Gillespie JS, Cavanagh HMA, Behan WMH, et al. Increased transcription of interleukin-6 in the brains of mice with chronic enterovirus infection. *J Gen Virol* 1993; 74: 741-743.

[20] Lynch WP, Robertson SJ, Portis JL. Induction of focal spongioform neurodegeneration in developmentally resistant mice by implantation of murine retrovirus-infected microglia. *J Virol* 1995;69:1408-1419.

[21] Heyes M, Saito K, Lacker A, et al. Sources of the neurotoxin quinolinic acid in the brain of HIV-1-infected patients and retrovirus-infected macaques. *FASEB J* 1998;12:881-896.

[22] Miniami M, Kuraishi Y, Satoh M. Effects of kainic acid on messenger RNA levels of IL-1beta, IL-6, TNF alpha and LIF in rat brain. *Biochem Biophys Res Commun* 1991;176:593-598.

[23] Blaylock RL. New Developments in the prevention and treatment of neurodegenerative diseases using nutraceuticals and metabolic stimulants. *J Am Nutr Assoc* 2002;5:15-32.

[24] Van Westerlaak MG, Jootsen EA, Gribnau AA, Cools AR, Bar PR. Chronic mitochondrial inhibition induces glutamate-mediated corticomotorneuron death in an organotypic culture model. *Exp Neurol* 2001;167:393-400.

[25] Weglicki WB, Phillips TM, et al. Magnesium deficiency elevates circulating levels of inflammatory cytokines and endothelin. *Mol Cell Biochem* 1992;110:169-173.

[26] Rockwell NJ. Cytokines-killer in the brain? *J Physiol* 1999;514:3-17.

[27] Park CH, Choi SH, Piao Y, et al. Glutamate and aspartate impair memory retention and damage hypothalamic neurons in adult mice. *Toxicol Lett* 2000;115:117-125.

[28] Mogenson GJ, Brudzynski SM, Wu M, Yang CR, Yim CCY. From motivation to action: regulation of limbic, nucleus accumbens, ventral pallidum, pedunculopontine nucleus circuitries involved in limbic-motor integration. In: Kalivas PW and Barnes CD, eds. *Limbic Circuits and Neuropsychiatry.* Boca Raton, Fla.: CRC Press; 1993:193-236.

[29] Blaylock RL. The central role of excitotoxicity in autism spectrum disorders. *J Am Nutr Assoc* 2003;6:7-19.

[30] Conner TJ, Leonard BE. Depression stress immunological activation: the role of cytokines in depressive disorders. *Life Sci* 1998;2:583-606.

[31] Poutiainen E, Hokkanen L, Niemi M-L, Forkkil M. Reversible cognitive decline during high-dose alpha-interferon treatment. *Pharmacol Biochem Behav* 1994;47:901-905.

[32] Denicoff KD, Rubinow DR, Papa MZ, et al. The neuropsychiatric effects of treatment with IL-2 and lymphokine-activated killer cells. *Ann Intern Med* 1987;107: 93-300.

[33] Turowski RC, Triozzi PL. Central nervous system toxicities of cytokine therapy. In: Plotnikoff NP, Faith RE, Murgo AJ, Good RA, eds. *Cytokines Stress and Immunity*. Boca Raton, Fla.: CRC Press; 1999:93-114.

[34] Renault PF, Hoofnagle JH, Park Y, et al. Psychiatric complications of long-term interferon-alpha therapy. *Arch Intern Med* 1987;147:1577-1580.

[35] Iaffaiolo RV, Fiorenza L, D'avino M, et al. Neurotoxic effects of long-term treatment with low-dose alpha 2b interferon. *Curr Ther Res* 1990;48:403-407.

[36] Meyers CA, Scheiel RS, Forman AD. Persistent neurotoxicity of systemically administered interferon-alpha. *Neurology* 1991;41:672-676.

[37] Adams F, Quesada JR, Gutterman JU. Neuropsychiatric manifestations of human leukocyte interferon therapy in patients with cancer. *JAMA* 1984;252:938-941.

[38] Kurzrock R, Quesada JR, Talpaz M, et al. Phase I study of multiple dose intramuscularly administered recombinant gamma interferon. *J Clin Oncol* 1986;4:1101-1109.

[39] Hu S, Sheng WS, Ehrlich LC, Peterson PK, Chao CC. Cytokine effects on glutamate uptake by human astrocytes. *Neuroimmunomodulation* 2000;7:153-159.

[40] Nicolson GL, Nasrally MY, Haier J, Pomfret J. High frequency of systemic mycoplasma infections in Gulf War veterans and civilians with amyotrophic lateral sclerosis (ALS). *J Clin Sci* 2002; 9:525-529.

[41] Moreno-Fuenmayor H, Borjas L, Arrieta A, Valera V, Socorro-Candanoza L. Plasma excitatory amino acids in autism. *Invest Clin* 1996;37:13-128.

[42] Wu J, Gao J, Zhao XN, Zhang ZX. Enhancing effects of Herpesvirus hominis on sodium glutamate neurotoxicity. *Zhongguo Yao Li Bao* 1994;15:161-164.

[43] Arai K, Matsuki N, Ikegaya Y, Nishiyama N. Deterioration of spatial learning performances in lipopolysaccharide-treated mice. *Jpn J Pharmacol* 2001;87:95-201.

[44] Broderick PA. Interleukin 1 alpha alters hippocampal serotonin and norepinephrine release during open-field behavior in Sprague-Dawley animals: differences from the Fawn-Hooded animal model of depression. *Prog Neuropsychopharmacol Biol Psychiatry* 2002;26:1355-1372.

[45] Warren RP, Singh VK. Elevated serotonin levels in autism: association with the major histocompatibility complex. *Neuropsychobiology* 1996;34:72-75.

[46] Lellouch-Tubiana A, Fohlen M, Robain O, Rozenberg F. Immunocytochemical characterization of long-term persistent immune activation in human brain after herpes simplex encephalitis. *Neuropath Appl Neurobiol* 2000;26:285-294.

[47] Anderson T, Schultzberg M, Schwartz R, et al. NMDA-receptor antagonist prevents measles virus-induced neurodegeneration. *Eur J Neurosci* 1991;3:66-71.

[48] Booss J, Davis LE. Smallpox and smallpox vaccination: neurological implications. *Neurology* 2003;60:1241-1245.

[49] Rook GAW, Zumla A. Gulf War syndrome: is it due to a systemic shift in cytokine balance toward a Th2 profile? *Lancet* 1997;349:1831-1833.

[50] Everson MP, Kotler S, Blackburn WD. Stress and immune dysfunction in Gulf War veterans. *Ann NY Acad Sci* 1999;876:413-418.

[51] Asa PB, Wilson RB, Garry RF. Antibodies to squalene in recipients of anthrax vaccine. *Exp Mol Pathol* 2002;73:19-27.

[52] Warren RP, Singh VK, Averett RE, et al. Immunogenetic studies in autism and related disorders. *Mol Chem Neuropathol* 1996;28:77-81.

[53] Singh VK, Warren RP, Odell JD, Warren WL, Cole P. Antibodies to myelin basic protein in children with autistic behavior. *Brain Behav Immunol* 1993;7(1):97-103.

[54] Malek-Ahmadi Cytokines and etiopathogenesis of pervasive developmental disorders. *Med Hypotheses* 2001;56:321-324.

Cellular/Molecular

Cannabinoid CB_2 Receptors and Fatty Acid Amide Hydrolase Are Selectively Overexpressed in Neuritic Plaque-Associated Glia in Alzheimer's Disease Brains

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The endocannabinoid system is still poorly understood. Recently, the basic elements that constitute it, i.e., membrane receptors, endogenous ligands, and mechanisms for termination of the signaling process, have been partially characterized. There is a considerable lack of information, however, concerning the distribution, concentration, and function of those components in the human body, particularly during pathological events. We have studied the status of some of the components of the endocannabinoid system, fatty acid amide hydrolase and cannabinoid CB_1 and CB_2 receptors, in postmortem brains from patients with Alzheimer's disease. Using specific polyclonal antibodies, we have performed immunohistochemical analysis in hippocampus and entorhinal cortex sections from brains of Alzheimer's disease patients. Our results show that both fatty acid amide hydrolase and cannabinoid CB_2 receptors are abundantly and selectively expressed in neuritic plaque-associated astrocytes and microglia, respectively, whereas the expression of CB_1 receptors remains unchanged. In addition, the hydrolase activity seems to be elevated in the plaques and surrounding areas. Thus, some elements of the endocannabinoid system may be postulated as possible modulators of the inflammatory response associated with this neurodegenerative process and as possible targets for new therapeutic approaches.

Key words: Alzheimer; astrocyte; astroglia; cannabinoids; immunoreactivity; microglia; neuropathology

Introduction

The endocannabinoid system (ECS) performs many biologically important functions (Porter and Felder, 2001). The isolation and cloning of two different types of cannabinoid receptors, termed CB_1 and CB_2, partially unveiled the molecular mechanisms that mediate the well known effects of natural cannabinoids (for review, see Pertwee, 1997). Since then, putative endogenous ligands for those receptors have been isolated, and the mechanisms for termination of the biological signal have been identified. Thus, the ethanolamine of arachidonic acid [termed "anandamide" (AEA)] and 2-arachidonoyl-glycerol (2-AG) are the two main endogenous ligands (or "endocannabinoids") isolated so far (Mechoulam et al., 1998). On the other hand, a specific uptake mechanism and subsequent degradation of these compounds through the action of an amide hydrolase [fatty acid amide hydrolase (FAAH)] have been described (for review, see Giuffrida et al., 2001). Very recently, the possible existence of other subtypes of cannabinoid receptors, "non-CB_1 and non-CB_2," has been raised (Breivogel at al., 2001).

The distribution of the different elements of the ECS has been widely studied. Thus, it is currently accepted that although the cannabinoid CB_1 receptors are present in different tissues (predominantly of nervous origin), the cannabinoid CB_2 receptors are restricted to cell types related to the immune function (Grundy et al., 2001). CB_1 receptors are widely expressed in the CNS, being specially abundant in basal ganglia, hippocampus, cerebellum, and cortical structures (Herkenham et al., 1991; Mailleux and Vanderhaeghen, 1992a; Glass et al., 1997). This pattern of distribution matches well with the known effects of cannabinoids on motor and cognitive functions (Pertwee, 1997).

The presence of CB_2 receptors has been reported in spleen macrophages, tonsils, B cells and natural killer cells, monocytes, neutrophils, and T cells (Lynn and Herkenham, 1994; Galiegue et al., 1995), as well as in different cell lines (Grundy et al., 2001). Because of their presence in immune tissues, much work has been focused on the possible role of CB_2 receptors in mediating inflammatory responses (for review, see Parolaro et al., 2002); however, contradictory results have been obtained, partially because of the variety of experimental models and the doses of cannabinoids that were used (Grundy et al., 2001).

On the other hand, FAAH is considered to be one of the key elements in the regulation of the ECS function. It mediates the termination of the signal of AEA and possibly 2-AG (Deutsch et

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al., 2000, 2002), although it has been suggested that 2-AG is degraded *in vivo* primarily by the action of a monoglyceride lipase (Dinh et al., 2002). FAAH is an integral membrane protein and was originally cloned as the degrading enzyme of the sleep-inducing factor *cis*-9-octadecenoamide (oleamide) (Maurelli et al., 1995). Its cellular distribution in the rat brain has been studied by the use of different antibodies (Egertova et al., 1998; Tsou et al., 1998b) and by *in situ* hybridization (Thomas et al., 1997). Results were similar in these studies, showing that pyramidal cortical neurons, hippocampal pyramidal cells, and Purkinje cerebellar neurons exhibit the most intense immunostaining. FAAH displays a similar pattern of distribution in the human brain, being present in both neuronal and glial elements and showing a significant overlap with CB_1 receptors, mainly in areas related to motor control and memory (Romero et al., 2002).

Few data exist regarding the changes that the ECS may exhibit in normal or pathological aging. Autoradiographic studies in the past decade reported that CB_1 receptors are decreased in aged rats (Mailleux and Vanderhaeghen, 1992b; Romero et al., 1998) and that pathological conditions in the human affecting basal ganglia structures dramatically decrease the density of these receptors (Glass et al., 1993; Richfield and Herkenham, 1994). From these studies and from the known distribution of CB_1 and FAAH, the possible therapeutic interest of cannabinoid agonists and antagonists in motor diseases has been suggested (Fernandez-Ruiz et al., 2002). Thus, it seems of great importance to establish the status of the ECS in other pathological conditions affecting the human CNS, such as Alzheimer's Disease (AD).

Materials and Methods

Tissues. Postmortem brain tissues were obtained from four control brains (age range 64–75 years) and seven AD patients (age range 68–82 years) within a 12 hr postmortem interval. Control subjects had no background of neuropsychiatric disease, and the full neuropathological examination performed in every case on paraffin-embedded tissue excluded any significant pathological finding. On the other hand, all AD subjects met the CERAD (consortium to establish a registry for Alzheimer's disease) clinical and neuropathological criteria for the diagnosis of definite AD (Mirra et al., 1991). AD diagnosis was confirmed by Gallyas and silver-methenamine stainings in paraffin-embedded tissue sections from the same cases used in the immunohistochemical studies.

Brain hemispheres were separated and processed for freezing (for Western blotting and FAAH activity experiments) or for fixation (for immunohistochemistry). Thus, in each case, one of the hemispheres was fixed by immersion in 4% buffered formaldehyde, and select, small tissue blocks containing the areas of interest for this study (hippocampus and entorhinal and parahippocampal cortices) were transferred to 50 mM potassium PBS (KPBS) and cut on a Leica Vibratome. These regions were chosen because they are known to show high densities of β-amyloid peptide (Aβ)-containing neuritic plaques in AD (Morrison and Hof, 2002). Free-floating sections (50 μm thick) were used for immunohistochemical and staining procedures. To obtain a more efficient immunostaining, tissue sections were subjected to an antigen retrieval procedure (Shi et al., 2001). Briefly, sections were placed in a stainless steel pressure cooker containing a boiling solution (0.01 M sodium citrate, pH 6). After they were heated under pressure for 2 min, samples were removed and washed extensively in KPBS.

Western blotting. The protocol used is basically as described previously (Romero et al., 2002). Human brain was obtained at autopsy and a 1 gm piece of cerebral cortical gray matter was homogenized in 10 ml of M-PER mammalian protein extraction reagent (Pierce, Rockford, IL). The homogenate was shaken gently for 10 min and then centrifuged at 27,000 × *g* for 15 min. The supernatant was isolated, and protein was determined using the BCA protein assay kit (Pierce).

Brain protein extract (50 μg) was reduced and denatured and separated by electrophoresis through a 10.5 × 10 cm, 0.75-mm-thick 15% polyacrylamide preparative gel. After separation, the proteins in the gel were transferred to nitrocellulose membrane. The nitrocellulose was washed with PBS containing 0.2% Tween 20 (PBST), and remaining binding sites on the membrane were blocked by overnight incubation in PBST containing 2% nonfat dried milk at 4°C. Incubation of primary antibodies was performed at 1:300 dilution in PBST containing 2% nonfat dried milk overnight at 4°C. In some experiments, the antibodies were preincubated with 8 μg/ml of the same immunizing peptides used for the generation of the antibodies. After the nitrocellulose membrane was washed with PBST, it was incubated with an alkaline phosphatase-conjugated goat anti-rabbit secondary antibody (Sigma, St. Louis, MO), 1:2000 in PBST containing 2% nonfat dried milk for 1 hr at room temperature. The nitrocellulose membrane was washed extensively with PBST, followed by PBS. Finally, the immune complex was visualized by incubating in the presence of nitroblue tetrazolium–5-bromo-4-chloro-3-indoyl phosphate chromogen.

Immunohistochemistry. The protocol used is basically as described previously (Tsou et al., 1998a; Romero et al., 2002), with slight modifications. Briefly, floating sections were washed in KPBS (50 mM), and endogenous peroxidase was blocked by incubation in peroxidase-blocking solution (Dako, Copenhagen, Denmark) for 20 min at room temperature. The sections were then washed in 50 mM KPBS and incubated with the corresponding antibody. The antibodies used included the following: polyclonal anti-CB_1 receptor (1:2000; Affinity Bioreagents), polyclonal anti-CB_2 receptor (1:1500; Affinity Bioreagents, Golden, CO), polyclonal anti-FAAH (Romero et al., 2002) (1:1000), monoclonal anti-CD68 (1:100; Dako) for microglia, and monoclonal anti-GFAP (1:200, Dako) for astrocytes. After incubation with the corresponding primary antibody, the sections were washed in 50 mM KPBS and incubated with biotinylated goat anti-rabbit antibody (1:200), for polyclonal primary antibodies, or biotinylated horse anti-mouse antibody, for monoclonal primary antibodies, at room temperature for 1 hr followed by avidin–biotin complex (Vector Elite, Burlingame, CA), following the manufacturer's instructions. Visible reaction product was produced by treating the sections with 0.04% diaminobenzidine (DAB) (Dako), 2.5% nickel sulfate, and 0.01% H_2O_2 dissolved in 0.1 M sodium acetate.

For double-labeling studies, sections were sequentially incubated with anti-FAAH antibody, treated in the same way as described above, except that the staining was visualized with DAB in the absence of nickel sulfate (rendering brown color) followed by incubation with anti-Aβ monoclonal antibody (1:200; Dako) and signal was revealed with Vector SG (Vector; rendering blue color). The same procedure was used for CB_2/Aβ double-labeling experiments.

Sections were mounted on gelatin-coated slides, dehydrated, and sealed with coverslips. The observations and photography of the slides were done using a Nikon Eclipse E600 microscope and a Nikon FDX-35 camera. Controls for the immunohistochemistry included the preabsorption and coincubation of the antibodies with the corresponding immunogenic proteins (CB_1, fusion protein against amino acids 1–100 of human-CB_1 at 5 μg/ml; CB_2, fusion protein against amino acids 1–33 of human-CB_2 at 5 μg/ml; FAAH, fusion protein against amino acids 561–579 of rat-FAAH at 1.25 μg/ml) and incubation in the absence of primary antibody.

Fatty acid amide hydrolase assay. Frozen tissue sections (50 μm thick) were obtained after cryosectioning. After they were stained with methylene blue, individual neuritic plaques were dissected from entorhinal and parahippocampal cortices under the microscope and transferred to PBS. For control data, portions of similar size were dissected from 50-μm-thick frozen sections of control brain. Afterward, FAAH assays were performed as described (Edgemond et al., 1998). Briefly, plaques were hand homogenized in 50 μl of Tris buffer (50 mM, pH 7.4) containing EDTA (1 mM) and $MgCl_2$ (3 mM). The entire lysate was incubated with [^{14}C]AEA (2000 dpm), labeled on its ethanolamine moiety, for 15 min. The incubation was quenched by the addition of 0.2 ml of chloroform/methanol (1:2) followed by extraction as described (Edgemond et al., 1998). Control incubations were performed in the absence of tissue.

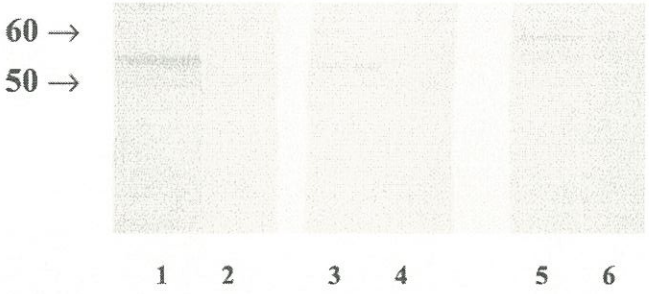

Figure 1. Western blots of CB_1 (lanes 1, 2), FAAH (lanes 3, 4), and CB_2 (lanes 5, 6) immunoreactivities in human temporal cortex from an AD patient. Single bands of ~50 kDa (CB_1 and FAAH) or 60 kDa (CB_2) were observed (lanes 1, 3, and 5, respectively). No immunoreactivities were detected when the primary antibodies were preincubated with the respective immunizing peptides (lanes 2, 4, and 6).

Results

AD diagnosis was confirmed by neuropathological examination on paraffin sections by Gallyas and silver methenamine stainings, revealing the presence of abundant neuritic plaques in entorhinal and parahippocampal cortices (data not shown). Western blots confirmed the presence of CB_1, CB_2, and FAAH in AD homogenates as well as the specificity of the antibodies used (Fig. 1). The bands observed matched the manufacturer's reported weights of the different proteins and previously published data (Tsou et al., 1998b; Romero et al., 2002).

FAAH immunoreactivity in sections from healthy individuals (Fig. 2*A*) revealed the same pattern described previously (Romero et al., 2002), with a predominant labeling of neuronal elements (Fig. 2*A*, inset). Specifically, an intense and regular staining was located on pyramidal neurons in cell bodies and proximal processes. In AD samples, FAAH was detected mainly in cell bodies and processes of hypertrophied astrocytes surrounding neuritic plaques (Fig. 2*B*,*C*) in entorhinal and parahippocampal areas. In contrast, GFAP immunostaining was detectable in both protoplasmic and fibrous astrocytes (Fig. 2*D*). Scattered neurons in the dentate gyrus showed marked FAAH immunoreactivity (data not shown). In support of the immunohistochemical data, FAAH enzyme activity could be detected in five of five individual plaques but never (0 of 5) in tissue pieces of the same size taken from cortices of healthy brain (Fig. 2*F*). Finally, the staining was completely prevented by preabsorption and coincubation with the immunizing peptide (Fig. 2*E*).

No staining for the CB_2 receptor was observed in the same regions of samples from healthy individuals (Fig. 3*A*). In AD samples, CB_2 receptor immunoreactivity was limited to grouped cells, with morphological properties characteristic of neuritic plaque-associated microglia (Fig. 3*B*,*C*). CD68 immunostaining (a commonly used phenotypic marker for all types of microglia) revealed a more abundant signal, including plaque-associated and non-associated microglial cells (Fig. 3*D*). The immunizing peptide reversed the staining for CB_2 (Fig. 3*E*).

Subsequent to these observations and to better characterize the cellular location of FAAH and CB_2 in AD tissue sections, double-immunostaining experiments were conducted. Thus, FAAH immunoreactivity could be clearly seen in hypertrophic astrocytes surrounding Aβ-enriched neuritic plaques (Fig. 4*A*,*B*). In addition, CB_2 immunoreactivity could be circumscribed to Aβ neuritic plaque-associated microglia only (Fig. 4*C*,*D*).

Control brains showed a neuronal pattern of staining for CB_1 receptors, with pyramidal cortical neurons exhibiting a high intensity of labeling (Fig. 5*A*). No changes in the density or location of CB_1 receptors could be seen in the vicinity of neuritic plaques (Fig. 5*B*,*C*). The immunizing peptide was also effective in preventing the immunostaining for CB_1 receptors (Fig. 5*D*).

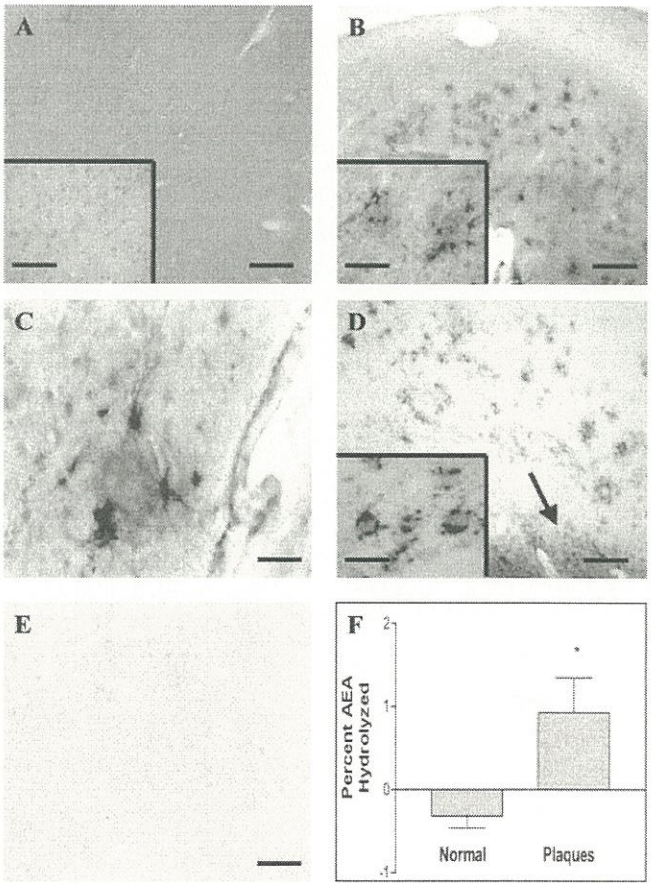

Figure 2. *A–F*, FAAH (*A–C*, *E*) and GFAP (*D*) immunoreactivities in parahippocampal cortex and FAAH activity in neuritic plaques (*F*). *A*, FAAH staining in a healthy individual sample. Note the neuronal pattern of staining (inset). *B*, Low and high (inset) magnifications of FAAH immunoreactivity in parahippocampal cortex of an AD case. Note the intense signal for FAAH in hypertrophied astrocytes surrounding neuritic plaques. *C*, Detail of FAAH immunoreactivity in hypertrophied astrocytes. *D*, Low and high (inset) magnification of GFAP immunoreactivity in an AD case. Note that the signal is detectable in both protoplasmic and fibrous (arrow) astrocytes. *E*, FAAH staining after preabsorption and coincubation of the antibody with the immunizing peptide. Note the absence of any detectable signal. *F*, FAAH activity. Individual plaques were dissected under microscope, homogenized, and assayed for FAAH activity using the conversion of [^{14}C]AEA to [^{14}C]ethanolamine during a 15 min incubation. Shown is the mean of five individual determinations; groups were significantly different using unpaired *t* tests with $p < 0.05$. Scale bars: *A*, *B*, *D*, 800 μm; *E*, inset in *A*, 400 μm; insets in *B* and *D*, 200 μm; *C*, 100 μm.

Discussion

AD is a chronic degenerative disorder of the brain and accounts for the most common form of dementia in the elderly (Strohmeyer and Rogers, 2001). The histopathology of AD is currently well known, with hallmarks including senile plaques, neuritic tangles, loss of neurons, damaged synaptic connections, and reactive gliosis (Giulian, 1999). Reactive gliosis involves both microglia, which attack the senile plaque, and astroglia, which surround the plaque complex and play a critical role in AD inflammation (for review, see Wyss-Coray and Mucke, 2002). Thus, the formation of complex protein aggregates containing Aβ is thought to induce a chronic inflammatory response that

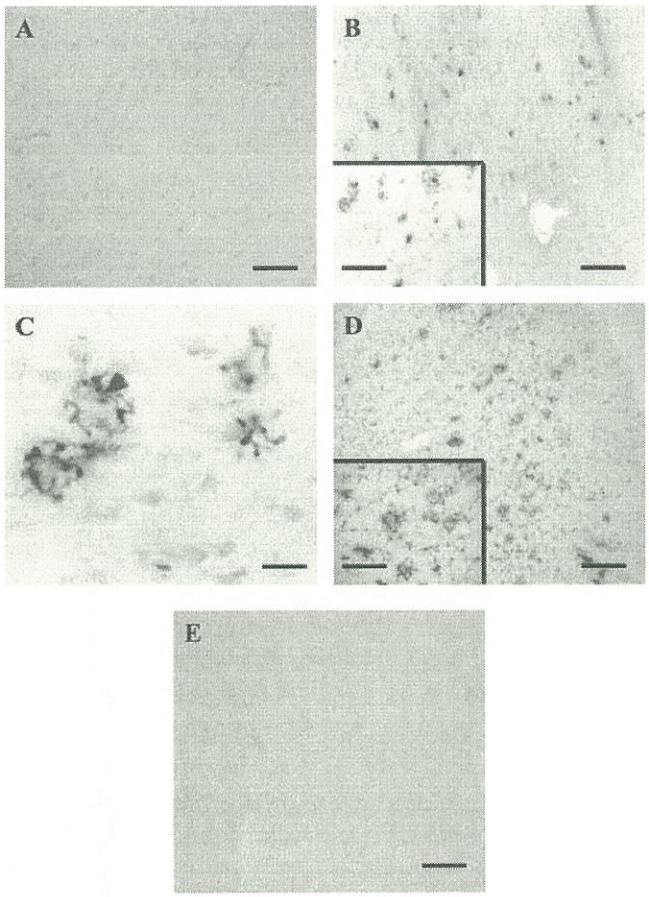

Figure 3. CB_2 (*A–C*, *E*) and CD68 (*D*) stainings in parahippocampal cortex. *A*, CB_2 staining in a healthy individual sample. No detectable signal could be seen. *B*, Low and high (inset) magnifications of CB_2 immunoreactivity in parahippocampal cortex of an AD case. Note the intense signal for CB_2 in microglial cells located on neuritic plaques. *C*, Detail of CB_2 immunoreactivity in neuritic plaque-associated microglia. *D*, Low and high (inset) magnification of CD68 immunoreactivity in an AD case. *E*, CB_2 staining after preabsorption and coincubation of the antibody with the immunizing peptide. Note the absence of any detectable signal. Scale bars: *A*, *B*, *D*, *E*, 800 μm; insets in *B* and *D*, 200 μm; *C*, 100 μm.

leads, among other events, to the activation of both microglia and astroglia. These are known to play a relevant pathophysiological role, because they produce abundant proinflammatory substances that initiate a secondary damaging process (Strohmeyer and Rogers, 2001). The aim of this study was to determine the status of some components of the endocannabinoid system, CB_1 and CB_2 receptors and FAAH, in the brains of AD patients and to explore their possible role in this neurodegenerative disorder.

We report that FAAH protein and activity and CB_2 receptor protein are selectively overexpressed in glial cells that are linked to the inflammatory process that accompanies Alzheimer's disease. To our knowledge, this is the first observation in human tissue that suggests a role of the ECS in the progression of this neurodegenerative disease. We use the term "selectively" in two senses: first, although CB_2 receptors and FAAH exhibit upregulation in glial cells associated with senile plaques, CB_1 receptor density is not modified in the vicinity of these pathological structures. Second, FAAH expression appears to be restricted to reactive astrocytes, and CB_2 receptors are expressed only in activated microglial cells. Whether this upregulation is specific of AD or is common to other pathologies that exhibit reactive gliosis is being investigated currently in our laboratory.

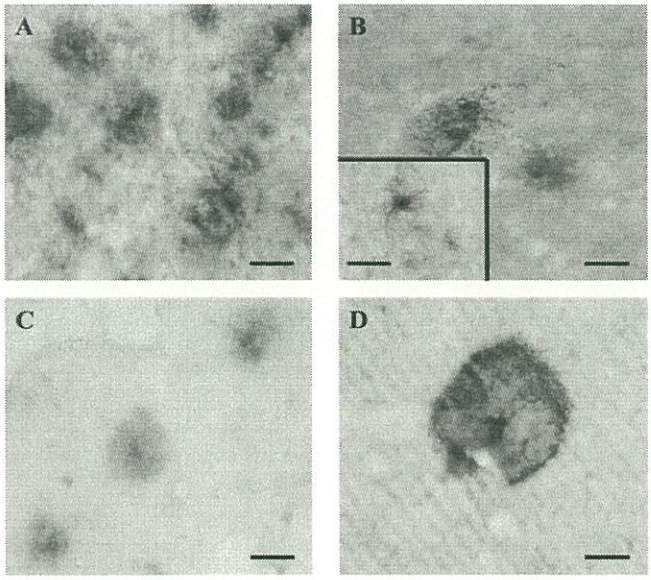

Figure 4. FAAH and CB_2 are expressed in glial cells associated with β-amyloid-enriched neuritic plaques. *A*, *B*, FAAH (brown) and β-amyloid peptide (blue) stainings. Note that FAAH-positive cells are astrocytes surrounding β-amyloid-enriched plaques. *C*, *D*, CB_2 (brown) and β-amyloid peptide (blue) stainings. CB_2 immunostaining is limited to plaque-associated microglial cells.

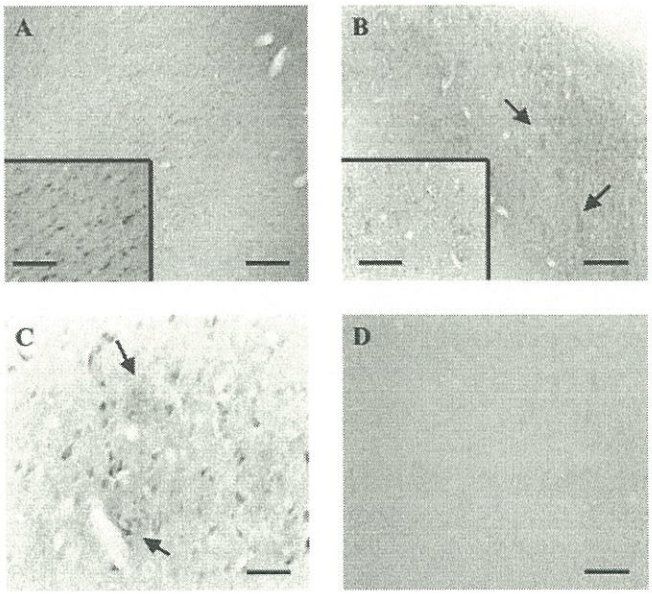

Figure 5. CB_1 staining in parahippocampal cortex. *A*, CB_1 staining in a healthy individual sample. Pyramidal cortical cells showed moderate to intense staining level (inset). *B*, Low and high (inset) magnifications of CB_1 immunoreactivity in parahippocampal cortex of an AD case. Note the general lower intensity of the signal for CB_1 and how the neuritic plaques can be observed easily (arrows). *C*, Detail of CB_1 immunoreactivity, showing no changes in the vicinity of neuritic plaques (arrows). *D*, CB_1 staining after preabsorption and coincubation of the antibody with the immunizing peptide. Note the absence of any detectable signal. Scale bars: *A*, *B*, *D*, 800 μm; inset in *B*, 400 μm; inset in *A*, 200 μm; *C*, 100 μm.

A previous article by Westlake et al. (1994) failed to establish a link between changes in CB_1 receptors or mRNA levels in tissues from Alzheimer's disease patients and the specific pathological events that take place in this illness. In agreement with this report, we have not found changes in the distribution of CB_1 receptors in the vicinity of neuritic plaques; however, it must be noted that the

autoradiographic analysis in the cited study was done using the synthetic cannabinoid CP-55,940 as radioligand. This compound is an agonist for both CB_1 and CB_2 receptor subtypes (Pertwee, 1997), and only decreases in cannabinoid receptor density were detected by these authors. This is in contrast with our observations: although CB_1 receptors were unaltered in the vicinity of the neuritic plaques in our AD samples, CB_2 receptors were dramatically overexpressed in the activated microglia. We do not have a plausible explanation for this discrepancy other than the different methodologies used in each study. It could be argued that the lack of cellular resolution in the autoradiographic method could mask the specific increase in CB_2 receptors that we have observed. Finally, although we did not observe significant changes in CB_1, this subtype of cannabinoid receptors has been implicated in the regulation of microglial function (Waksman et al., 1999), and thus an important role for this subtype of cannabinoid receptors in the inflammatory events related to AD cannot be ruled out.

The presence of FAAH in astrocytes has been observed previously in human CNS (Romero et al., 2002), pointing to a possible role for this enzyme in the regulation of blood vessel tone in the brain and in the regulation of synaptic transmission. It has also been reported that rat astrocytes accumulate and produce anandamide and other acylethanolamides and contain CB_1 receptors (Beltramo and Piomelli, 2000; Walter et al., 2002). Furthermore, we found detectable levels of FAAH activity in the vicinity of individually dissected neuritic plaques, in contrast to control brains. The absence of any activity in control samples may be attributable to the extremely small size of the tissue that was dissected and used in the enzymatic assay.

Because AEA and, at least partially, 2-AG are substrates for FAAH and are both converted to arachidonic acid, the massive presence of FAAH in astrocytes surrounding neuritic plaques suggests that astrocytes, via FAAH, could be a significant source of arachidonic acid and related proinflammatory substances in the vicinity of these plaques, with harmful effects. It is important to note that the use of anti-inflammatory compounds is currently one of the most promising lines of research for the treatment of AD; thus, the beneficial effects of cyclooxygenase inhibitors (such as ibuprofen or aspirin) have recently been reported (In'T Veld et al., 2001; Zandi et al., 2002). In light of these observations, we speculate that inhibition of FAAH activity could be beneficial in preventing the inflammatory process associated with Aβ deposition.

To our knowledge, this report is the first evidence for the presence of CB_2 receptors in the human CNS. It has been reported that CB_2 receptors are expressed in granule and Purkinje cells of the mouse cerebellum (Skaper et al., 1996) and that rat microglial cells express CB_2 receptors and that this expression is upregulated when the microglia become activated (Carlisle et al., 2002). Furthermore, these receptors have recently been reported to play an important role in microglial migration (Walter et al., 2003). It is important to note that we detected CB_2 receptors only in microglial cells, which is in agreement with the well known immunomodulatory effects of CB_2 activation. Thus, many studies have shown that CB_2 receptor activation leads to a myriad of changes in the production of inflammation-related substances, although with results that vary depending on the experimental model used and the concentration of cannabinoids used (Grundy et al., 2001). In any case, the selective presence of CB_2 receptors in microglial cells opens new perspectives on the role of CB_2 receptors in the human CNS and suggests that the modulation of their activity may have therapeutic implications.

References

Beltramo M, Piomelli D (2000) Carrier-mediated transport and enzymatic hydrolysis of the endogenous cannabinoid 2-arachidonylglycerol. NeuroReport 11:1231–1235.

Breivogel CS, Griffin G, Di Marzo V, Martin BR (2001) Evidence for a new G protein-coupled cannabinoid receptor in mouse brain. Mol Pharmacol 60:155–163.

Carlisle SJ, Marciano-Cabral F, Staab A, Ludwick C, Cabral GA (2002) Differential expression of the CB_2 cannabinoid receptor by rodent macrophages and macrophage-like cells in relation to cell activation. Int Immunopharmacol 2:69–82.

Deutsch DG, Glaser ST, Howell JM, Kunz JS, Puffenbarger RA, Hillard CJ, Abumrad N (2000) The cellular uptake of anandamide is coupled to its breakdown by fatty acid amide hydrolase (FAAH). J Biol Chem 276:6967–6973.

Deutsch DG, Ueda N, Yamamoto S (2002) The fatty acid amide hydrolase (FAAH). Prostaglandins Leukot Essent Fatty Acids 66:201–210.

Dinh TP, Carpenter D, Leslie FM, Freund TF, Katona I, Sensi SL, Kathuria S, Piomelli D (2002) Brain monoglyceride lipase participating in endocannabinoid inactivation. Proc Natl Acad Sci USA 99:10819–10824.

Edgemond WS, Greenberg MJ, McGinley PJ, Muthian S, Campbell WB, Hillard CJ (1998) Synthesis and characterization of diazomethylarachidonyl ketone: an irreversible inhibitor of *N*-arachidonylethanolamide amidohydrolase. J Pharmacol Exp Ther 286:184–190.

Egertova M, Giang DK, Cravatt BF, Elphick MR (1998) A new perspective on cannabinoid signaling: complementary localization of fatty acid amide hydrolase and the CB_1 receptor in rat brain. Proc R Soc Lond B Biol Sci 265:2081–2085.

Fernandez-Ruiz J, Lastres-Becker I, Cabranes A, Gonzalez S, Ramos JA (2002) Endocannabinoids and basal ganglia functionality. Prostaglandins Leukot Essent Fatty Acids 66:257–267.

Galiegue S, Mary S, Marchand J, Dussossoy D, Carriere D, Carayon P, Bouaboula M, Shire D, Le Fur G, Casellas P (1995) Expression of central and peripheral cannabinoid receptors in human immune tissues and leukocyte subpopulations. Eur J Biochem 232:54–61.

Giuffrida A, Beltramo M, Piomelli D (2001) Mechanisms of endocannabinoid inactivation: biochemistry and pharmacology. J Pharmacol Exp Ther 298:7–14.

Giulian D (1999) Microglia and the immune pathology of Alzheimer's disease. Am J Hum Genet 65:13–18.

Glass M, Faull RL, Dragunow M (1993) Loss of cannabinoid receptors in the substantia nigra in Huntington's disease. Neuroscience 56:523–527.

Glass M, Dragunow M, Faull RL (1997) Cannabinoid receptors in the human brain: a detailed anatomical and quantitative autoradiographic study in the fetal, neonatal and adult human brain. Neuroscience 77:299–318.

Grundy RI, Rabuffetti M, Beltramo M (2001) Cannabinoids and neuroprotection. Mol Neurobiol 24:29–51.

Herkenham M, Lynn AB, Johnson MR, Melvin LS, De Costa BR, Rice KC (1991) Characterization and localization of cannabinoid receptors in rat brain: a quantitative *in vitro* autoradiographic study. J Neurosci 11:563–583.

In'T Veld BA, Ruitenberg A, Hofman A, Launer LJ, van Duijn CM, Stijnen T, Breteler MB, Stricker BHC (2001) Nonsteroidal anti-inflammatory drugs and the risk of Alzheimer's disease. N Eng J Med 345:1515–1521.

Lynn AB, Herkenham M (1994) Localization of cannabinoid receptors and nonsaturable high-density cannabinoid binding sites in peripheral tissues of the rat: implications for receptor-mediated immune modulation by cannabinoids. J Pharmacol Exp Ther 268:1612–1623.

Mailleux P, Vanderhaeghen JJ (1992a) Distribution of neuronal cannabinoid receptor in the adult rat brain: a comparative receptor binding radioautography and in situ hybridization histochemistry. Neuroscience 48:655–668.

Mailleux P, Vanderhaeghen JJ (1992b) Age-related loss of cannabinoid receptor binding sites and mRNA in the rat striatum. Neurosci Lett 147:179–181.

Maurelli S, Bisogno T, De Petrocellis L, Di Luccia A, Marino G, Di Marzo V (1995) Two novel classes of neuroactive fatty acid amides are substrates for mouse neuroblastoma "anandamide amidohydrolase". FEBS Lett 377:82–86.

Mechoulam R, Fride E, Di Marzo V (1998) Endocannabinoids. Eur J Pharmacol 359:1–18.

Mirra SS, Heyman A, McKeel D, Sumi SM, Crain BJ, Brownlee LM, Vogel FS,

Hughes JP, van Belle G, Berg L (1991) The consortium to establish a registry for Alzheimer's disease (CERAD), Part II. Standardization of the neuropathologic assessment of Alzheimer's disease. Neurology 41:479–486.

Morrison JH, Hof PR (2002) Selective vulnerability of corticocortical and hippocampal circuits in aging and Alzheimer's disease. Prog Brain Res 136:467–486.

Parolaro D, Massi P, Rubino T, Monti E (2002) Endocannabinoids in the immune system and cancer. Prostaglandins Leukot Essent Fatty Acids 66:319–332.

Pertwee RG (1997) Pharmacology of cannabinoid CB_1 and CB_2 receptors. Pharmacol Ther 74:129–180.

Porter AC, Felder CC (2001) The endocannabinoid nervous system: unique opportunities for therapeutic intervention. Pharmacol Ther 90:45–60.

Richfield EK, Herkenham M (1994) Selective vulnerability in Huntington's disease: preferential loss of cannabinoid receptors in lateral globus pallidus. Ann Neurol 36:577–584.

Romero J, Berrendero F, Garcia-Gil L, De la Cruz P, Ramos JA, Fernandez-Ruiz JJ (1998) Loss of cannabinoid receptor binding and messenger RNA levels and cannabinoid agonist-stimulated [^{35}S]guanylyl-5′*O*-(thio)-triphosphate binding in the basal ganglia of aged rats. Neuroscience 84:1075–1083.

Romero J, Hillard CJ, Calero M, Rabano A (2002) Fatty acid amide hydrolase localization in the human central nervous system: an immunohistochemical study. Brain Res Mol Brain Res 100:85–93.

Shi SR, Cote RJ, Taylor CR (2001) Antigen retrieval techniques: current perspectives. J Histochem Cytochem 49:931–937.

Skaper SD, Buriani A, Dal Toso R, Petrelli L, Romanello S, Facci L, Leon A (1996) The ALIAmide palmitoylethanolamide and cannabinoids, but not anandamide, are protective in a delayed postglutamate paradigm of excitotoxic death in cerebellar granule neurons. Proc Natl Acad Sci USA 93:3984–3989.

Strohmeyer R, Rogers J (2001) Molecular and cellular mediators of Alzheimer's disease inflammation. J Alz Dis 3:131–157.

Thomas EA, Cravatt BF, Danielson PE, Gilula NB, Sutcliffe JG (1997) Fatty acid amide hydrolase, the degradative enzyme for anandamide and oleamide, has selective distribution in neurons within the rat central nervous system. J Neurosci Res 50:1047–1052.

Tsou K, Brown S, Sanudo-Pena MC, Mackie K, Walker JM (1998a) Immunohistochemical distribution of cannabinoid CB_1 receptors in the rat central nervous system. Neuroscience 83:393–411.

Tsou K, Nogueron MI, Muthian S, Sanudo-Pena MC, Hillard CJ, Deutsch DG, Walker JM (1998b) Fatty acid amide hydrolase is located preferentially in large neurons in the rat central nervous system as revealed by immunohistochemistry. Neurosci Lett 254:137–140.

Waksman Y, Olson JM, Carlisle SJ, Cabral GA (1999) The central cannabinoid receptor (CB_1) mediates inhibition of nitric oxide production by rat microglial cells. J Pharmacol Exp Ther 288:1357–1366.

Walter L, Franklin A, Witting A, Moller T, Stella N (2002) Astrocytes in culture produce anandamide and other acylethanolamides. J Biol Chem 277:20869–20876.

Walter L, Franklin A, Witting A, Wade C, Xie Y, Kunos G, Mackie K, Stella N (2003) Nonpsychotropic cannabinoid receptors regulate microglial cell migration. J Neurosci 23:1398–1405.

Westlake TM, Howlett AC, Bonner TI, Matsuda LA, Herkenham M (1994) Cannabinoid receptor binding and messenger RNA expression in human brain: an in vitro receptor autoradiography and in situ hybridization histochemistry study of normal aged and Alzheimer's brains. Neuroscience 63:637–652.

Wyss-Coray T, Mucke L (2002) Inflammation in neurodegenerative disease–A double-edged sword. Neuron 35:419–432.

Zandi PP, Anthony JC, Hayden KM, Mehta K, Mayer L, Breitner JC (2002) Reduced incidence of AD with NSAID but not H2 receptor antagonists: the Cache County Study. Neurology 59:880–886.

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Involvement of cannabinoid CB2 receptor in alcohol preference in mice and alcoholism in humans.

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We tested if cannabinoid type 2 receptor (CB2) in the central nervous system plays a role in alcohol abuse/dependence in animal model and then examined an association between the CB2 gene polymorphism and alcoholism in human. Mice experiencing more alcohol preference by drinking showed reduced Cb2 gene expression, whereas mice with little preference showed no changes of it in ventral midbrain. Alcohol preference in conjunction with chronic mild stress were enhanced in mice treated with CB2 agonist JWH015 when subjected to chronic stress, whereas antagonist AM630 prevented development of alcohol preference. There is an association between the Q63R polymorphism of the CB2 gene and alcoholism in a Japanese population (P=0.007; odds ratio 1.25, 95% CI, (1.06-1.47)). CB2 under such environment is associated with the physiologic effects of alcohol and CB2 antagonists may have potential as therapies for alcoholism.

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Methods to study the behavioral effects and expression of CB2 cannabinoid receptor and its gene transcripts in the chronic mild stress model [Methods Mol Med. 2006]

Discovery of the presence and functional expression of cannabinoid CB2 receptors in brain. [Ann N Y Acad Sci. 2006]

A role for cannabinoid receptors, but not endogenous opioids, in the antinociceptive activity of the CB2-selective agonist, GW405833. [Eur J Pharmacol. 2005]

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Lien alcoolisme et fonction CB2

Molecule of the Year: Cannabiodiol

By FRED GARDNER

THC -delta-9 tetrahydrocannabinol- is not the only active ingredient in cannabis. At least five other cannabinoids exhibit biological activity, and so do some terpenes and flavonoids. All these compounds are found in the resin stored in the plant's glandular trichomes. They are chemically related.

THC predominates in plants bred for psychoactivity (as cannabis plants have been bred for generations in California and elsewhere). Cannabidiol -CBD- is the predominant cannabinoid in plants typically bred for fiber. There are only trace quantities of CBD in high-THC plants because one form of the same gene codes for THC synthase and the other codes for CBD synthase. Thus growers selecting for high THC content get low CBD.

http://budz.wordpress.com/2007/12/24/molecule-of-the-year-cannabiodiol/ 18/03/2008

California growers hoping to develop plants with a high CBD-to-THC ratio have been stymied by lack of access to an analytical test lab. In surreptitious tests, "high grade" buds were reportedly in the range of 15-20% THC and 0.1% CBD.

The U.S. Drug Enforcement Administration has placed CBD on Schedule I even though CBD has no known adverse effects and doesn't induce "euphoria." The most dire effects attributed to marijuana -tachycardia (accelerated heartbeat), panic, confusion, anxiety, even psychosis- are effects of THC that CBD has been shown to mitigate!

By listing CBD as a Schedule 1 substance and denying growers the means to develop high-CBD plant strains, the government is protecting the American people from an immunomodulator with anti-inflammatory, anti-convulsant, anti-psychotic, anti-oxidant, and neuro-protective properties. In whose interests could that possibly be?

BF Mechoulam on CBD END BF

The chemist who worked out the precise structure of CBD 45 years ago, Raphael Mechoulam, gave a "review talk" at this year's meeting of the International Association for Cannabis as Medicine. Mechoulam had just gotten his PhD in chemistry in the Fall of '62 and was looking for a research project that might lead to tenure at the Weizmann Institute. He chose to analyze the components of cannabis, he said, thinking "it's a minor project, it will be finished off in six months."

Hashish of Lebanese origin was obtained from the police -"There is a fantastic collaboration between Arabs and Jews in smuggling," Mechoulam observed- and a dozen constituents were then identified by two types of chromatography. (Some cannabis constituents had been identified previously, including CBD, which Roger Adams of the University of Illinois isolated in the early 1940s.)

It was generally assumed throughout the '60s and '70s that the cannabinoids exerted effects not by binding to a specific receptor but "nonspecifically" by altering the lipid structure of cellular membranes. Mechoulam established that the action was specific by purifying THC and showing that only the natural version of the molecule -and not its synthetic mirror image- was exerting the effect. In 1988 Alynn Howlett found that THC was indeed activating a receptor. It was dubbed "CB1" and was found in those areas of the brain involved in movement, stress, cognitive function - "everywhere it would be expected," said Mechoulam, given what was known about the effects of cannabis on people.

Unlike THC, CBD hardly binds to the CB1 receptor. It binds to a second cannabinoid receptor -CB2-originally found in spleen cells by S. Munro of Cambridge University in 1993 and subsequently found in the stomach, liver, heart, kidney, lymph and immune cells, bones, endocrine glands, and throughout the peripheral nervous system.

In his IACM talk Mechoulam reviewed research in recent years that has shed light on aspects of CBD's mechanism of action. Its lipid-solubility enables it to get into places in the brain that conventional neurotransmitters cannot reach. It is a potent anti-oxidative agent. It turns out to be an antagonist to a recently discovered receptor called GPR-55 to which THC and 2-AG bind as agonists. It blocks the uptake of adenosine, an inhibitory neurotransmitter that may promote sleep. It blocks the formation of various cytokines (signaling compounds not released by nerves or glands) under certain circumstances. It activates the serotonin receptors. No wonder, then, that CBD plays a role in many clinical conditions.

Conditions treatable by CBD

Mechoulam described an experiment led by Paul Consroe and colleagues in Brazil in which CBD was tested as a treatment for intractable epilepsy. Patients stayed on the anticonvulsants they had been on (which hadn't eliminated their seizures) and added 200mg/day of CBD or a placebo. Of the seven patients getting CBD over the course of several months, only one showed no improvement; three became seizure-free; one experienced only one or two seizures, Mechoulam recalled; and two experienced reduced severity and occurrence of seizures.

"So it seemed a very promising approach," said Mechoulam, "but unfortunately, nothing has been done ever since. To the best of my knowledge, nobody has done any work on cannabidiol in the clinic on epilepsy, and I wonder why."

A colleague of Mechoulam's, Marc Feldman at Imperial College, London, tested CBD on mice who had a version

of rheumatoid arthritis and found that it reduced inflammation by almost 50% at the right dose -5mg/kg of body weight. But this "beautiful antiinflammatory reaction was lost if we went up to, say, 25 mg/kg," Mechoulam said. Drug developers must bear in mind and cope with the fact that cannabinoids have a finite "therapeutic window" -they are ineffective at low and high doses.

Mechoulam has been testing CBD on mice bred to have a version of type-1 diabetes that manifests around age 14 weeks. He and his co-workers treated these mice with CBD for their first 6-7 weeks of life, then tested them 6-7 weeks later and found that only 30% had developed diabetes (compared to 90-100% given placebo).

In a follow-up experiment the mice weren't given a course of CBD until age 14 weeks, when they were developing diabetes. They were then tested at age 24 weeks, and again only 30% of the treated mice were found to have diabetes. In other words, CBD did not just prevent onset but blocked development of diabetes.

Examination of the insulin-producing islets showed that only 8% were intact in the untreated diabetic mice, whereas 77% were intact in the mice treated with CBD. "I believe that here we have something very promising," Mechoulam said. "We plan to have a clinical trial starting next week treating patients, and hopefully at the next meeting I will tell you that all of them are cured."

Cardiologists working with mice at Hebrew University have found that CBD treatment at the time of a heart attack can reduce infarct size by about 66%. "So now they're pushing me, 'let's have more CBD,'" Mechoulam said. "We should try it with humans in a few years."

He went on: "What about sleep? I'm jumping from thing to thing to show you that CBD does quite a lot of things and I'm not sure that all of them are according to the same mechanism." Mechoulam was part of a group led by Eric Murillo-Rodriguez that administered CBD to rats and determined that while THC caused sleepiness, CBD increased wakefulness and significantly decreased REM sleep. According to Mechoulam, "When one says 'cannabis causes sleep,' one should think really of two compounds, one that causes sleep and one that causes awakening."

The anti-nausea and memory extinction effects of CBD "seem to be closely related," Mechoulam said. He described the problem of anticipatory nausea, for which no good drugs are available. (The effects of chemotherapy can be so nauseating that patients start vomiting when they see the doctor or nurse who is going to administer the treatment.) Linda Parker at the University of Guelph conditioned shrews to start vomiting by administering lithium fluoride at a certain location. When the shrews were subsequently placed in that location they began vomiting. But if given CBD, they could be moved to the dreaded location without vomiting. "The conditioned-wretching reaction was completely abolished," Mechoulam declared. [THC is anti-emetic, too; the advantage of CBD in this instance may be legal rather than medical.]

Mechoulam is hopeful that CBD can help tone down other kinds of conditioning. He described an experiment in which rats had a choice of two paths, one leading to cocaine, one to no reward. Rats like cocaine (and amphetamine) and will learn to choose the path leading to it. But if injected with CBD, they no longer show a preference for cocaine! Mechoulam characterized post-traumatic stress disorder, certain phobias and forms of chronic pain as "human situations which are conditioned" and might be amenable to treatment with CBD. "I know that many patients with PTSD take cannabis, self administered," Mechoulam said. He has been trying to interest the Israeli Ministry of Health in testing CBD and THC at various ratios to treat PTSD.

Fred Gardner will be opening for a band called Lake Street at the Rockitt Room (formerly the Last Day Saloon, Clement St. off 6th Ave.) Sunday, Nov. 25, 8 p.m. He can be reached at fred@plebesite.

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FACILITATION OF CONTEXTUAL FEAR MEMORY EXTINCTION AND ANTI-ANXIOGENIC EFFECTS OF AM404 AND CANNABIDIOL IN CONDITIONED RATS

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Departamento de Farmacologia, Centro de Ciências Biológicas, Universidade Federal de Santa Catarina, UFSC, Florianópolis-SC, Brazil

Studies suggest an important role of the endocannabinoid (eCB) system in the modulation of emotional states and extinction of aversive memories in animals. Particularly, enhancement of eCB transmission by the inhibition of uptake/metabolism seems to be an interesting pharmacological approach. The aim of this study was to investigate the central effects of the eCB uptake/metabolism inhibitor AM404 and the phytocannabinoid with different mechanisms of action cannabidiol (CBD) on the extinction of contextual fear memories in rats. Male Wistar rats were conditioned and injected i.c.v. with AM404 or CBD 24 h later. The animals were subjected to three consecutive 9-min non-reinforced exposures to the conditioning context (extinction sessions). A 3-min drug-free test of contextual memory was performed 24 h after the last extinction session to investigate long-lasting effects. Injection of AM404 (1.0 μg/μl, i.c.v.) and CBD (2.0 μg/μl, i.c.v.) facilitated the extinction of contextual fear memory, with long-lasting effects. This response was antagonized by the CB1-selective antagonist SR141716A (0.2 mg/kg, i.p.), but not by the TRPV1-selective antagonist capsazepine (5.0 μg/μl, i.c.v.), thus suggesting the involvement of CB1 cannabinoid receptors in the facilitation of extinction by these drugs. Anxiolytic-like effects might have contributed to the facilitation of extinction as suggested by an antianxiogenic effect in the fear-potentiated plus-maze test. These results complement other lines of evidence suggesting a role of the eCB system in the modulation of the emotional states and highlight that CBD, a non-psychoative phytocannabinoid could be an interesting pharmacological approach to reduce the anxiogenic effects of stress and promote the extinction of fear memories, such as PTSD.

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1: Psychopharmacology (Berl). 1982;76(3):245-50. Links

Action of cannabidiol on the anxiety and other effects produced by delta 9-THC in normal subjects.

Zuardi AW, Shirakawa I, Finkelfarb E, Karniol IG.

The object of the experiment was to verify whether cannabidiol (CBD) reduces the anxiety provoked by delta 9-THC in normal volunteers, and whether this effect occurs by a general block of the action of delta 9-THC or by a specific anxiolytic effect. Appropriate measurements and scales were utilized and the eight volunteers received, the following treatments in a double-blind procedure: 0.5 mg/kg delta 9-THC, 1 mg/kg CBD, a mixture containing 0.5 mg/kg delta 9-THC and 1 mg/kg CBD and placebo and diazepam (10 mg) as controls. Each volunteer received the treatments in a different sequence. It was verified that CBD blocks the anxiety provoked by delta 9-THC, however this effect also extended to marihuana-like effects and to other subjective alterations induced by delta 9-THC. This antagonism does not appear to be caused by a general block of delta 9-THC effects, since no change was detected in the pulse-rate measurements. Several further effects were observed typical of CBD and of an opposite nature to those of delta 9-THC. These results suggest that the effects of CBD, as opposed to those of delta 9-THC, might be involved in the antagonism of effects between the two cannabinoids.

PMID: 6285406 [PubMed - indexed for MEDLINE]

Related Links

Effects on variable-interval performance in rats of delta 9-tetrahydrocannabinol and cannabidiol, separately and in combination. [Pharmacol Biochem Behav. 1983]

Pharmacological interaction of the effects of delta 9-trans-tetrahydrocannabinol and cannabidiol on serum corticosterone levels in rats. [Arch Int Pharmacodyn Ther. 1984]

Influence of cannabidiol on delta-9-tetrahydrocannabinol effects. [Arch Int Pharmacol Ther. 1976]

Characteristics of the stimulus produced by the mixture of cannabidiol with delta 9-tetrahydrocannabinol. [Arch Int Pharmacodyn Ther. 1981]

Randomized, double-blind, placebo-controlled study about the effects of cannabidiol (CBD) on the pharmacokinetics of Delta9-tetrahydrocannabinol (THC) after oral application of THC verses standardized cannabis extract. [Ther Drug Monit. 2005]

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Br J Pharmacol. 2008 May 12; : 18469842 (P,S,E,B,D)

Cannabidiol, extracted from Cannabis sativa, selectively inhibits inflammatory hypermotility in mice.

R Capasso, F Borrelli, G Aviello, B Romano, C Scalisi, F Capasso, A A Izzo

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Background and purpose:Cannabidiol is a Cannabis-derived non-psychotropic compound that exerts a plethora of pharmacological actions, including anti-inflammatory, neuroprotective and antitumour effects, with potential therapeutic interest. However, the actions of cannabidiol in the digestive tract are largely unexplored. In the present study, we investigated the effect of cannabidiol on intestinal motility in normal (control) mice and in mice with intestinal inflammation.Experimental approach:Motility in vivo was measured by evaluating the distribution of an orally administered fluorescent marker along the small intestine; intestinal inflammation was induced by the irritant croton oil; contractility in vitro was evaluated by stimulating the isolated ileum, in an organ bath, with ACh.Key results:In vivo, cannabidiol did not affect motility in control mice, but normalized croton oil-induced hypermotility. The inhibitory effect of cannabidiol was counteracted by the cannabinoid CB(1) receptor antagonist rimonabant, but not by the cannabinoid CB(2) receptor antagonist SR144528 (N-[-1S-endo-1,3,3-trimethyl bicyclo [2.2.1] heptan-2-yl]-5-(4-chloro-3-methylphenyl)-1-(4-methylbenzyl)-pyrazole-3-carboxamide), by the opioid receptor antagonist naloxone or by the alpha(2)-adrenergic antagonist yohimbine. Cannabidiol did not reduce motility in animals treated with the fatty acid amide hydrolase (FAAH) inhibitor N-arachidonoyl-5-hydroxytryptamine, whereas loperamide was still effective. In vitro, cannabidiol inhibited ACh-induced contractions in the isolated ileum from both control and croton oil-treated mice.Conclusions and implications:Cannabidiol selectively reduces croton oil-induced hypermotility in mice in vivo and this effect involves cannabinoid CB(1) receptors and FAAH. In view of its low toxicity in humans, cannabidiol may represent a good candidate to normalize motility in patients with inflammatory bowel disease.British Journal of Pharmacology advance online publication, 12 May 2008; doi:10.1038/bjp.2008.177.

Other papers by authors:

Br J Pharmacol. 2008 Jan 28; : 18223666 (P,S,E,B,D)

The role of endocannabinoids in the regulation of gastric emptying: alterations in mice fed a high-fat diet.

V Di Marzo, R Capasso, I Matias, G Aviello, S Petrosino, F Borrelli, B Romano, P Orlando, F Capasso, A A Izzo

1Endocannabinoid Research Group, Institute of Biomolecular Chemistry, National Research Council, Pozzuoli, Naples, Italy.

Neurogastroenterol Motil. 2007 Sep 4; : 17931335 (P,S,E,B,D)

The hallucinogenic herb Salvia divinorum and its active ingredient salvinorin A reduce inflammation-induced hypermotility in mice.

R Capasso, F Borrelli, J Zjawiony, L Kutrzeba, G Aviello, G Sarnelli, F Capasso, A A Izzo

Phytomedicine. 2006 Jun ;12 (6-7):501-5, discussion 505 16008126 (P,S,E,B)

Senna and the formation of aberrant crypt foci and tumors in rats treated with azoxymethane.

F Borrelli, R Capasso, G Aviello, G Di Carlo, A A Izzo, N Mascolo, F Capasso

Department of Experimental Pharmacology, University of Naples Federico II, Naples, Via D. Montesano 80131 Naples, Italy.

Arch Toxicol. 2006 Oct 25; : 17063369 (P,S,E,B,D)

Senna: a laxative devoid of carcinogenic effects.

F Borrelli, G Aviello, R Capasso, F Capasso

Department of Experimental Pharmacology, University of Naples Federico II, Via D. Montesano 49, 80131, Naples, Italy. franborr@unina.it.

Neurogastroenterol Motil. 2006 Jan ;18 (1):69-75 16371085 (P,S,E,B,D)

The hallucinogenic herb Salvia divinorum and its active ingredient salvinorin A inhibit enteric cholinergic transmission in the guinea-pig ileum.

R Capasso, F Borrelli, F Capasso, D J Siebert, D J Stewart, J K Zjawiony, A A Izzo

Department of Experimental Pharmacology, University of Naples Federico II, Naples, Italy.

Br J Pharmacol. 2000 Apr ;129 (8):1627-32 10780987 (P,S,E,B) Cited:1

Central and peripheral cannabinoid modulation of gastrointestinal transit in physiological states or during the diarrhoea induced by croton oil.

A A Izzo, L Pinto, F Borrelli, R Capasso, N Mascolo, F Capasso

Department of Experimental Pharmacology, University of Naples 'Federico II', via D. Montesano 49, 80131 Naples, Italy. aaizzo@unina.it

Eur J Pharmacol. 1999 Jul 21;377 (2-3):215-8 10456433 (P,S,E,B)

Contractile effect of (+)-glaucine in the isolated guinea-pig ileum.

A A Izzo, F Borrelli, F Capasso, R Capasso, L Pinto, A Cristoni, N Mascolo

Department of Experimental Pharmacology, University of Naples Federico II, Italy.

Br J Cancer. 2004 Jul 5;91 (1):194 15199387 (P,S,E,B)

Eating garlic and onion: a matter of life or death.

A A Izzo, R Capasso, F Capasso

1Department of Experimental Pharmacology, University of Naples Federico II, via D. Montesano 49, Naples 80131, Italy.

Fitoterapia. 2002 Nov ;73 Suppl 1 :S36-43 12495708 (P,S,E,B)

Effect of a propolis extract and caffeic acid phenethyl ester on formation of aberrant crypt foci and tumors in the rat colon.

F Borrelli, A A Izzo, G Di Carlo, P Maffia, A Russo, F M Maiello, F Capasso, N Mascolo

Department of Experimental Pharmacology, University of Naples 'Federico II', Via D. Montesano 49, 80131, Naples, Italy. franborr@unina.it

Life Sci. 2001 Sep 7;69 (16):1871-7 11693267 (P,S,E,B)

Effect of bisacodyl and cascara on growth of aberrant crypt foci and malignant tumors in the rat colon.

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1: Braz J Med Biol Res. 2006 Apr;39(4):421-9. Epub 2006 Apr 3.

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Cannabidiol, a Cannabis sativa constituent, as an antipsychotic drug.

Zuardi AW, Crippa JA, Hallak JE, Moreira FA, Guimarães FS.

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A high dose of delta9-tetrahydrocannabinol, the main Cannabis sativa (cannabis) component, induces anxiety and psychotic-like symptoms in healthy volunteers. These effects of delta9-tetrahydrocannabinol are significantly reduced by cannabidiol (CBD), a cannabis constituent which is devoid of the typical effects of the plant. This observation led us to suspect that CBD could have anxiolytic and/or antipsychotic actions. Studies in animal models and in healthy volunteers clearly suggest an anxiolytic-like effect of CBD. The antipsychotic-like properties of CBD have been investigated in animal models using behavioral and neurochemical techniques which suggested that CBD has a pharmacological profile similar to that of atypical antipsychotic drugs. The results of two studies on healthy volunteers using perception of binocular depth inversion and ketamine-induced psychotic symptoms supported the proposal of the antipsychotic-like properties of CBD. In addition, open case reports of schizophrenic patients treated with CBD and a preliminary report of a controlled clinical trial comparing CBD with an atypical antipsychotic drug have confirmed that this cannabinoid can be a safe and well-tolerated alternative treatment for schizophrenia. Future studies of CBD in other psychotic conditions such as bipolar disorder and comparative studies of its antipsychotic effects with those produced by clozapine in schizophrenic patients are clearly indicated.

PMID: 16612464 [PubMed - indexed for MEDLINE]

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Cannabis-based medicines--GW pharmaceuticals: high CBD, high THC, medicinal cannabis--GW pharmaceuticals, THC:CBD. [Drugs R D. 2003]

Anxiolytic-like effect of cannabidiol in the rat [illegible] [Prog Neuropsychopharmacol Biol Psychiatry. 2006]

Cannabidiol monotherapy for treatment-resistant schizophrenia. [J Psychopharmacol. 2006]

The nonpsychoactive cannabis constituent cannabidiol is an oral anti-arthritic therapeutic in murine collagen-induced arthritis. [Proc Natl Acad Sci U S A. 2000]

Randomized, double-blind, placebo-controlled study about the effects of cannabidiol (CBD) on the pharmacokinetics of Delta9-tetrahydrocannabinol (THC) after oral application of THC verses standardized cannabis extract. [Ther Drug Monit. 2005]

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Neurobiology of Disease

Nonpsychoactive Cannabidiol Prevents Prion Accumulation and Protects Neurons against Prion Toxicity

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Prion diseases are transmissible neurodegenerative disorders characterized by the accumulation in the CNS of the protease-resistant prion protein (PrPres), a structurally misfolded isoform of its physiological counterpart PrPsen. Both neuropathogenesis and prion infectivity are related to PrPres formation. Here, we report that the nonpsychoactive cannabis constituent cannabidiol (CBD) inhibited PrPres accumulation in both mouse and sheep scrapie-infected cells, whereas other structurally related cannabinoid analogs were either weak inhibitors or noninhibitory. Moreover, after intraperitoneal infection with murine scrapie, peripheral injection of CBD limited cerebral accumulation of PrPres and significantly increased the survival time of infected mice. Mechanistically, CBD did not appear to inhibit PrPres accumulation via direct interactions with PrP, destabilization of PrPres aggregates, or alteration of the expression level or subcellular localization of PrPsen. However, CBD did inhibit the neurotoxic effects of PrPres and affected PrPres-induced microglial cell migration in a concentration-dependent manner. Our results suggest that CBD may protect neurons against the multiple molecular and cellular factors involved in the different steps of the neurodegenerative process, which takes place during prion infection. When combined with its ability to target the brain and its lack of toxic side effects, CBD may represent a promising new anti-prion drug.

Key words: prion; cannabinoid; neuroprotection; scrapie-infected mice; cell-free conversion; microglia

Introduction

Scrapie in sheep, bovine spongiform encephalopathy in cattle, and Creutzfeldt–Jakob disease (CJD) in humans belong to a group of fatal neurodegenerative disorders called transmissible spongiform encephalopathies (TSEs) or prion diseases. No therapeutic treatments against TSEs are currently available. The urgent need to find effective anti-prion therapies has been strengthened by the emergence of variant CJD (vCJD) caused by contaminated beef consumption and the fact that vCJD can be transmitted via blood transfusion (Llewelyn et al., 2004). A critical event in TSE pathogenesis is the conversion of the normal protease-sensitive host prion protein (PrPsen) to an aggregated and protease-resistant form, PrPres. Both PrP isoforms are required for infection and pathogenesis (Sailer et al., 1994). Although PrPres has been recovered in various tissues such as spleen, tonsils, and muscles, tissue damage is most severe in the CNS of the prion-affected host. Intraneuronal vacuolization, severe neuronal cell death, microglia activation, and astrogliosis are the main hallmarks of TSEs. In affected brains, the amount and location of PrPres deposits are clearly linked to histopathological lesions. Thus, the presence of PrPres is considered indicative of TSE disease.

One possible approach to TSE therapy is the inhibition of PrPres formation in the CNS. A wealth of experimental data indicates that cyclic compounds are capable of inhibiting PrPres formation *in vitro* (Caughey et al., 1998). Nevertheless, no compounds have been identified that have a therapeutic benefit after infection has reached the CNS. One possibility is that the blood–brain barrier (BBB) restricts the access of many potential anti-TSE inhibitors (Priola et al., 2000). Therefore, in searching for compounds that could be used in the treatment of prion diseases, we focused on cyclic molecules exhibiting particular properties such as the ability to cross easily the BBB, weak toxicity, and few side effects. Cannabinoids possess all of these characteristics, making them of interest as potential anti-prion drugs. Moreover, cannabinoids are neuroprotectant in a wide variety of *in vitro* and *in vivo* models of neuronal injury including neurodegenerative disorders (Lastres-Becker et al., 2005). These effects have been ascribed, among others, to antioxidant properties, NMDA antagonism, decrease in glutamate release, and blockade of microglia migration and activation (Mechoulam et al., 2002).

This background prompted us to assay a series of cannabinoid derivatives for their ability to prevent PrPres accumulation in two well established scrapie-infected cell models. We report that cannabidiol (CBD), a nonpsychoactive component of *Cannabis sativa*, inhibited PrPres formation in cells and exhibited neuroprotective activity against PrPres-induced neurotoxicity. Moreover,

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CBD increased survival times and inhibited PrPres accumulation in the brains of scrapie-infected mice. Based on an *in vitro* conversion assay, we demonstrated that the involved mechanisms are more complicated than direct interactions between CBD and PrPres and/or PrPsen, thus defining a new class of anti-TSE compounds. Given that a number of clinical trials have underscored the potential of cannabinoids as antiemetics and analgesics as well as in the treatment of multiple sclerosis, epilepsy, and glaucoma (Ben Amar, 2006), cannabinoids would be available for immediate TSE clinical trials.

Materials and Methods

Materials. An Opti-MEM, RPMI 1640, Neurobasal, B27, G418, and penicillin–streptomycin (P/S) mixture was purchased from Invitrogen (San Diego, CA). L-Glutamine, fetal calf serum (FCS), and PBS were from BioWhittaker (Verviers, Belgium). Horseradish peroxidase-conjugated goat anti-mouse antibodies were from Jackson ImmunoResearch (West Grove, PA). All the cannabinoids were purchased from Sigma (St. Louis, MO), except CBD was from GW Pharmaceuticals (Wiltshire, UK), dissolved at 10^{-2} M in ethanol, and stored at −20°C until use.

Cell cultures. N11 microglia were grown in RPMI 1640 containing 10% FCS and P/S. Epithelial cells (Rov9) chronically infected with natural sheep scrapie (Rov9sc$^+$) were grown in DMEM supplemented with 10% FCS, P/S, and 1 μg/ml doxycycline. Neuroblastoma cells chronically infected with the murine Chandler strain (N2asc$^+$) were grown in Opti-MEM supplemented with 10% FCS, P/S, and 1 μg/ml G418. PrPres-cured neuroblastoma cells (N2asc$^-$) were obtained by treatment with Congo red (1 μg/ml) for several passages.

Assay for PrP-res accumulation from N2asc$^+$ and Rov9sc$^+$ cultures. N2asc$^+$ and Rov9sc$^+$ cells were seeded at 10% confluent density in the appropriate medium and treated with the indicated concentration of drugs for 4 d (passage 1). Control experiments were performed in the presence of ethanol alone; the amount of ethanol was fixed to 1% in all conditions. Cultures were split every 4 d at a 1:4 dilution and incubated in the presence of the drug for the indicated number of passages. Confluent cultures were homogenized in lysis buffer (50 mM Tris-HCl, pH 7.4, containing 150 mM NaCl, 0.5% Triton X-100, 0.5% sodium deoxycholate, and 5 mM EDTA) and centrifuged at 3000 × *g* for 5 min. For detection of PrPsen, one-tenth of a postnuclear supernatant was mixed with the denaturing loading buffer. For detection of PrPres, lysates were digested with 20 μg of proteinase K (PK) per milligram of total protein for 30 min at 37°C before centrifugation at 20,000 × *g* for 90 min. Pellets were resuspended in denaturing loading buffer, boiled, and loaded onto a 12% polyacrylamide gel. Mouse and sheep PrPres were assayed with the SAF83 or SAF70 monoclonal antibodies, respectively. Blots were developed using an enhanced chemiluminescence system (ECL; Amersham Biosciences, Piscataway, NJ) with a LAS3000 detector (Fuji, Tokyo, Japan). To correct for any loading artifact, blots with non-PK-digested proteins were reprobed with the anti-Erk antibody. Densitometry analyses were performed with NIH Image software on the PK-digested immunopositive band corresponding to the glycosylated PrP form, and results were expressed as a percentage of control levels.

In vivo *CBD treatments.* Tga20 mice, which overexpress murine PrP, and C57BL/6 mice were intraperitoneally infected with 100 μl of a 2% homogenate prepared from the brains of terminally ill 139A scrapie-infected C57BL/6 mice. As a negative control, Prnp$^{0/0}$ mice were infected under the same experimental conditions. Mice were treated intraperitoneally three times per week for the indicated period of time with 200 μl of 20 or 60 mg/kg CBD diluted 1:1:2 in an ethanol/cremophor/NaCl 0.9% mixture. A control group of scrapie-infected animals was treated only with the vehicle mixture. To evaluate the toxicity of CBD, scrapie-free mice were treated with the higher CBD dose (i.e., 60 mg/kg) three times per week for 12 weeks. As indicated, treatments began on the day of scrapie inoculation ($t = 0$) or 30 and 120 d postinoculation (dpi). Animals were monitored every 2 d, and the onset of clinical scrapie was defined when mice showed at least three of the following signs: ataxia, kyphosis, generalized tremor, swaying gait, tail stiffness.

Metabolic labeling of PrPsen. Mouse PrP expressing the epitope to the monoclonal antibody 3F4 but without the glycophosphatidyl-inositol membrane [Mo3F4(GPI^{NEG})] has been described previously (Priola et al., 2001). Mo3F4(GPI^{NEG}) was expressed in mouse fibroblast cells, and a single cell clone expressing high levels of Mo3F4(GPI^{NEG}) PrP, Mo3F4(GPI^{NEG})-F3, was derived. Mo3F4(GPI^{NEG})-F3 cells were metabolically labeled with 1 mCi of Tran^{35}S methionine/cysteine (Perkin-Elmer, Wellesley, MA), and Mo3F4(GPI^{NEG}) PrP-sen was immunoprecipitated with the 3F4 antibody as described previously (Kocisko et al., 1994).

Cell-free conversion assay. Enriched PrPres isolated from the brains of mice infected with the Chandler scrapie strain was partially unfolded in 2.5 M guanidine hydrochloride for 1 h at 37°C. For each conversion reaction, 100 ng of unfolded PrPres was mixed with 10,000 cpm (~1 ng) of radiolabeled and immunoprecipitated Mo3F4(GPI^{NEG}) PrPsen. Cannabinoids freshly diluted in ethanol were added to the conversion reaction to the indicated final concentration. Because ethanol alone can inhibit PrPres formation (S. A. Priola, unpublished data), the final amount of ethanol for all reactions, including the "no inhibitor" and "no PrPres" controls, was kept constant at 2.5% of the total reaction volume. This

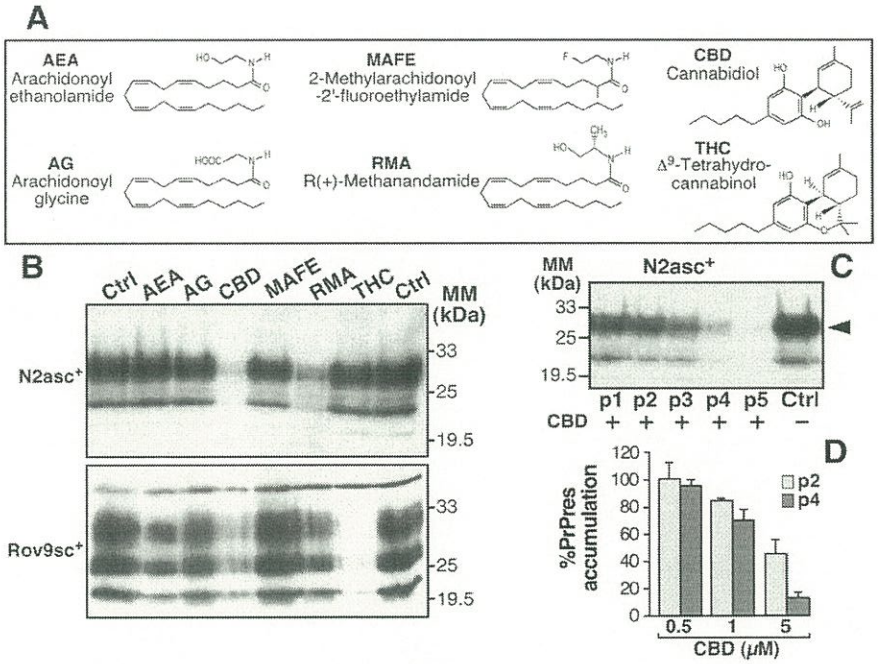

Figure 1. Effects of CBD and cannabinoid analogs on PrPres accumulation in mouse- and sheep-infected cell models. ***A***, Names, abbreviations, and structures of cannabinoid derivatives used in the current study. ***B***, N2asc$^+$ (top) and Rov9sc$^+$ (bottom) cells were treated with 5 μM of each tested drug or with ethanol alone (Ctrl). Cell cultures were passaged four consecutive times. ***C***, N2asc$^+$ treated with 5 μM of CBD for the indicated number of passages (p1–p5). Cultures were homogenized in lysis buffer, PK digested, and subjected to Western blotting. PK-digested mouse or sheep PrPres was assayed with SAF83 (***B***, top, ***C***) and SAF70 (***B***, bottom) antibodies, respectively. Immunoblots (***B***, ***C***) are representative of typical experiments. Densitometry analyses were performed on the PK-digested immunopositive band indicated by the arrowhead, and are results expressed as a percentage of control levels. ***D***, Dose–response of CBD on the PrPres amount at the second (p2, light gray) and fourth (p4, dark gray) passages of N2asc$^+$. The data represent the mean of four independent experiments ± SD. MM, Molecular mass.

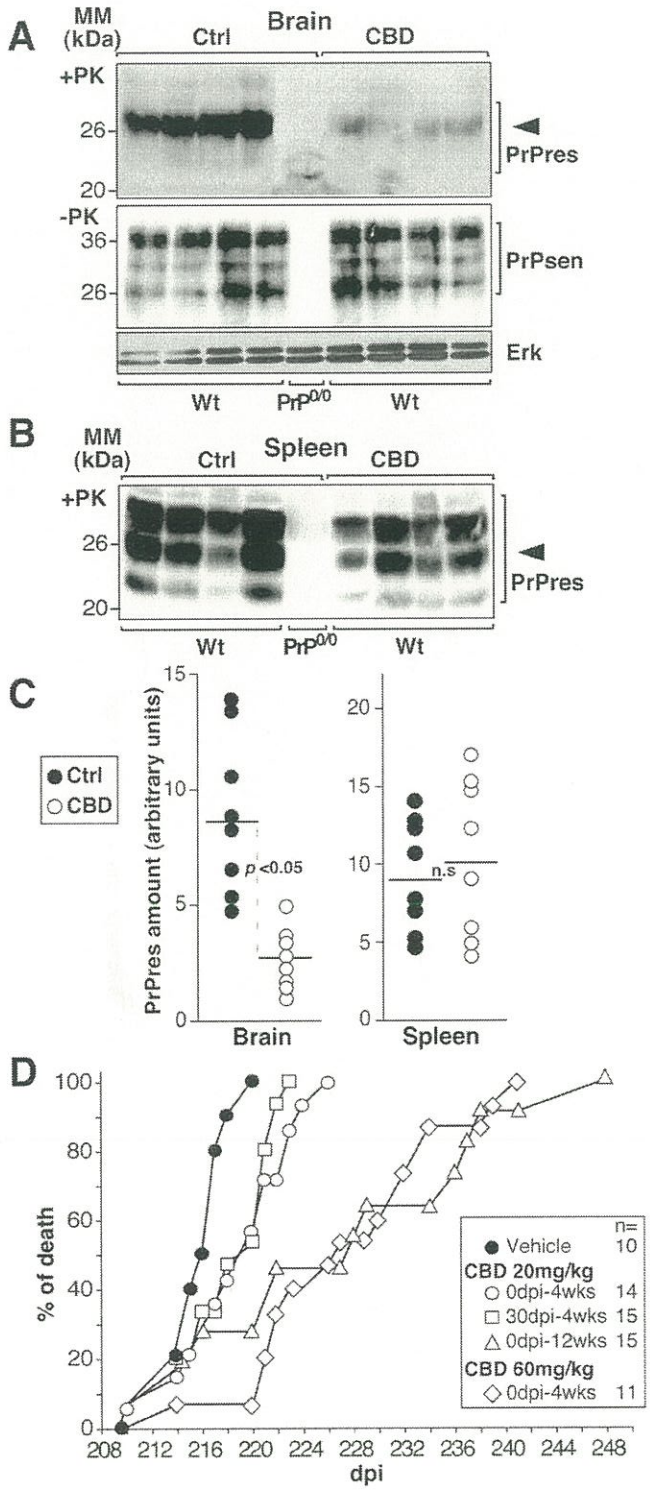

Figure 2. CBD prevents cerebral PrPres formation and prolongs prion disease incubation time in scrapie strain-infected mice. ***A***, ***B***, Western blot analyses performed on brain (***A***) or spleen (***B***) homogenates of scrapie-infected C57BL/6 wild-type (Wt) or $PrP^{0/0}$ mice treated three times per week intraperitoneally for 3 weeks with 20 mg/kg CBD; the control (Ctrl) group of animals ($n = 8$) was treated with the vehicle alone. The treatments began the day of scrapie infection. Forty days after infection, mice were killed, and tissues were homogenized in lysis buffer. Detection of PrPsen or Erk (***A***, middle and bottom) and PrPres (***A***, top, ***B***) were performed on undigested and PK-treated lysates, respectively. Four hundred micrograms (***A***, top, ***B***) and 25 μg (***A***, middle and bottom) of total proteins were loaded per lane. Densitometry analysis was performed on the band indicated by the arrowhead, and the amount of PrP was calculated as follows: mean intensity/surface × correction factor for load as expressed in arbitrary units. MM, Molecular mass. ***C***, Each dot represents the normalized PrPres content of a single vehicle-treated (Ctrl; ●) or CBD-treated (○) wild-type mouse. The dark line is the mean value. ***D***, Days to death of infected C57BL/6 mice treated with the vehicle alone (●) or with CBD at the indicated concentration (open symbols). The starting point in days postinfection (dpi) and the duration of the treatment in weeks (wks) are indicated. Individual data points represent the percentage of dead mice out of the total number of mice infected for each group. Multiple mice may be represented by a single data point. The legend is shown on the right, and the number of mice is indicated. Significance (p) was evaluated using the nonparametric Mann–Whitney test. Differences were considered significant for p values <0.05. n.s., Not significant.

amount of ethanol has a minimal effect on PrPres formation (Priola, unpublished data). Reactions with and without inhibitor were incubated in reaction buffer (0.75 M guanidine hydrochloride, 1.25% Sarkosyl, 5 mM cetyl pyridinium chloride, and 50 mM sodium citrate buffer, pH 6.0) for 2 d at 37°C. One-tenth of the reaction was methanol precipitated and used to assay the amount of total radiolabeled PrP. To determine the amount of radiolabeled PrPres formed, the remainder of the reaction was digested with 12 μg/ml PK for 1 h at 37°C. Proteolysis was stopped by the addition of 10 mM Pefabloc and 400 ng of bovine thyroglobulin, and proteins were methanol precipitated. Radiolabeled products were analyzed by SDS-PAGE and quantified using the Storm phosphorimager system (Amersham Biosciences).

Flow cytometry analysis. N2a cells were cultured in the presence of 5 μM CBD or with ethanol alone and trypsinated and resuspended in PBS, pH 7.4, supplemented with 0.1% BSA and 0.1% NaN_3. Alternatively, cells were fixed in Lyse/Fix Buffer (BD Biosciences, Franklin Lakes, NJ) and permeabilized in Perm Buffer III (BD Biosciences) according manufacturer recommendations. Intact or permeabilized cells were incubated with anti-PrP SAF83 antibody (1:200) in PBS–0.1% BSA for 30 min at room temperature and incubated with Alexa488-conjugated secondary antibody (1:400). Flow cytometry analysis was performed on a FACS-Calibur and the CELLQuest software. Cells were gated according to size and scatter to eliminate dead cells and debris from analysis. Experiments were repeated three times for consistency.

Confocal laser microscopy. N2a cells were cultured for four passages in the presence of 5 μM CBD or with ethanol alone and transferred to glass coverslips. For cell-surface PrPsen detection, coverslips were washed twice with cold PBS and fixed in 2% paraformaldehyde for 10 min at room temperature. After 20 min in PBS–5% BSA, each coverslip was incubated for 30 min in PBS–5% BSA containing the anti-PrP antibody SAF83 (1:200). Where indicated, the incubation with primary antibodies was done on permeabilized cells (i.e., in PBS–5%BSA supplemented with 0.1% Triton X-100). Cells were rinsed three times in PBS and incubated with the appropriate conjugated secondary antibodies (1:200) in PBS–5% BSA. Coverslips were mounted on glass slides with Fluoprep (bioMérieux, Marcy l'Etoile, France) containing 1 μg/ml DAPI (diamidino-4′,6-phénylindol-2 dichlorhydrate) to stain nuclei. Cells were observed under a Leica (Nussloch, Germany) laser-scanning confocal microscope (SP5) equipped with a DM-IRBE inverted microscope and an argon–krypton laser. Images were acquired as single transcellular optical sections and averaged over at least four scans per frame.

Scrapie and scrapie-free homogenate preparation. N2asc$^+$ homogenates (hgtsc$^+$), used as a source of PrPres, were obtained in detergent-free conditions (Marella and Chabry, 2004). The preparation was up to 75% PrPres with a final concentration of ~20 pg/μl. N2asc$^-$ homogenates (hgtsc$^-$) from uninfected cells were PrPres free and were used as negative controls.

Neurotoxicity assay on primary cultures of neurons. Cortical neurons from embryonic day 14 mice were prepared as described previously (Marella and Chabry, 2004). Cells were plated at a density of 5×10^4 cells/well in 96-well tissue-plastic dishes. Neurons were grown in Neurobasal medium supplemented with B27 and 10 μM cytosine β-D-arabinofuranoside to prevent glial growth. Cultures used after 6–8 d of differentiation were 95% neurons. Cultures were incubated with the indicated concentration of drug or ethanol alone before the addition of 5 or 10 μl of hgtsc$^-$ or hgtsc$^+$ (≈1 and 2 ng/ml PrPres, respectively). After overnight incubation, microglia were added to neurons at the ratio 1:10.

Table 1. CBD treatment of 139A scrapie strain-infected Tga20 mice

Treatment	Mice (scrapie sick/total)	Mean incubation time to illness (days ± SD)	Delay (days)	Significance *p* value (Mann–Whitney test)	Mean incubation time to death (days ± SD)	Delay (days)	Significance *p* value (Mann–Whitney test)
Vehicle only	10/10	85.3 ± 2.7	NA	NA	88.6 ± 1.7	NA	NA
CBD (60 mg/kg) starting at 0 dpi	9/9	91.0 ± 4.5	5.7	0.021	94.0 ± 4.3	5.4	0.022
CBD (60 mg/kg) starting at 30 dpi	10/10	87.0 ± 2.2	1.7	NS	90.2 ± 2.5	1.6	NS

NA, Not applicable; NS, not significant.

Neuronal viability was determined using the 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxy-phenyl)-2-(4-sulfophenyl)-2*H*-tetrazolium (MTS) method (AqueousOne; Promega, Madison, WI).

Microglia migration assay. The cell migration assay was performed as described previously (Marella et al., 2005). Briefly, neurons (5×10^5 cells/well) plated in 24 wells were incubated with PBS, hgtsc$^-$, or hgtsc$^+$ (≈0.2 ng/ml PrPres) in the absence or presence of CBD for 24 h at 37°C. N11 microglia were added to the top of a Boyden's chamber (5×10^4 cells/200 μl) and allowed to migrate through polyester filters for 6 h. Cultures were then fixed with 3% paraformaldehyde and stained with crystal violet, and the cells were counted (five random fields per filter) under an inverted microscope.

Results

Effects of cannabinoid derivatives on PrPres accumulation in mouse and sheep scrapie-infected cells

We screened a series of cannabinoid derivatives for their ability to prevent PrPres accumulation in scrapie-infected cells (Fig. 1*A*). The chosen cannabinoid derivatives belonged to three different groups: endocannabinoids [arachidonoyl ethanolamide (AEA), arachidonoylglycine (AG)], natural components of *C. sativa* [Δ^9-tetrahydrocannabinol (THC), CBD], and synthetic nonmetabolized molecules [2-methylarachidonoyl-2′-fluoroethylamide (MAFE), *R*(+)-methanandamide (RMA)]. We used two well established scrapie-infected cell models, namely neuroblastoma cells chronically infected with the Chandler murine strain of scrapie (N2asc$^+$) (Nishida et al., 2000) and epithelial cells infected with natural sheep scrapie (Rov9sc$^+$) (Vilette et al., 2001). N2asc$^+$ and Rov9sc$^+$ cells were treated continuously with 5 μM of each tested drug over four passages of the cells. In N2asc$^+$ and Rov9sc$^+$ cells, the level of PrPres accumulation was drastically reduced in the presence of CBD as estimated by Western blot analysis (Fig. 1*B*). THC was also able to reduce the level of PrPres, but only in sheep Rov9sc$^+$ cells (Fig. 1*B*, bottom). In both scrapie-infected cell types, RMA induced a slight decrease in the PrPres content compared with the untreated control, whereas AEA, AG, and MAFE had no effect (Fig. 1*B*).

To assay the effect of CBD on PrPres accumulation over time, Chandler-infected N2asc$^+$ were treated with 5 μM CBD for five consecutive passages, and the PrPres level was analyzed for each passage. From the second passage, the amount of PrPres was significantly reduced in cells treated with 5 μM CBD. Over the course of successive passages, the PrPres amount decreased progressively until it was barely detectable by the fifth passage (Fig. 1*C*). The reduction in PrPres accumulation was dependent on both the number of passages of treated cells and the CBD concentration (Fig. 1*D*). Similar effects of CBD were observed on N2asc$^+$ infected with another murine scrapie strain named 22L (data not shown). In summary, CBD is a unique cannabinoid derivative able to strongly prevent PrPres formation, regardless of scrapie strain, in both mouse and sheep scrapie-infected cells.

CBD reduces the cerebral accumulation of PrPres and prolongs the survival time of scrapie-infected mice

We next addressed whether or not CBD could affect *in vivo* PrPres accumulation. This was investigated by inoculating mice with the 139A murine scrapie strain, followed by treatment with 20 mg/kg CBD. Forty days after infection, mice were killed, and Western blot analysis was performed on brain and spleen, the crucial organs involved in TSE pathology, to detect and quantify the amount of PrPres (Fig. 2). As expected, no PrP immunoreactivity was detected in the brain or spleen of infected *Prnp*$^{0/0}$ mice (Fig. 2) because they fail to accumulate PrPres and are known to be resistant to prion infection (Büeler et al., 1993). In the brains of CBD-treated mice, PrPres was barely detectable, whereas substantial amounts of PrPres were present in the brain of presymptomatic untreated control mice (Fig. 2*A*, top). Measurement of the amount of PrPsen in brain lysates revealed no significant change between control and CBD-treated mice, suggesting that CBD did not affect the level of PrPsen expression *in vivo* (Fig. 2*A*, middle). Surprisingly, no significant difference in PrPres accumulation was observed in spleen homogenates from control and CBD-treated animals (Fig. 1*B*). Quantification of the Western blot data confirmed that the amount of cerebral PrPres accumulation was significantly different in CBD-treated animals compared with control animals ($p < 0.05$, Mann–Whitney test) (Fig. 1*C*), whereas no significant difference was detected in the spleen.

To determine whether CBD could affect prion disease *in vivo*, we infected wild-type mice (Fig. 1*D*) by intraperitoneal inoculation with a high dose of 139A murine scrapie strain. This strain was chosen because its incubation period after peripheral inoculation is shorter and less variable than other strains (Carp et al., 1997). Starting on the day of infection (0 dpi) and continuing three times per week over a 4 week period, mice were treated with 20 mg/kg CBD. Treatment with CBD significantly increased the survival time of the infected wild-type mice compared with the vehicle-treated group ($p = 0.02$, Mann–Whitney test). Treatment over a longer period of time (up to 12 weeks) or treatment with a higher dose of CBD (60 mg/kg over 4 weeks) also led to a significant increase in the survival time of infected mice ($p = 0.003$ and 0.0003, respectively). To determine whether CBD also inhibited prion disease progression during the later stages of infection, we began treatment of infected mice at either 30 or 120 dpi. When 20 mg/kg CBD treatment was initiated at 30 dpi, the survival time of mice was significantly increased compared with the vehicle-treated group ($p = 0.03$). However, no significant difference was observed when CBD treatment was started at 120 dpi (data not shown). Overall, CBD delayed prion disease onset in scrapie-infected mice in a time- and concentration-dependent manner.

Because of their high level of PrP expression, Tga20 mice have shorter incubation times than wild-type mice and thus were used as a rapid assay for testing inhibition of scrapie disease (Table 1). As above, Tga20 mice were infected intraperitoneally with the 139A scrapie strain and treated with 60 mg/kg CBD intraperitoneally three times per week over 4 weeks. When treatment was applied starting the day of infection, CBD significantly delayed both the appearance of clinical signs of the disease (delay, 5.7 d; $p = 0.021$) and death (delay, 5.4 d; $p = 0.022$) compared with the vehicle-treated group. When CBD treatment started at 30 dpi

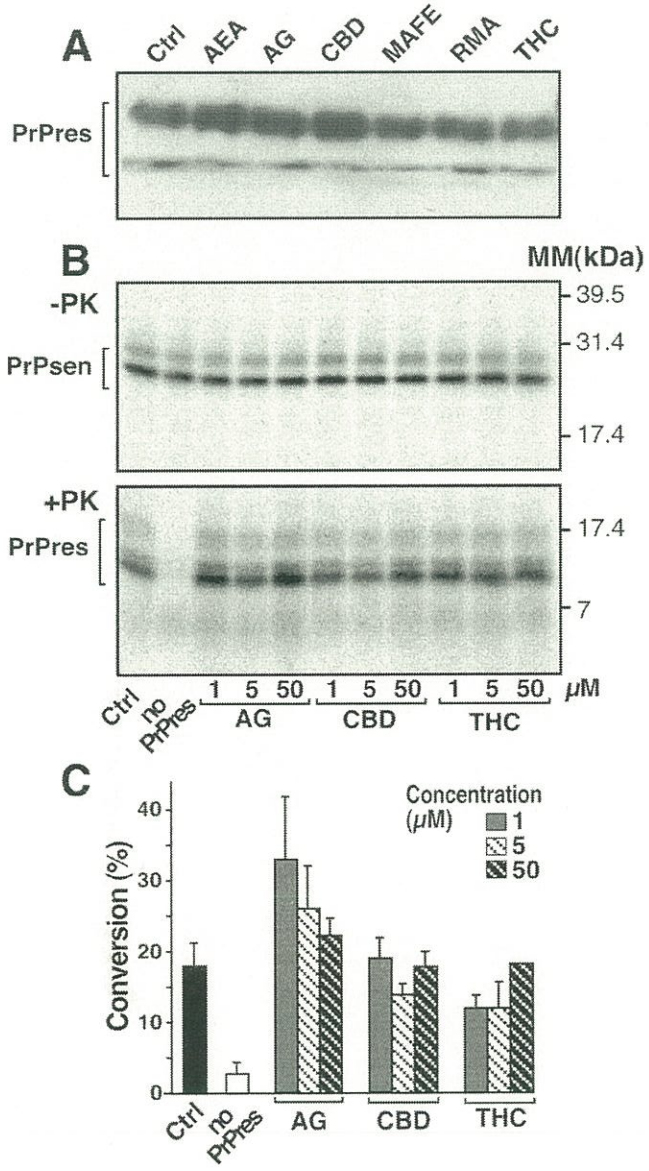

Figure 3. Cannabinoid derivatives do not interfere with sedimentation and cell-free formation of PrPres. ***A***, PK-digested N2asc$^+$ lysates were incubated in the presence of ethanol (Ctrl) or 10 μM of the indicated cannabinoid for 48 h at 4°C and prepared for Western blotting. ***B***, Cell-free formation of PrPres using ^{35}S-Mo3F4(GPI^{NEG}) PrPsen and PrPres derived from mice infected with the mouse Chandler scrapie strain, the parent strain of 139A. An autoradiograph of an SDS-PAGE gel from a representative experiment is shown. The top panel (−PK) shows the total amount of ^{35}S-PrP in the reaction, whereas the bottom panel (+PK) shows the amount of ^{35}S-Mo3F4(GPI^{NEG})PrPres generated. The amount of inhibitor present in each reaction is indicated, and molecular mass (MM) markers are shown on the right. The brackets indicate the protein bands that were quantified for the data in ***C***. ***C***, Quantification of the amount of ^{35}S-PrPres formed in the presence of different concentrations of cannabinoid derivatives. Error bars represent the SEM for $n = 3$. For each individual sample, percentage conversion = amount of ^{35}S-Mo3F4(GPI^{NEG})PrPres/amount of total ^{35}S-PrP(10) × 100. Using a one-way ANOVA with Dunnett's post-test, there was no significant difference between the positive control (no inhibitor) reaction and reactions containing 50 μM of inhibitor ($p > 0.05$), whereas the control and "no PrPres" groups were significantly different ($p < 0.01$).

(approximately one-third of the incubation time), no significant delay in the progression of the disease was observed. During the time course of these experiments, no significant side effects were seen in noninfected, CBD-treated mice (data not shown). In summary, our data demonstrate that, when applied the day of infection or as late as 1 month after infection, CBD slows down PrPres accumulation in the brains of prion-infected mice and delays the onset of terminal prion disease.

CBD does not interfere with PrPres *in vitro*

Scrapie-infected mice treated with CBD had significantly lower levels of PrPres accumulation in the brain (Fig. 2*A*), whereas scrapie-infected cells exposed to CBD had significantly decreased levels of PrPres (Fig. 1). These data suggested that CBD could exert its anti-scrapie effect *in vivo* either by destabilizing preexisting PrPres aggregates or by preventing PrPres formation. We first tested the capacity of cannabinoids to destabilize PrPres aggregates *in vitro* by assaying the change in sedimentation properties of PrPres in the presence of cannabinoids (Fig. 3*A*). PK-digested homogenates prepared from N2asc$^+$ were incubated with 10 μM of each compound for 2 d. At the end of the incubation time, the homogenates were assayed for PrPres by Western blotting. No difference in the amount of PrPres was observed in cannabinoid-treated N2asc$^+$ homogenates versus untreated homogenates (Fig. 3*A*).

Next, the ability of CBD to directly inhibit PrPres formation was tested in a cell-free assay (Kocisko et al., 1994). AG, CBD, and THC were added in increasing concentrations to a reaction mixture containing PrPres derived from Chandler scrapie-infected mice and ^{35}S-Mo3F4(GPI^{NEG}) PrPsen, a protein known to efficiently convert to PrPres *in vitro* (Priola et al., 2001). The amount of ^{35}S-Mo3F4(GPI^{NEG})PrPres generated was then determined. A representative reaction is shown in Figure 3*B*. The addition of up to 50 μM CBD did not appear to significantly decrease the amount of ^{35}S-Mo3F4(GPI^{NEG})PrPres formed compared either with reactions with no inhibitor added or with reactions containing AG and THC, which have no anti-scrapie properties (Fig. 3*C*). These results suggest that, mechanistically, CBD does not decrease PrPres formation via direct interactions with either PrPsen or PrPres.

CBD has no influence on the expression level and subcellular location of PrPsen

It is well established that the relative level of PrPres production correlates with the level of PrPsen expression (Daude et al., 2003). Thus, we analyzed the level of PrPsen in N2a cells treated with AG, CBD, or THC over four passages. None of the tested cannabinoids, even CBD, was able to modify the PrPsen expression level (Fig. 4*A*). If PrPsen trafficking to the outlet plasma membrane is blocked, PrPres formation is inhibited (Gilch et al., 2001). To examine the possibility that CBD could change the subcellular distribution of PrPsen and thus affect PrPres formation, cell-surface and intracellular PrPsen was observed on unpermeabilized and permeabilized N2a cells, respectively, using both fluorescence-activated cell sorting and confocal microscopy techniques. Cells treated with CBD for two and four passages expressed the same amount of surface-bound and intracellular PrPsen as AG-treated cells (Fig. 4*B*). Confocal microscopy observation confirmed that CBD treatment had no significant influence on the plasma membrane expression of PrPsen compared with AG (Fig. 4*C*, top panels) or vehicle alone (data not shown). As well, no differences in PrPsen-positive intracellular compartments could be detected in the presence of CBD (Fig. 4*B*,*C*, bottom panels). Thus, CBD appears to have little or no influence on the expression level and cell trafficking of PrPsen.

Neurotoxicity and microglial cell migration induced by PrPres are hampered by CBD

Because some cannabinoids have been shown to exert neuroprotective effects after various experimental brain injuries, it was of interest to test the neuroprotective properties of CBD against PrPres-induced neuronal cell death in primary neuronal cell culture (Fig. 5). Neurons exposed to hgtsc$^+$ for 2 d were clearly damaged as reflected by the disappearance of normal cell bodies and the presence of fragmented neurites compared with neurons exposed to the PrPres-free N2asc$^-$ homogenate (hgtsc$^-$). The addition of CBD appeared to decrease PrPres-induced neurotoxicity because the number and morphology of the neurons was similar to that observed in cells exposed to hgtsc$^-$ (Fig. 5*A*). Neuronal viability was also monitored by measuring the reduction in mitochondrial activity using the MTS assay. CBD treatment resulted in a concentration-dependent increase in the number of viable neurons for both concentrations of hgtsc$^+$ tested (Fig. 5*B*).

We previously demonstrated that neurons trigger microglial cell migration in response to PrPres exposure (Marella and Chabry, 2004). Because CBD has been shown to regulate microglia migration (Walter et al., 2003), it was of interest to determine whether or not CBD could affect this PrPres-induced chemotactic mechanism. The migration of N11 microglia toward a chamber containing neurons incubated with PBS, hgtsc$^-$ or hgtsc$^+$ was monitored in the presence of increasing concentrations of CBD (Fig. 5*C*). Hgtsc$^+$-exposed neurons induced an increase in the number of migrating microglia compared with hgtsc$^-$ and PBS-exposed neurons. Importantly, no effect on the basal migration rate of microglial cells was observed with CBD alone, indicating that CBD by itself does not have *in vitro* chemotactic properties (Fig. 5*C*). Thus, CBD was able to impair PrPres-induced microglial cell migration in a concentration-dependent manner.

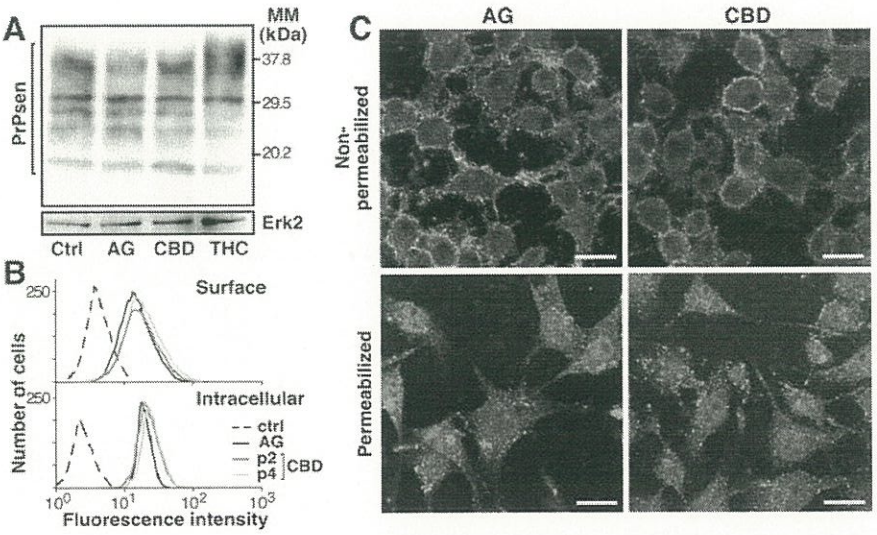

Figure 4. CBD does not affect either the level of PrPsen expression or its subcellular location. ***A***, N2asc$^+$ cells were treated for four consecutive passages with 5 μM of the indicated cannabinoids or ethanol alone (Ctrl). Cell lysates were subjected to SDS-PAGE and Western blotting using the anti-PrP antibody SAF83. Equal loading of proteins was confirmed by reprobing the same blot with anti-Erk2 antibody. MM, Molecular mass. ***B***, Flow cytometry analysis performed on intact (top) or permeabilized (bottom) N2a cells treated with 5 μM AG or CBD for two (p2) or four (p4) passages of the cells. Surface-bound and intracellular PrPsen was revealed with monoclonal antibody SAF83 as described in Materials and Methods. The dashed line represents the fluorescence intensity measured when cells were incubated with the Alexa488-conjugated secondary antibody alone. ***C***, Confocal microscopy images performed on unpermeabilized (top) or permeabilized (bottom) N2a cells treated with 5 μM of AG or CBD as indicated. Surface-bound and intracellular PrP (green) was revealed with monoclonal antibody SAF83 and Alexa488-conjugated secondary antibody. Identical photomultiplicator values and parameters of the laser-scanning confocal microscope were used for all pictures. Nuclei were stained by DAPI (diamidino-4′,6-phénylindol-2 dichlorhydrate) and appear in blue. Scale bars, 20 μm. ***A–C*** are representative experiments.

Discussion

Although the etiology of prion diseases remains uncertain, there is no doubt that pathogenesis is directly related to the formation of PrPres in the brains of TSE-affected patients and animals. Here, we demonstrate that CBD increases the survival time of scrapie-infected mice, most likely by preventing cerebral accumulation of PrPres. Given that CBD inhibited PrPres accumulation in cells infected with both murine and sheep scrapie strains, it is likely that it will be effective against different TSE agents in different species.

The mechanism by which CBD inhibits PrPres formation remains unclear. However, we were able to establish clearly that CBD does not modify the cellular trafficking and processing of PrPsen in neuroblastoma cells. Moreover, CBD does not appear to exert its anti-prion activity through the destabilization of preexisting PrPres aggregates. PrPres can assume a variety of abnormal aggregated states with different particle sizes, conformations, and membrane interactions (Caughey and Lansbury, 2003). In some cases, the low level of PrPres amyloid fibrils and/or plaques recovered in TSE-affected brains is not easily reconcilable with the dogma that large aggregates are responsible for neurodegeneration (Westaway et al., 1994; Chesebro et al., 2005). In fact, it is conceivable that the formation of large fibrils may be a way to sequester small neurotoxic entities in an inactivated and inert form. Thus, the precise nature of the PrPres moieties leading to neuronal malfunction and death during TSE pathogenesis remains uncertain. Growing evidence supports the idea that small PrPres oligomers and/or protofibrils may account for both the massive amount of neuronal cell death and infectivity of TSEs (Chiesa and Harris, 2001). Consequently, destabilization of large aggregates of PrPres leading to small oligomer formation could be more harmful than beneficial. In this context, the inability of CBD to break up PrPres aggregates in smaller pieces could actually be an advantage.

Compounds with cyclic structures containing hydrophobic aromatic chains or rings such as Congo red (Caughey and Race, 1992), quinacrine (Doh-Ura et al., 2000), and cyclic tetrapyrolles (Caughey et al., 1998) have been shown to decrease PrPres formation *in vitro*. These molecules bind strongly to PrP isoforms and hamper the changes in protein conformation required for PrPres formation. Based on the structural analogy, we hypothesized that CBD might act directly as an inhibitor of the conversion reaction by binding to PrPsen, PrPres, or both. Unexpectedly, we demonstrated that CBD does not inhibit PrPres formation in a cell-free assay in which the primary components are PrPsen and PrPres. It is therefore unlikely that CBD binds PrP isoforms. Rather, CBD may exert its anti-prion properties indirectly via more complicated cellular mechanisms that may be cell-type specific. The fact that inhibition of PrPres accumulation

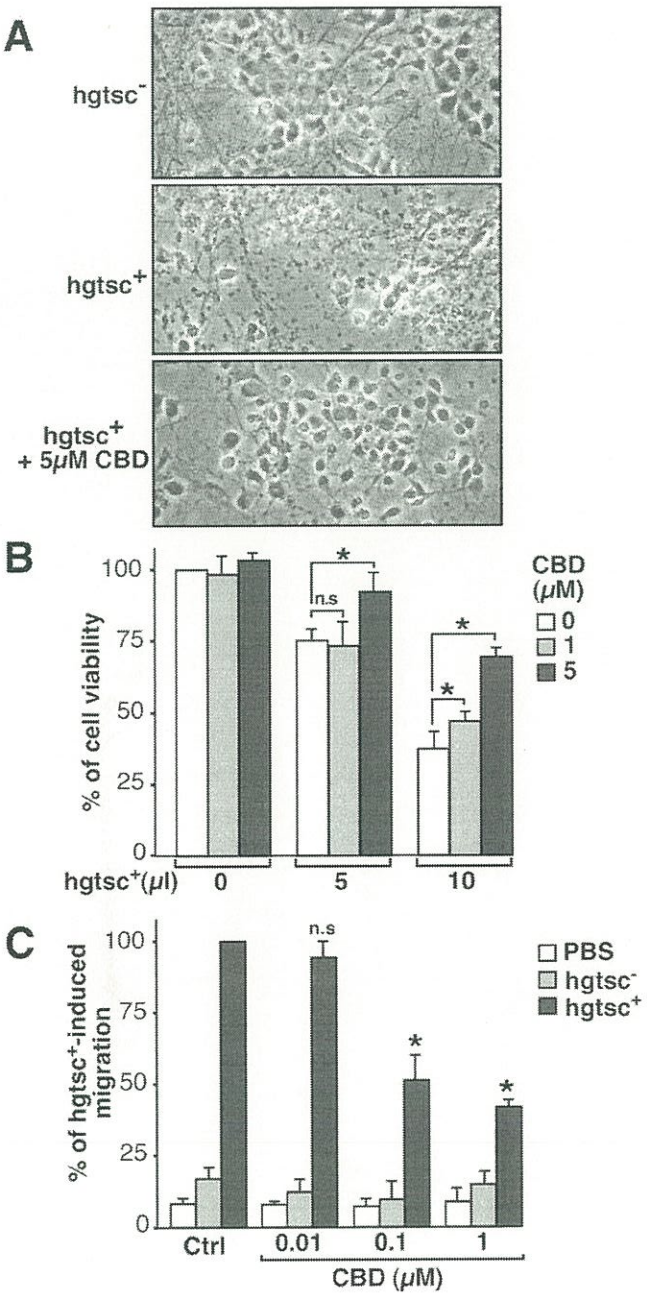

Figure 5. CBD prevents PrPres-induced neurotoxicity and microglial cell migration. ***A***, Phase-contrast micrographs of representative microscopic fields. Primary cultures of neurons were incubated with 10 μl of hgtsc $^-$ or hgtsc $^+$ alone or in the presence of 5 μM CBD for 48 h at 37°C. Magnification, 25×. ***B***, Neuronal viability was determined as a function of the concentration of hgtsc $^+$ incubated on neurons in the absence (white) or presence of 1 μM CBD (light gray) or 5 μM CBD (dark gray). The data are expressed as the percentage of viable neurons compared with untreated neurons and are the mean of three independent experiments with triplicate samples ± SD. ***C***, Neurons were incubated with 5 μl of PBS, hgtsc $^-$, or hgtsc $^+$ for 24 h in the absence (Ctrl) or presence of the indicated concentrations of CBD. Histograms represent the means of two independent experiments ± SD with triplicate samples. Results are expressed as a percentage of the migration observed after exposure to 5 μl of hgtsc $^+$. Statistically significant differences were obtained between CBD-treated and untreated neurons exposed to hgtsc $^+$ (*$p < 0.01$ using the unpaired Student's *t* test; n.s, Not significant).

by CBD in scrapie-infected cells is slow, requiring passage of the cells several times in the presence of the drug to see an effect, is consistent with this idea. Moreover, the idea of a mechanism of inhibition common to some cell types but not others is supported by the finding that CBD prevents PrPres formation in the brain, but not the spleen, of scrapie-infected mice. Alternatively, CBD may also hamper neuroinvasion from the periphery to the CNS. In this instance, because of its high lipophilicity, CBD could interact with neuronal cell membranes leading to membrane modifications unfavorable to PrPres formation. Indeed, compounds that decrease the membrane cholesterol level, such as lovastatin (Taraboulos et al., 1995), have been shown to inhibit PrPres formation in scrapie-infected cells.

Several lines of evidence support the idea that reactive microglial cells could be, at least in part, responsible for the observed brain pathology in TSEs and other neurological disorders (Brown, 2001). The presence of reactive microglial cells adjacent to PrPres deposits is almost universally observed in TSE-affected brains (Guiroy et al., 1994). Moreover, PrPres aggregation and microglia activation occur concomitantly and precede neuronal cell death (Giese et al., 1998) and clinical signs of the disease (Betmouni et al., 1996). We previously demonstrated that neurons exposed to PrPres trigger the recruitment of microglia to the vicinity of the PrPres deposits. Once in direct contact with PrPres, reactive microglial cells would release diffusible neurotoxic factors, such as nitric oxide, that could lead to cell death (Marella and Chabry, 2004). Interestingly, it has recently been shown that cannabinoids prevent Alzheimer's disease pathology via the blockade of microglial activation (Ramirez et al., 2005). Similarly, we demonstrated here that CBD protected neurons against PrPres toxicity and prevented PrPres-induced microglial cell migration. Thus, CBD may modulate glial cell function, leading to a reduction in the inflammatory response that usually accompanies neurodegeneration.

Another aspect of CBD-based treatment may be related to the NMDA receptor antagonism properties of CBD (Grundy et al., 2001). PrPres may activate either directly or indirectly the NMDA receptor channel, resulting in sustained intraneuronal calcium elevation and ultimately neuronal death (Brown et al., 1997). Indeed, NMDA receptor antagonists such as memantine and MK-801 [(+)-5-methyl-10,11-dihydro-5*H*-dibenzo [a,d] cyclohepten-5,10-imine maleate] display cytoprotective effects against PrPres-induced neuronal cell death (Müller et al., 1993). Together, our results suggest that CBD has the properties necessary to protect neurons against the multiple molecular and cellular factors involved in the different steps of the neurodegenerative processes that take place during TSE infection.

Overall, CBD is a promising therapeutic drug against the TSEs because it combines several crucial characteristics. It has a low toxicity and lack of psychotropic side effects as well as *in vivo* neuroprotective, anti-inflammatory, and anti-PrPres properties. Because CBD easily crosses the BBB, it also has the potential to be effective after prion infection has reached the CNS. Finally, prolonged treatments with CBD do not induce tolerance, a phenomenon frequently observed with THC. Additional investigations should be performed to define the optimal dose, route, frequency, and duration of the *in vivo* CBD treatment necessary to prevent TSE infection in different scrapie-infected animal models.

References

Ben Amar M (2006) Cannabinoids in medicine: a review of their therapeutic potential. J Ethnopharmacol 105:1–25.

Betmouni S, Perry VH, Gordon JL (1996) Evidence for an early inflammatory response in the central nervous system of mice with scrapie. Neuroscience 74:1–5.

Brown DR (2001) Microglia and prion disease. Microsc Res Tech 54:71–80.

Brown DR, Herms JW, Schmidt B, Kretzschmar HA (1997) PrP and beta-amyloid fragments activate different neurotoxic mechanisms in cultured mouse cells. Eur J Neurosci 9:1162–1169.

Büeler H, Aguzzi A, Sailer A, Greiner RA, Autenried P, Aguet M, Weissmann C (1993) Mice devoid of PrP are resistant to scrapie. Cell 73:1339–1347.

Carp RI, Meeker H, Sersen E (1997) Scrapie strains retain their distinctive characteristics following passages of homogenates from different brain regions and spleen. J Gen Virol 78:283–290.

Caughey B, Lansbury PT (2003) Protofibrils, pores, fibrils, and neurodegeneration: separating the responsible protein aggregates from the innocent bystanders. Annu Rev Neurosci 26:267–298.

Caughey B, Race RE (1992) Potent inhibition of scrapie-associated PrP accumulation by Congo red. J Neurochem 59:768–771.

Caughey WS, Raymond LD, Horiuchi M, Caughey B (1998) Inhibition of protease-resistant prion protein formation by porphyrins and phthalocyanines. Proc Natl Acad Sci USA 95:12117–12122.

Chesebro B, Trifilo M, Race R, Meade-White K, Teng C, LaCasse R, Raymond L, Favara C, Baron G, Priola S, Caughey B, Masliah E, Oldstone M (2005) Anchorless prion protein results in infectious amyloid disease without clinical scrapie. Science 308:1435–1439.

Chiesa R, Harris DA (2001) Prion diseases: what is the neurotoxic molecule? Neurobiol Dis 8:743–763.

Daude N, Marella M, Chabry J (2003) Specific inhibition of pathological prion protein accumulation by small interfering RNAs. J Cell Sci 116:2775–2779.

Doh-Ura K, Iwaki T, Caughey B (2000) Lysosomotropic agents and cysteine protease inhibitors inhibit scrapie-associated prion protein accumulation. J Virol 74:4894–4897.

Giese A, Brown DR, Groschup MH, Feldmann C, Haist I, Kretzschmar HA (1998) Role of microglia in neuronal cell death in prion disease. Brain Pathol 8:449–457.

Gilch S, Winklhofer KF, Groschup MH, Nunziante M, Lucassen R, Spielhaupter C, Muranyi W, Riesner D, Tatzelt J, Schatzl HM (2001) Intracellular re-routing of prion protein prevents propagation of PrP(Sc) and delays onset of prion disease. EMBO J 20:3957–3966.

Grundy RI, Rabuffetti M, Beltramo M (2001) Cannabinoids and neuroprotection. Mol Neurobiol 24:29–51.

Guiroy DC, Wakayama I, Liberski PP, Gajdusek DC (1994) Relationship of microglia and scrapie amyloid-immunoreactive plaques in kuru, Creutzfeldt-Jakob disease and Gerstmann-Straussler syndrome. Acta Neuropathol (Berl) 87:526–530.

Kocisko DA, Come JH, Priola SA, Chesebro B, Raymond GJ, Lansbury PT, Caughey B (1994) Cell-free formation of protease-resistant prion protein. Nature 370:471–474.

Lastres-Becker I, Molina-Holgado F, Ramos JA, Mechoulam R, Fernandez-Ruiz J (2005) Cannabinoids provide neuroprotection against 6-hydroxydopamine toxicity *in vivo and in vitro*: relevance to Parkinson's disease. Neurobiol Dis 19:96–107.

Llewelyn CA, Hewitt PE, Knight RS, Amar K, Cousens S, Mackenzie J, Will RG (2004) Possible transmission of variant Creutzfeldt-Jakob disease by blood transfusion. Lancet 363:417–421.

Marella M, Chabry J (2004) Neurons and astrocytes respond to prion infection by inducing microglia recruitment. J Neurosci 24:620–627.

Marella M, Gaggioli C, Batoz M, Deckert M, Tartare-Deckert S, Chabry J (2005) Pathological prion protein exposure switches on neuronal mitogen-activated protein kinase pathway resulting in microglia recruitment. J Biol Chem 280:1529–1534.

Mechoulam R, Panikashvili D, Shohami E (2002) Cannabinoids and brain injury: therapeutic implications. Trends Mol Med 8:58–61.

Müller WEG, Ushijima H, Schröder HC, Forrest JMS, Schatton WFH, Rytik PG, Heffner-Lauc M (1993) Cytoprotective effect of NMDA receptor antagonists on prion protein (PrionSc)-induced toxicity in rat cortical cell cultures. Eur J Pharmacol 246:261–267.

Nishida N, Harris DA, Vilette D, Laude H, Frobert Y, Grassi J, Casanova D, Milhavet O, Lehmann S (2000) Successful transmission of three mouse-adapted scrapie strains to murine neuroblastoma cell lines overexpressing wild-type mouse prion protein. J Virol 74:320–325.

Priola SA, Raines A, Caughey WS (2000) Porphyrin and phthalocyanine antiscrapie compounds. Science 287:1503–1506.

Priola SA, Chabry J, Chan K (2001) Efficient conversion of normal prion protein (PrP) by abnormal hamster PrP is determined by homology at amino acid residue 155. J Virol 75:4673–4680.

Ramirez BG, Blazquez C, Gomez del Pulgar T, Guzman M, de Ceballos ML (2005) Prevention of Alzheimer's disease pathology by cannabinoids: neuroprotection mediated by blockade of microglial activation. J Neurosci 25:1904–1913.

Sailer A, Bueler H, Fischer M, Aguzzi A, Weissmann C (1994) No propagation of prions in mice devoid of PrP. Cell 77:967–968.

Taraboulos A, Scott M, Semenov A, Avraham D, Laszlo L, Prusiner SB (1995) Cholesterol depletion and modification of COOH-terminal targeting sequence of the prion protein inhibit formation of the scrapie isoform. J Cell Biol 129:121–132.

Vilette D, Andreoletti O, Archer F, Madelaine MF, Vilotte JL, Lehmann S, Laude H (2001) Ex vivo propagation of infectious sheep scrapie agent in heterologous epithelial cells expressing ovine prion protein. Proc Natl Acad Sci USA 98:4055–4059.

Walter L, Franklin A, Witting A, Wade C, Xie Y, Kunos G, Mackie K, Stella N (2003) Nonpsychotropic cannabinoid receptors regulate microglial cell migration. J Neurosci 23:1398–1405.

Westaway D, DeArmond SJ, Cayetano-Canlas J, Groth D, Foster D, Yang S-L, Torchia M, Carlson GA, Prusiner SB (1994) Degeneration of skeletal muscle, peripheral nerves, and the central nervous system in transgenic mice overexpressing wild-type prion proteins. Cell 76:117–129.

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California Medical Survey: The Adverse Effects of Marijuana

by Fred Gardner, Counterpunch
November 19th, 2006

In the past 10 years, California doctors have authorized cannabis use by at least 350,000 patients. What have they learned about its adverse effects?

According to a survey of 19 doctors associated with the Society of Cannabis Clinicians, side-effects are relatively rare, mild, and transient. There have been no deaths, no major adverse events attributed to cannabis -with one exception involving a claim by an establishment psychiatrist that cannabis induced and exacerbated psychosis in an 18-yea old whom she had on a regimen of Lexapro and Zyprexa.

Comments by the SCC doctors follow.

Frank Lucido, MD: Reported adverse effects are rare, in part because the patient coming a medical cannabis consultation has already found cannabis to be of benefit. (I have had perhaps 10 patients in 10 years who had never tried cannabis or who hadn't used it in ma years and were uncertain if it would effectively treat their current illness or symptoms.) Two patients have discontinued use in response to decreased productivity. The overwhelming majority report that they are MORE productive when their symptoms are controlled with cannabis.

Robert Sullivan, MD: None common (c. 1%), none "serious." Weight gain, tolerance, anxiety (related to potential theft from an outdoor garden), dry mouth, short-term memo decrease, anxiety, red eyes. All described in response to my inquiry (not spontaneous). None resulted in stopping cannabis use.

Marian Fry, MD: The most significant negative reactions are due to fear of incarceration and the results of abuse by officers unwilling to honor California law.

William Toy, MD: The most important adverse effects are respiratory problems caused b smoking. Most patients who have respiratory problems use vaporizers or edible forms of cannabis. We go out of our way to get patients on vaporizers and we now have only a sn percentage of smokers -mostly people who have been smoking marijuana for 30-40 year Most in this group use very little, maybe one or two doses a day.

Philip A. Denney, MD: Virtually none reported by patients except contacts with the lega system. Patients are able to stop using easily in order to pass drug tests or when traveling Overdose from edible cannabis -an unpleasant drowsiness lasting six to eight hours- is ra and transient.

David Bearman, MD: Occasional complaints of cough. Many more complaints about Marinol than cannabis -dysphoria, ineffective, costs too much.

Tom O'Connell, MD: The most common is the "paranoid" reaction, in which, characteristically, a user who is "high" develops the uncomfortable feeling that everyone

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he/she sees KNOWS they are high and is critical of them for it. It almost always occurs i situation where the person may be forced to deal unexpectedly with the public. It certain needs further study. In any event, patients deterred from using pot aren't lining up for approvals to do so.

William Courtney, MD: A significant number of my middle-aged patients are no longer enamored of the psychoactive effects that previously were the highlight of their cannabis use. For them, what was euphoric has now become dysphoric. Such patients tolerate the anxiogenic properties in order to enjoy the anti-spasmodic or analgesic effects -much as patient on chemotherapy reluctantly accepts the nausea in exchange for the anti-tumor effects. While a few patients have discovered that there are strains that provide relief without dysphoria, others are excited by the possibility of daytime CBD analgesia or autoimmune modulation without alteration of their sensorium.

Dr. A.: We've had several reports of hypotensive reaction -a sudden drop in blood pressu which results in fainting. It's very rare and, as reported by my patients, is a one-time thin It typically happens after a big meal, when the GI tract is opened up and absorbing a lot blood.

Jeffrey Hergenrather, MD: Is there a downside to the use of cannabis? The sense of intoxication rarely lasts longer than an hour and tends to be more troubling to the novice than to the experienced user. For some people cannabis can induce dry mouth, red eyes, unsteady gait, mild in-coordination, and short-term memory loss, all of which are transie These effects are reportedly trivial compared to those brought on by pharmaceutical alternatives.

Cannabis use is steadily finding acceptance in society. Still, for many it remains awkwar not totally impractical in the workplace. People whose jobs require multi-tasking such as pilots, drivers, dispatchers, switchboard operators, and many professionals find the intoxicating effects of cannabis inappropriate in the workplace, and therefore reserve the use for after work.

The survey, conducted by your correspondent for the upcoming issue of O'Shaughnessy' (and previewed exclusively on CounterPunch), does not pretend to be rigorous. It involv the patient population least likely to experience adverse events and a setting in which adverse events might be downplayed (examinations in which the patient is seeking the doctor's approval to use). As Dr. Lucido and others point out, in the first 10 years of lega created by Prop 215, almost all the patients seeking physician approval to use cannabis h been self-medicating previously with positive results. Truly naïve patients have been rar and those experiencing unwanted side-effects would be unlikely to return to the doctor fi renewal, i.e., their complaints would go unreported.

The charge that cannabis use caused and then increased the severity of a psychotic break an 18-year-old was made by a Stanford University psychiatrist, Dr. P., who filed a complaint with the state medical board against the doctor who had approved it. "I believ THC caused his depression to worsen, interferes with antidepressant meds, and clearly caused his psychosis," Dr. P advised the board. "He is also psychologically and physical dependent on the substance. He refuses to quit. He even admitted to seeking the medical marijuana justification in order to use regularly 'legally.'"

The assumption that marijuana causes physical dependence is without scientific foundati Dr. P.'s use of the term "even admitted" reveals a prosecutorial frame of mind. She seem appalled to learn what all cannabis consultants know and what should come as no surpri to any person with common sense: feeling legitimate relieves anxiety! Dr. P.'s treatment the mutual patient involved anti-marijuana exhortations and the pushing of her preferred corporate drugs. Lexapro is an SSRI antidepressant made by Forest Pharmaceuticals. Lik all SSRIs it is slowly but surely being linked to suicide in the medical literature (while th drug companies and their paid researchers in the psychiatric establishment challenge eac piece of evidence).

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Dr. P.'s allegation that marijuana use precipitated and aggravated the patient's break with reality can't be proved or disproved. Some published studies indicate an "association" between marijuana use and schizophrenia, but not necessarily a causal relationship. (A person seeing demons or hearing voices may use cannabis because he finds that it quiets them.) Schizophrenia occurs in about 1% of adult populations in all countries and cultures regardless of the prevalence of cannabis use. The use of Marinol (synthetic THC) by teenage cancer patients has not resulted in an increased incidence of schizophrenia.

Ironically, the component of the cannabis plant thought to have sedative and anti-psychotic properties -Cannabidiol (CBD)- is present only in trace amounts in the strains available to California patients. As indicated by Dr. Courtney, the SCC doctors are frustrated that they don't know the cannabinoid contents of the herbs their patients are using. They all wish a high-CBD strain was available. They would have learned a lot in 10 years about how it differs from high-THC cannabis. Prohibition sabotages research.

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LESTER GRINSPOON

The Boston Globe

Marijuana as wonder drug

By Lester Grinspoon | March 1, 2007

A NEW STUDY in the journal Neurology is being hailed as unassailable proof that marijuana is a valuable medicine. It is a sad commentary on the state of modern medicine -- and US drug policy -- that we still need "proof" of something that medicine has known for 5,000 years.

The study, from the University of California at San Francisco, found smoked marijuana to be effective at relieving the extreme pain of a debilitating condition known as peripheral neuropathy. It was a study of HIV patients, but a similar type of pain caused by damage to nerves afflicts people with many other illnesses including diabetes and multiple sclerosis. Neuropathic pain is notoriously resistant to treatment with conventional pain drugs. Even powerful and addictive narcotics like morphine and OxyContin often provide little relief. This study leaves no doubt that marijuana can safely ease this type of pain.

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As all marijuana research in the United States must be, the new study was conducted with government-supplied marijuana of notoriously poor quality. So it probably underestimated the potential benefit.

This is all good news, but it should not be news at all. In the 40-odd years I have been studying the medicinal uses of marijuana, I have learned that the recorded history of this medicine goes back to ancient times and that in the 19th century it became a well-established Western medicine whose versatility and safety were unquestioned. From 1840 to 1900, American and European medical journals published over 100 papers on the therapeutic uses of marijuana, also known as cannabis.

Of course, our knowledge has advanced greatly over the years. Scientists have identified over 60 unique constituents in marijuana, called cannabinoids, and we have learned much about how they work. We have also learned that our own bodies produce similar chemicals, called endocannabinoids.

The mountain of accumulated anecdotal evidence that pointed the way to the present and other clinical studies also strongly suggests there are a number of other devastating disorders and symptoms for which marijuana has been used for centuries; they deserve the same kind of careful, methodologically sound research. While few such studies have so far been completed, all have lent weight to what medicine already knew but had largely forgotten or ignored: Marijuana is effective at relieving nausea and vomiting, spasticity, appetite loss, certain types of pain, and other debilitating symptoms. And it is extraordinarily safe -- safer than most medicines prescribed every day. If marijuana were a new discovery rather than a well-known substance carrying cultural and political baggage, it would be hailed as a wonder drug.

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and synthesize analogs, and to package them in non-smokable forms. In time, companies will almost certainly come up with products and delivery systems that are more useful and less expensive than herbal marijuana. However, the analogs they have produced so far are more expensive than herbal marijuana, and none has shown any improvement over the plant nature gave us to take orally or to smoke.

We live in an antismoking environment. But as a method of delivering certain medicinal compounds, smoking marijuana has some real advantages: The effect is almost instantaneous, allowing the patient, who after all is the best judge, to fine-tune his or her dose to get the needed relief without intoxication. Smoked marijuana has never been demonstrated to have serious pulmonary consequences, but in any case the technology to inhale these cannabinoids without smoking marijuana already exists as vaporizers that allow for smoke-free inhalation.

Hopefully the UCSF study will add to the pressure on the US government to rethink its irrational ban on the medicinal use of marijuana -- and its destructive attacks on patients and caregivers in states that have chosen to allow such use. Rather than admit they have been mistaken all these years, federal officials can cite "important new data" and start revamping outdated and destructive policies. The new Congress could go far in establishing its bona fides as both reasonable and compassionate by immediately moving on this issue.

Such legislation would bring much-needed relief to millions of Americans suffering from cancer, AIDS, multiple sclerosis, arthritis, and other debilitating illnesses.

Lester Grinspoon, an emeritus professor of psychiatry at Harvard Medical School, is the coauthor of "Marijuana, the Forbidden Medicine." ■

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Marijuana: The Forbidden Medicine

By the Authors Q&A About this Site Reader Contributions

To navigate our site, please move your cursor over the topics listed in the graphic shown above.

Welcome to a place to learn about patients' experiences with medical marijuana. Please share your own medical marijuana story with us.

- **Lester Grinspoon, M.D.**, Associate Professor of Psychiatry (Emeritus) at Harvard Medical School and
- **James Bakalar, J.D.**, Lecturer in Law in the Department of Psychiatry at Harvard Medical School.

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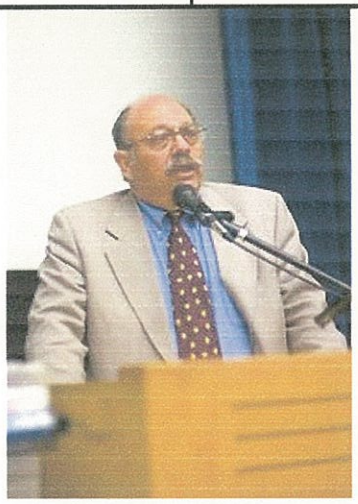

Dr. David Bearman is one of the most clinically knowledgable physicians in the U.S. in the field of medicinal marijuana. He has spent 40 years working in substance and drug abuse treatment and prevention programs. Dr. Bearman was a pioneer in the free and community clinic movement. His career includes public health, administrative medicine, provision of primary care, pain management and cannabinology.

His almost 40 year professional experience in the drug abuse treatment and prevention field includes being the Co-Director of the Haight-Ashbury Drug Treatment Program, being a member of Governor Reagan's Inter Agency Task Force on Drug Abuse, a member of both the Santa Barbara and the San Diego County Drug Abuse Technical Advisor Committees, and a consultant to Hoffman-LaRoche, Santa Barbara County Schools and the National PTA.

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Dr. Bearman's new book, *Demons, Discriminaton and Dollars*,
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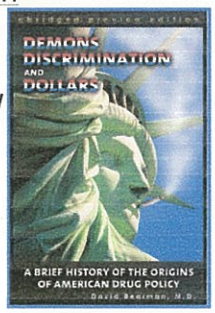

Webmaster: Dr. Robert Lewis. For more information about this or other sites, Dr. Lewis may be contacted at drbobmeister@gmail.com.

David Bearman, M.D.

Summary of Dr. David Bearman's experience as a Public Health Official and Community Volunteer:

ADMINISTRATIVE POSITIONS: Zona Seca Medical Director 2001-June 2006, currently Medical Consultant; Santa Barbara Regional Health Authority, Senior Health Care Advisor and Grants Development, 199-2001, and Medical Director and Director of Medical Services Department 1983-1997. Sutter County Health Department, Director and Health Officer, 1982-1983; San Diego State University, Director of Health Services, 1974-1982; Isla Vista Medical Clinic, Director/Administrator/Founder 1970-1974; the United States Public Health Service, Medical Officer 1967-1970.

ELECTED OFFICES HELD: Director, Goleta West Sanitary District, 1985-1990, 1995-present; Director, Goleta Water District, 1989-1993; Representative, Isla Vista Community Council, 1971-1973.

BOARDS AND COMMITTEES: California health Insurance Organization, Medical Director Committee; Medical of Governor Reagan's California Interagency Task Force on Drug Abuse, Secretary Cal State University Medical Director Association (1974-1982), Member Nominating Committee ACHA 1980 & 1981; Member California State Universities Alcohol Advisory Committee; Board Member Isla Vista Health Projects, Board Member National Free Clinic Council, Board Member Northeast Mental Health Center in San Francisco, Member of Santa Barbara Chapter American Red Cross, Advisory Committee; Santa Barbara County Chapter of American Diabetes Association, Board Member; Santa Barbara County Drug Abuse Advisory Committee, Santa Barbara County Drug Abuse Master Plan Committee, Santa Barbara County Aging and Long-Term Care committee, Santa Barbra County AIDS Advisory Committee, Member San Diego County Drug Abuse Technical Advisory Committee, Member Santa Barbara County Drug Abuse Advisory Committee; Seattle Open Door Clinic, Founding Board Member; Member State Advisory Committee on Primary Care Clinics Member; Southern California Council of Free Clinics, Past VP.

PUBLICATIONS: Dr. Bearman has contributed articles on public health in the following publications: New Physician, Journal of Psychoactive Drugs, Harvard Business Review, Coastlines, Journal of Social Issues, American Journal of Pain Management, San Diego Medical Society Magazine, Santa Barbara Medical Society Bulletin, California Physician, Seattle Helix, Daily Nexus, Santa Barbara News Press, Western Goleta Free Press, Isla Vista Town Crier, Santa Barbara Regional Health Authority Members Newsletter and Provider Newsletter.

CONFERENCES DIRECTED:

- Counter Culture Conference (1972)
- Parenting and Drug Abuse (9180)
- PCCHA (1982)
- Patients Out of Time (2006) Co-Coordinator

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BIO

Dr. Bearman received his M.D. from the University of Washington School of Medicine. He graduated with honors from the University of Wisconsin with an undergraduate degree in Psychology. He was the Director of Medical Services for the Santa Barbara Regional Health Authority (SBRHA) since its inception in 1983 through June 1997, and was then promoted to Senior Health Care Advisor/Grants Development Director. In 1999 as a result of Dr. Bearman's efforts, the California Healthcare Foundation allocated ten (10) million dollars to several health entities located in Santa Barbara County for a county-wide medical data exchange system. SBRHA is the oldest County Organized Health System (COHS) in California and the U.S.

As Medical Director for SBRHA's first 14 years he developed and implemented Quality Assurance (QA) Utilization Review (UR) system and on-site review; created Peer Review and Quality Improvement Committee and developed Targeted Case Management Program. Dr. Bearman's responsibility as Medical Director included quality related activities such as addressing grievances, analyzing data, developing quality based distribution criteria, addressing facility related audits, and supervising regular QI studies.

He has a long and illustrative background in the field of drug abuse treatment and prevention, been prominent in the community clinic movement having started in Seattle the 3rd Free Clinic in the Country, directed the Haight Ashbury Drug Treatment Program, and founded the Isla Vista Medical Clinic in 1970. He has been Medical Director of Santa Barbara County Methadone Maintenance Clinic and Ventura County Opiate Detox Program, taught courses on substance abuse at UCSF, UCSB, and SDSU, been a consultant to Hoffman LaRoche, NIDA and the National PTA, directed several conferences, delivered numerous professional talks, consulted widely, and been an expert witness in over 100 civil, criminal, and family court cases and currently is Medical Consultant to Zona Seca.

Previously, he was Director and Health Officer of the Sutter County Health Department, Director of Student Health at San Diego State University, Director/Administrator/Founder of the Isla Vista Medical Clinic, and a Medical Officer in USPHS. At the USPHS he worked with the Model Cities Program to help develop several innovative health delivery systems including a comparison study of the cost of fee for service and managed care in Seattle in the early 1970's.

Dr. Bearman is the author of numerous articles on Medicaid managed care, drug abuse treatment and prevention, and human sexuality. He has published in the Journal of Psychoactive Drugs, The Harvard Business Review, The New Physician and The Journal of Social Issues. He has consulted with a variety of providers on managed care and has given numerous presentations on the topic at such conferences as California Children's Services, National Managed Health Care Congress, Alameda Health Consortium, Health Care Association of Southern California, Fresno Medical Group, EPA/MCH Consortium, and San Diego Health Access Group.

He is also active in local politics, having been elected to the Goleta Water Board and several times elected to the Goleta West Sanitary District. His civic service includes serving on the Board of Advisors Santa Barbara Red Cross, Board Santa Barbara Diabetes Association, Board of Isla Vista Health Projects and served on Santa Barbara Long-Term Care Task Force and West Santa Barbara Health Task Force.

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Cannabis and ADD

The primary symptoms of the most commonly recognized combined-type of ADHD are (1) motor overactivity, (2) inattention, and (3) impulsivity (American Psychiatric Association, 1994). Symptoms may decrease after adolescence, although they often persist into adulthood. Our understanding of the pathophysiology of ADHD and the mechanisms of therapeutic action of stimulants is clearly still in its infancy.

During the past several years, cannabinoid biology has witnessed marked advances that has propelled endocannabinoid research to the forefront of biomedical research. In order to appreciate the role of cannabinoids in treating ADD, ADHD, and adult ADD, a review of these conditions, a brief overview of neuroantomy and the endocannabinoid neurochemical anandamide.

• ADHD is Serious and Current Treatment Options are Controversial

ADHD is a serious condition. There is controversy over the use of stimulants as treatment. There is agreement that we need to continue to search for other effective treatment with fewer side effects. Cannabis has been suggested as being both a sequelae of ADD, ADHD and adult ADD as well as a form of self-medication.

Let's review the medicinal potential for cannabinoids in the treatment of ADD, ADHD and adult ADD. A good place to start as we assess the efficacy of cannabis or other cannabinoid for ADD. The NIH Consensus statement of the NIH Development Conference of November 1998:

> "Attention deficit hyperactivity disorder or ADHD is a commonly diagnosed behavioral disorder of childhood that represents a costly major public health problem. Children with ADHD have pronounced impairments and can experience long-term adverse effects on academic performance, vocational success, and social-emotional development which have a profound impact on individuals, families, schools, and society. Despite progress in the assessment, diagnosis and treatment of ADHD, this disorder and its treatment have remained controversial, especially the use of psychostimulants for both short- and long-term treatment."

They point out that in regard to treatment, that there is:

> "No consensus regarding which ADHD patients should be treated with psychostimulants. These problems point to the need for improved assessment, treatment, and follow-up of patients with ADHD."

They go on to say that there is:
"One of the major controversies regarding ADHD concerns the use of psychostimulants to treat the condition. Because psychostimulants are more readily available and are being prescribed more frequently, concerns have intensified over their potential overuse and

abuse." When adverse drug reactions do occur, they are usually related to dose. Effects associated with moderate doses may include decreased appetite and insomnia. Further, "A wide variety of treatments have been used for ADHD including, but not limited to, various psychotropic medications, psychosocial treatment, dietary management, herbal and homeopathic treatments, biofeedback, meditation, and perceptual stimulation/training." Even more importantly the NIH consensus statement says that: "There are no conclusive data on treatment in adolescents and adults with ADHD."

The consensus statement is clear on the scope of the problem:
"In the larger world, these individuals consume a disproportionate share of resources and attention from the health care system, criminal justice system, schools, and other social service agencies. Methodological problems preclude precise estimates of the cost of ADHD to society. However, these costs are large."

The statement continues:
"The impact of ADHD on individuals, families, schools, and society is profound and necessitates immediate attention. A considerable share of resources from the health care system and various social service agencies is currently devoted to individuals with ADHD."

The risks of treatment, particularly the use of stimulant medication, are of considerable professional and lay interest. Substantial evidence exists of wide variations in the use of psychostimulants across communities and physicians, suggesting no consensus among practitioners regarding which ADHD patients should be treated with psychostimulants.

Existing diagnostic and treatment practices, in combination with the potential risks associated with medication, point to the need for improved awareness by the health service sector concerning an appropriate assessment, treatment, and follow-up.

• What is ADHD?

Lately several physicians who recommend/approve medicinal cannabis have seen an increase in the number of patients coming in who have been diagnosed with Attention Deficit Disorder (ADD) or Attention Deficit with Hyperactivity Disorder (ADHD). Before we discuss some possible ways of dealing with this problem I'd like to discuss what we mean by these diagnoses. The first and foremost thing we need to remember is that these diagnoses don't correspond to any recognized pathology. For example, if we say somebody has appendicitis, we can look at the removed appendix under the microscope and see some specific changes like a lot of a certain type of cells that create an inflammatory response. Somebody with asthma will have certain easily identifiable changes in their lungs, etc. A person with ADD/ADHD does not have any such changes as far as we know. The cause of ADHD is unknown, although in some cases there appears to be a genetic component. It has been shown that people with ADHD have less activity in areas of the brain that control attention. What ADD/ADHD have is a certain group of symptoms, like difficulty concentrating, hyperactivity, behavior issues, etc. But there appears to be a deficiency in free dopamine.

Attention Deficit Hyperactivity Disorder (ADHD), was formerly called hyperkinesis or minimal brain dysfunction. It is described as a chronic, neurologically based syndrome characterized by any or all of three types of behavior: hyperactivity, distractibility, and impulsivity. Hyperactivity refers to feelings of restlessness, fidgeting, or inappropriate activity (running, wandering) when one is expected to be quiet; distractibility to heightened distraction by irrelevant sights and sounds or carelessness and inability to carry simple tasks to completion; and impulsivity to socially inappropriate speech (e.g., blurting out something without thinking) or striking out. Unlike similar behaviors caused by emotional problems or anxiety, ADHD does not fluctuate with emotional states. While the three typical behaviors occur in nearly everyone from time to time, in those with ADHD they are excessive, long-term, and pervasive and create difficulties in school, at home, or at work. ADHD is usually diagnosed before age seven. It is often accompanied by a learning disability. More recently there has also been described adult ADHD.

• **Treatment**

While we strongly suspect an important role of dopamine deficiency, we don't really know the cause of the symptoms, and there is no routine test for dopamine levels, so the diagnosis becomes what we call a diagnosis of exclusion. That is, we make sure the person does not have some other identifiable condition, such as depression or some learning disabilities or a physical problem causing the symptoms, and if they don't, and have a certain number of symptoms from a predefined list, we label them with ADD/ADHD and give a drug that tends to make them a bit more manageable. The accepted drug treatments tell that Ritalin cures nothing but in many cases many make the patient more manageable.

Conventional ADD treatment usually includes behavioral therapy and emotional counseling combined with sympathominetic medications such as methyphenidate hydrochloride (Ritalin) or dextroamphetamine (Dexedrine), Atomoxetine (Strattera), Amphetamine mixture (Adderal) or long-acting methylphenidate (e.g., Metadate LD, Concerta, Ritalin LA), that in many cases make the patient more manageable. They also have many unacceptable side effects.

The first dictum of medicine is "first, do no harm." Another bedrock principal is that a prescriber must balance off the side effects of the treatment with the benefits. With ADD, the use of Ritalin and other stimulants has been routinely and repeatedly criticized because of its side effects profile. All of my patients with ADD have been critical of either the side effects of these drugs, the lack of effectiveness or both. They have found cannabis to be both more effective and have far fewer side effects.

No less an authority than DEA Administrative Law Judge, Francis L. Young, in 1988, after a two-year hearing to reschedule cannabis said:

> "Nearly all medicines have toxic, potentially lethal effects. But marijuana is not such a substance. There is no record in the extensive medical literature describing a proven, documented cannabis-induced fatality ... Simply stated, researchers have been unable to give animals enough marijuana to induce death .. In practical terms, marijuana cannot induce a lethal response as a result of drug-related toxicity ... In strict medical terms marijuana is far safer than many foods we commonly consume ... Marijuana, in its natural form, is one of the safest therapeutically active substances known to man."

When a medication gives you a symptom that you did not want, we call that symptom a side effect. When it comes to treatment of ADHD for many, cannabinoids have far fewer and less annoying side effects than the stimulants that are often used to treat AD/HD and other conditions. The most common stimulants are methyphenidate (Ritalin, Concerta, Metadate-ER) and amphetamine (Dexedrine, Dexedrine Spansules, Adderall). Some individuals who take stimulants experience mild problems, some much more significant, unpleasant side effects. Some are simply unable to tolerate stimulants. Many people simply stop their prescribed stimulant medication instead of working with their physician to find a way to decrease side effects. Cannabinoids offer another viable option.

A study reported in Clinician reviews in 2000 entitled "Treating ADHD May Prevent Substance Abuse" found that:

> "... untreated ADHD presents a significant risk factor for Substance Use Disorder (SUD) in adolescence, whereas treating ADHD may reduce this risk." (NOTE: I have no idea why substance use - as opposed to substance abuse- is a disorder.) At any rate, the authors "point to previous studies in which they found ADHD-SUD associations in adults with ADHD who had never been diagnosed or treated as children. Further examination is necessary in order to evaluate the risk factors for girls and nonwhite boys. However, these findings may reduce apprehension in treating children who have ADHD and promote earlier intervention. This, in turn, may prevent the academic, psychiatric, and interpersonal complications of ADHD in adolescents, and subsequently, in adults.

• Cannabis Studies

In some cases where Ritalin is ineffective or unacceptable, cannabis has been found to be helpful. Much of the evidence about the use of cannabis is anecdotal, however that is changing. On 11/19/2000 Daniel Q. Haney of the Associated Press wrote:

> "maybe the smoke is about to clear in the debate over medical marijuana. Few ideas, it seems, are so firmly held by the public and so doubted by the medical profession as the healing powers of pot. But at last, researchers are tiptoeing into this field, hoping to prove once and for all whether marijuana really is good medicine.

> To believers, marijuana's benefits are already beyond discussion: Pot eases pain, settles the stomach, builds weight and steadies spastic muscles. And that's hardly the beginning. They speak of relief from MS, glaucoma, itching, insomnia, arthritis, depression, childbirth, attention deficit disorder and ringing in the ears.
>
> Marijuana is a powerful and needed medicine, they say, tragically withheld by misplaced phobia about drug addiction."

He points out that while many are not impressed with these anecdotal reports stretching over centuries funding from the State of California to the Center for Medicinal Cannabis Research the questions as to cannabis' medical efficacy will be scientifically studied. In Haney's words:

> "Pot has many effects on the body, including some that are probably worthwhile. But does it substantially relieve human suffering, they ask? And if so, is it any better than medicines already in drugstores?
>
> For the first time in at least two decades, marijuana the medicine is being put to the test. Scientists say they will try to hold marijuana to the same standard as any other drug, to settle whether its benefits match its mystique.
>
> One way to buff up a pharmaceuticals' raffish image -- especially one that's a drug in more than one sense of the word -- is to call it something else. When the University of California at San Diego started the country's first institute to study the medical sues of marijuana this year, they named it the Center for Medicinal Cannabis Research. Cannabis is the botanical term for pot.
>
> "We talked about it a lot," says Dr. Igor Grant, the psychiatrist who heads the new center. "Marijuana is such a polarizing name. We don't want this institute to be caught in the crossfire between proponents and antagonists. Ultimately, if cannabis drugs become medicine, they will almost certainly be known by that name, not marijuana."

The center appears to be living up to its expectations. It was authorized to give out $9 million to California researchers over the three years from 2000-2003. This has been enough funding to underwrite 18 NIDA/FDA-approved studies. At least one or two are looking at cannabis and ADHD.

Research Implications

Here's my take on the implication of the brain studies related to cannabinoids.

• Cannabinoid Receptors

There are two cannabdinoid receptors in the body – CB_1 located in the brain, and CB_2 in the periphery.

• for CB_1 there are two natural ligands in the body anandamide (arachidonyl ethanolamide) and 2-AG (2 arachidonyl glyceride)

• palmitylethanolamide is the natural ligand for CB_2

• Cannabis/Tetrahydrocannabinol

There are over 400 different chemicals in marijuana, about 60 of which are known as cannabinoids. These chemicals are found nowhere else in nature. The most important cannabinoid in marijuana is known as delta-9-tetrahydrocannabinol (THC). THC is the main psychoactive (mind-altering) ingredient in marijuana. These plant cannabinoids can stimulate the body's endocannabinoid system.

• Endocannabinoid System

Cannabinoid CB(1) receptors are highly localized in the central nervous system. A 2000 report of the work of Martin, Ledent, et.al. in Feb. 2002 issue of Psycho Pharmacology concluded that endogenous cannabinoids through the activation of CB1 receptors are implicated in the control of emotional behavior and participate in the physiological processes of learning and memory.

The highest concentration of CB1 THC receptors in the brain are found in the hippocampus (where memory is formed), cerebellum (deals with coordinating movements and balance), the striatum, amygdala (emotion), cerebral cortex (higher centers of reasoning) and the basal ganglia. An important class of neurons that express high levels of CB(1) receptors are GABAergic interneurons in the hippocampus, amygdala and cerebral cortex. They may act as retrograde synaptic mediators of the phenomena of depolarization-induced suppression of inhibition or excitation in hippocampus and cerebellum. In other words, they may mediate by decreasing sensory input. Signaling by the endocannabinoid system represents a mechanism by which neurons can communicate backwards across synapses to modulate their inputs. Cannabinoid receptors are co-localized with dopamine receptors suggesting that cannabinoids influence dopaminergic processes.

The active ingredient in marijuana is delta-9-tetrahydrocannabinol (Δ^9-THC). It binds to CB_1 receptors (G-protein-coupled receptors) that are present on presynaptic membranes in several parts of the brain.

• Cannabinoids

It is possible that, in part at least, cannabis' effects are due to the cannabinoids, a major nonpsychotropic constituent of cannabis. It was recently discovered that the cannabinoids (as opposed to THC) effect the inhibition of anandamide uptake.

• Prefrontal Cortex and Midbrain

The prefrontal cortex (PFC) is essential for attentional control, organization and planning. Lesions to the PFC in humans can produce distractibility, hyperactivity, and impulsivity (Stuss, Eskes, & Foster, 1994). The PFC projects to many subcortical regions, including the dorsal and ventral striatum, thalamus, amygdala, substantia nigra, and ventral tegmental area (Alexander, DeLong, & Strick, 1986) all areas with high concentration of THC receptors. The motor dysregulation characteristic of ADHD and neuroimaging data suggest that dysfunction in striatum or in the cortical regulation of striatum is involved in the pathophysiology of ADHD. This dysregulation may be associated with lower than normal levels of free dopamine.

• Dopamine

That is the neuro anatomy but the power for the getting neural impulses around the brain are the neurotransmittors. These cross the synapse and stimulate receptors in the next neuron causing transmission of nerve impulses. Neurotransmitters include norepinephrone, seratonin, acetylcholine, dopamine and anandamide (the naturally occurring cannabinoid).

Catecholamines: (Dopamine), Norepinephrine, Epinephrine (adrenalin) control the so-called adrenergic systems. Some of these neurons radiate from the limbic system and discharge neurotransmitters in a diffuse manner into the frontal cortex, i.e. into broad areas of brain tissue as opposed to delivering the chemical to specific synapses. They thus account for "global vigilance" (staying awake), mood, fight or flight response, etc. Chocolate, coffee, nicotine, THC and stress all increase Dopamine. Thorazine and Haldol block Dopamine action (less learning, remembering and motivation).

Attention-Deficit Disorder (ADD) and Attention-Deficit Hyperactivity Disorder (ADHD) patients may have less dopamine produced than those who do not have this condition. Also a preliminary study reveals that adults with ADD/ADHD have 70 percent more dopamine transporters in their brains than normal subjects. These transporters tie up dopamine leaving less free dopamine available for neuro stimulation.

• Low Dopamine

In 2000 Grace proposed a model of dopaminergic dysfunction in ADHD at the cellular level that explain many of the symptoms of ADHD. He suggests that, possibly because of reduced stimulation from PFC, children with ADHD have low tonic dopaminergic activity. Low tonic stimulation of inhibitory autoreceptors produces high phasic activity in the nucleus accumbens, and possibly other subcortial sites as well, that may result in dysregulated motor and impulse control,

Dopamine receptor oversensitivity (whatever that is) also may cause the body to decrease the amount of dopamine being produced. A shortage of dopamine in the frontal lobe can contribute to poor working memory.

• Stimulants May Inhibit Dopamine Breakdown

Dopamine also contributes to the feelings of bliss and regulates feeling of pain in the body. There is strong evidence that the catecholamines dopamine and norepinephrine are important in the pathophysiology of ADHD, as well in the mechanism of therapeutic action of stimulant drugs. Because of the known effects of stimulants in blocking reuptake of catecholamines and (in the case of d-amphetamine) facilitating their release, it has traditionally been believed that the stimulants compensate for catecholamine deficiency in ADHD.

By blocking dopamine reuptake, stimulants increase tonic levels of dopamine in the extracellular space, increasing the stimulation of impulse-regulating presynaptic autoreceptors and thereby reducing phasic dopamine release.

Hyperactivity, and possibly poor motor impulse control, in ADHD may result from excess dopaminergic activity in the limbic system. The striatum and/or nucleus accumbens are possibilities. Stimulant drugs may reduce hyperactivity by reducing activation of the striatum, possibly through a mechanism that involves stimulation of inhibitory pre-synaptic autoreceptors.

• Cannabinoids Regulate Neural Traffic

There are essentially two kinds of brain cells, according to Stanford University neuroscientist Dan Madison. There are the principal cells that make up what he likened to a superhighway system of long-range information movement, and there are "interneurons," which are like traffic signals along that highway.

"Cannabinoids are a way for the principal cells to regulate the traffic lights," Madison said. After two years of laboratory study and frustrating dead ends, Wilson and Nicoll found that the role of the brain's cannabis is to make the feedback system work. Harvard researchers, working independently, found an essentially identical role for endogenous cannabinoids in another part of the brain, called the cerebellum, which helps to control motor function.

"It's a way for a nerve cell to adjust the gain or intensity of the information coming into it," Nicoll said. "It turns up the amplifier, in a way, and allows more input to get through."

These adjustments seem to have an important role in the brain's uncanny ability to synchronize the firing of nerve cells scattered throughout the brain linking behavior with mood and memory with vision or hearing. Thousands of signals thus become molded into vast oscillations, helping the brain bind together different aspects of perception into coherent state of mind -- a feeling of being in love, perhaps, when we look at someone.

• Retrograde Messenger System

Experiments in the last few years have shown that in any neural circuits where *endocannabinoids* are present these endocannabinoids *may participate in a retrograde messenger system whose goal is presynaptic inhibition*. Endocannabinoids serve as the

messengers in this system, and CB1 serves as the receptor that initiates the inhibition. This is especially important in signaling between neurons in the hippocampus, where strengthening and weakening of neural connections, thereby reorganizing neural circuits, is thought to be a cellular correlate of learning and memory.

Cannabis appears to treat ADD and ADHD by increasing the availability of dopamine. This then has the same effect but is a different mechanism of action than stimulants like Ritalin (methylphenidate) and dexedrine (1) amphetamine which act by binding to the dopamine and interfering with the metabolic breakdown of dopamine. Cannabis (THC) is an anandamide agonist, that is it stimulates the anandamide (CB1) receptor sites.

Researchers working on Tourette's Syndrome (TS) favorable response to Δ^9-THC said: "neuroanatomical structures which are probably involved in TS pathology are heavily associated with the CB_1 receptor system. Considering an involvement of the dopamine system in TS pathophysiology it can be speculated that tic improvement might be caused by an interaction between cannabinoid and dopamine mechanisms. I believe that this is literally true for the rather closely related ADHD.

• Research

What does research say about cannabinoids controlling ADD or hyperactivity. An animal model study, published in the May 2000 issue of the Journal of Neuroscience, reports that synthetic compounds developed to block the way anandamide – the body's own cannabis-like compound or cannabinoid – is inactivated or broken down could correct forms of hyperactivity, such as attention deficit disorder.

Research by Dr. Daniel Piomelli at UCI also suggests a possible mechanism of action for cannabis in treating ADD. Dr. Piomelli, professor of pharmacology, led a team that found that a chemical called AM404 reversed the normal inactivation of a naturally occurring chemical in the brain called anandamide, which is related to marijuana's active ingredient and opposes or counteracts the actions of dopamine. According to Reuters article, Piomelli's study showed that: A chemical that boosts a marijuana-like substance in the brain may insure new treatments for brain disorders such as schizophrenia, Parkinson's disease, and attention-deficit/hyperactivity disorder (ADHD). (Note: Arachidonoyl ethanolamide (AEA) was the first endogenous cannabinoid to be isolated and characterized as an agonist acting on the same receptors (CB_1 and CB_2) as tetrahydrocannabinols (THC). This means that stimulating the anandamide receptors could effectively treat ADD.

Piomelli and his colleagues found that AM404 targeted nerves that produced unusually high levels of dopamine and caused exaggerated movements and other problems in rats. Instead of directly encouraging the production of dopamine-curbing anandamide, AM404 was found to discourage the disintegration of existing anandamide. More anandamide was then available to bind to receptors on nerve cells and reduce the stimulation of nerve cells by dopamine.

What I believe is happening is two things. One is that release of anandamide slows down the rate of neurotransmission. This is one of Piomelli's principle findings. Others have suggested a second action of stimulating anandamide receptor sites and that is they fire Renshaw cells. Renshaw cells are in the midbrain and their neurons go downward in the brain. Their function is to turn off some of the cells which provide sensory input. In the _____ studies by reversing the inactivation of anandamide, AM404 is able to gently curb the exaggerated movements and other disorders caused by too much dopamine activity in nerve cells.

In the case of Parkinson's disease, patients have too little dopamine, while people with ADHD, schizophrenia or Tourette's syndrome may have too much. The hope is that AM404 will lay the groundwork for a new class of drugs that either boost or block dopamine, without the side effects linked to current treatments, Piomelli told Reuters Health in an interview. "Our results are interesting," he said, "because they show that you can modulate dopamine without acting on the dopamine system." Instead of directly encouraging the production of dopamine-curbing anandamide, AM404 was found to discourage the disintegration of existing anandamide. More anandamide was then available to bind to receptors on nerve cells and reduce the stimulation of nerve cells by dopamine.

Piomelli and his colleagues showed for the first time that in rats, anandamide naturally counters dopamine. Usually, though, anandamide is inactive in the brain. The California team's latest experiments in rats reveal that AM404 stops anandamide from being "drained from the brain," which allows it to suppress dopamine.

Although dopamine's role in brain disorders is not completely understood, an elevated level is a "common element" in conditions such as ADHD, schizophrenia and Tourette's syndrome, Piomelli explained. These disorders are all marked by hyperactive "intrusions" into normal brain function, he said. For example, people with Tourette's experience physical "tics," while schizophrenics suffer from delusions.

A UCI news release of May 1, 2000 states that:
"If further research proves successful, the chemical could be used to treat schizophrenia, Tourette's, Parkinson's, autism and attention-deficit disorder, all of which are currently treated by drugs that attack the dopamine system in the brain." Piomelli's research shows "you can modulate dopamine without acting on the dopamine system." These conditions are treated with drugs that affect the dopamine system. Piomelli points out that these existing treatments have side effects such as lethargy and impaired sexual activity. The potential for anandamide-boosting drugs to work against these disorders has some anecdotal backing. Anandamide's counterpart, marijuana, is used by many schizophrenics who report that it relieves their symptoms, Piomelli noted.

"But," he said, "we are not implying that marijuana is use for these conditions."

Piomelli is quoted by USA Today that, "Marijuana has a lot of pharmaceutical and pharmacological potential. The potential now is becoming very, very clear." Many decades-old prejudices are being lifted and that is reflected by the considerable funding that the federal government is giving to research marijuana.

Marijuana, according to Piomelli, is far less selective than anandamide in activating brain cells. Because pot smoking overstimulates the brain, he said, cells eventually become desensitized to any benefits the drug initially brings.

SOURCE: Journal of Neuroscience May 2000.

"I would be very surprised that if in the next 10 years there isn't an important new medicine developed from our better understanding of the cannabis system in the body," says Piomelli.

While Piomelli himself has discounted the use of cannabis for these disorders, this research clearly lays out a potential mechanism of action. An article by UCI staff writer Andreas Von Bubroff states that: "Anandamide is similar to marijuana's active ingredient, THC and belongs to a class of neurotransmitters called endogenous cannabinoids since it is naturally produced by some of the brain's nerve cells." It is known that cannabis is neuroprotective and in practice it has shown to provide relief for some epilepsy, Tourette's and some ADD sufferers. I myself have had several patients who have benefited from cannabis for ADD and with far fewer side effects than Ritalin.

Lastly, there is a six (6) page paper by Kurt E. Patterson discussing marijuana and ADD. In it he states that "There is some evidence available that medical marijuana has been found to be an effective medication for some types of ADD by other researchers in the field. (1) Unfortunately, ADD encompasses such a variety of conditions that the limited amount of research in the field leaves many of the effective therapeutic mechanisms under-investigated. Considering the regulatory difficulties in researching the effects of medical marijuana, it isn't surprising that the information regarding medical marijuana and ADD is largely anecdotal(2)."

• **Experiences**

What does 215 mean – not being an attorney it is difficult to parse the language where on the one hand the preamble talks "about" serious and on the other Prop 215 gives a list of conditions including nausea, glaucoma, migraine, pain and then adds for any other condition that a physician feels that cannabis may be useful for. The argument is made for a broad interpretation of 215 approvals and recommendations by no less an authority than the California DAs Association, California Sheriffs Association, and California Narcotics Officers Association in their ballot argument against 215. They argue that if 215 passed, cannabis could be recommended for anything. A broad reading is argued for by the brief of the CMA arguing that basically 215 protects a physician's first amendment right to communication with his patient concerning whether some medications best

prescription, herbal, vitamins or alternatives and complementing medicine may be helpful to that included.

Insofar as ADD goes it appears that it qualifies under a reasonable interpretation of either a narrow or broad interpretation of 215. NORML seems to be taking the narrow interpretation whereas CMA and the DA's take a broader interpretation.

ADD has been termed a serious enough condition that hundreds of thousands of school children are treated with (some would say subjected to) the not so benign medication Ritalin. ADD as well as ADHD and adult ADD or ADHD have been shown to be very disruptive on people's lives – their self-esteem, their ability to succeed in life, their ability to do well in school. There are numerous websites on ADD and ADHD which assert that this is a serious condition.

Several physicians in California who regularly made 215 recommendation (Dr. Frank Luceria, Dr. Tom O'Connell, Dr. Tod Mikuriya) have indicated that they have made many recommendations for the medical use of cannabis to treat ADD. Dr. Lester Grinspoon, emeritus professor of psychiatry at Harvard School of Medicine and author of Marijuana, The Forbidden Medicine, has a website which lists anecdotal reports of the medicinal benefits of cannabis. Out of a sample of 25 displayed 3, or 12% were describing cannabis' benefits for treatment of ADD.

As recently as March 5, 2002, a 48-Hours TV program chronicled the effectiveness of a medical recommendation for the treatment of ADD in an eight year old child. A California court determined that this constituted an appropriate treatment for this child.

There appears to be overwhelming anecdotal evidence not only is of benefit in treating ADD with cannabinoids but also that many view ADD as a serious condition. Further, it is clear that the FDA in the person of Administrative law judge has officially found that marijuana is safer than Ritalin.
In order to discuss this, let's have a brief and superficial review of how the brain works.

• Appendix

BRAIN
A) 4 big regions:
1) Cerebrum: the largest part of the brain, it contains deep grooves (called sulci) to increase surface area

A) Cortex: 3 functional categories: Sensory, Motor and Associative cortexes.

B) The Cerebrum is. The Cerebrum controls learning, intelligence, judgment and voluntary activities.

C) It is divided into two halves:

1) The right half is thought to house artistic ability and controls the left side of the body.

2) The left half houses mathematical ability and controls the right side of the body.

D) The Cerebrum has four "lobes"

1) Occipital lobe:
   a) located behind the parietal lobe and temporal lobe.
   b) Concerned with vision.

2) Frontal lobe:
   a) located in front of the central sulcus.
   b) The frontal lobe is concerned with functions such as reasoning, planning, part of speech and movement (motor cortex), emotions, and problem-solving.

3) Temporal lobe:
   a) located below the lateral fissure.
   b) Concerned with hearing and memory.

4) Parietal lobe:
   a) located behind the central sulcus.
   b) Concerned with perceptions related to touch, pressure, temperature and pain.

E) Basal Nuclei have motor control/pattern generators;

F) Limbic system for initial memory, emotion--

1) On top of the brainstem and buried underneath the cerebral cortex, there is a set of more evolutionary primitive brain structures called the limbic system.

2) The limbic system components are involved in many of our emotions and motivations, especially those related to survival such as fear, anger and emotions relating to sexual behavior, and feelings of pleasure such as those experienced from eating and sex.

3) There are two important limbic system structures.
   a) The amygdala which is involved in emotion or feelings
   b) The hippocampus which is involved in memory.

2) Diencephalon:
   a) thalamus (=sensory processing, relay) + pineal;
   b) hypothalmus with pituitary gland=reflex integrators for many autonomic reflexes such as temperature, reproduction, appetite...

3) Cerebellum: pattern generators/procedural memory for coordination of motor cortex: learns repetitive skilled motions. The Cerebellum is the second largest part of the brain. It coordinates muscles and maintains balance.

4) Brainstem -- Pons & Medulla Oblongata -- reflex integrators for autonomic cardiovascular, and respiratory reflexes. The Medulla connects brain to the spinal cord and controls involuntary actions, (i.e., heartrate, breathing, B.P.)

B. Divisions of the Brain -

1) Prosencephalon - (forebrain) integrates sensory information
2) Mesencephalon - (midbrain) coordinates sensory information
3) Rhombencephalon - (hindbrain) reflex actions.

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ADHD: Medicinal Marijuana Breakthrough

MONDAY, NOVEMBER 20, 2006

CANNABIS CURE FOR ATTENTION DEFICIT HYPERACTIVITY

By David Berman, MD

So you say your 15 year old doesn't pay attention in school, he fidgets in class, is sometimes disruptive and is just barely getting by grade wise. But wait. Recently he's been doing a lot better. He's keeping up with his homework and his grades have gone from C minuses to As and Bs. Then all of a sudden he's back to his old tricks, busted at school for marijuana.

Maybe we need to take a closer look and see if there really is something more significant going on. Maybe, just maybe, the scientists who are studying the endocannabinoid system and clinicians treating patients with cannabis, cannabinoids and anandamide blockers are right and he really is treating his ADHD. Your first reaction is to dismiss that idea. But read on and you won't be so quick to be influenced by 90 years of unscientific propaganda.

ADHD is a costly problem that strains medical/mental health, educational, and legal institutions. ADHD afflicts 3-5% of Americans. It is one of the most common psychiatric disorders of childhood and in adults frequently results in tragic consequences in professional and personal lives. ADHD is characterized by persistent impairments in attention (or concentration) and/or symptoms of hyperactivity and impulsivity.

The preponderance of studies show marijuana use is overwhelmingly prevalent with ADHD sufferers, either as a self-medicament or for recreation. While some apply preconceptions that marijuana exacerbates ADHD almost all California cannabinologists believe camnabis and cannabinoids have substantially improved the lives of ADHD sufferers, and with less negative side effects than common

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CANNABIS CURE FOR ATTENTION DEFICIT HYPERACTIVITY ...

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stimulant drug ADHD treatments.

As we have come to understand more about the brain and the role of dopamine and the endocannabinoid system we are starting to unravel how cannabis, anandamide and dranabinol act to free up dopamine and decrease the overstimulation of the midbrain. But before we jump to the present we need to recognize that ADHD sufferers react differently to stimulant drugs from the average population[4]. This was first noted in 1937 when Dr. Charles Bradley attempted to treat the first recognized cases of ADHD.(ADHD has gone by the names of Minimal Brain Dysfunction [MBD], hyperkinesis, etc.) After trying sedatives his patients became more hyperactive. Noting this paradox he tried amphetamine, which calmed them down.

From that point, stimulants (sympathomimetic drugs) became the mainstay for treating ADHD. It turns out that they work by tying up dopamine transporter thus freeing up dopamine, previously bound to the dopamine transporter, to engage in retrograde inhibition. Dopamine is essentially acting as a damper on neurotransmission by depolarizing the neuron that just released it. The stimulants main draw back is that they come with a host of unacceptable side effects-jitteriness, anxiety, sleep difficulty, appetite suppression and a propensity to be quick to anger.

It turns out that cannabis also frees up dopamine but it has a very benign side effect profile. Noting cannabis' vastly superior side effect profile DEA Administrative Law Judge, Francis L. Young, after a two-year hearing to reschedule cannabis in 1998 said:

"Nearly all medicines have toxic, potentially lethal effects. But marijuana is not such a substance. There is no record in the extensive medical literature describing a proven, documented cannabis-induced fatality .... In strict medical terms marijuana is far safer than many foods we commonly consume ... Marijuana, in its natural form, is one of the safest therapeutically active substances known to man."

The results in treating ADHD with cannabis are often spectacular .Patients report grades going from Cs and Ds to As and Bs. Dr. David Bearman, a physician practicing in Santa Barbara California, reports patients have said "I graduated from the Maritime Academy

because I smoked marijuana", and "I got my Ph. D because of smoking marijuana." Almost universally ADHD patients who therapeutically used cannabis reported it helped them pay attention in lecture, focus their attention instead of thinking of several ideas almost at the same time, helped them to stay on task and do their homework.

Marijuana Science & the Brain

In the 1940s tetrahydrocannabinol (THC) was identified as the major psychoactive in marijuana.In 1964 Israeli scientist Rapheal Mechulam, isolated the most pharmacologically active of the 483 chemical in cannabis,delta 9 THC. While the psychoactive effects of marijuana are still mostly attributed to THC[1] several cannabinoids, flavinoids and terpenoids also from cannabis, are thought to have therapeutic value.

In the 1990s, scientists discovered vast amounts of THC receptors in

the mammalian (e.g., cows, dolphins, humans) central nervous system. This system is now known as the endocannabinoid system and is a major part of the human brain. Its importance was accepted when it was learned that THC receptors exist in greater numbers than receptors for hundreds of drugs used in modern medicine. This initiated widespread medical interest in marijuana; new findings appear almost daily in the medical literature.

The first cannabinoid-receptors in the human brain were named CB1 and CB2. They are located in high concentration in the midbrain in the limbic system and in the forebrain of the cerebral cortex. Soon it was found that the human brain produces over 60 endocannabinoids (i.e., THC-like substances). Endocannabinoids exert most of their pharmacological actions by activating the CB1 receptor in the brain. While the brain produces its own cannabinoids, smoking marijuana also stimulates the body's endocannabinoid system.

70% of the brain's job is to inhibit sensory input from the other 30% Typical ADHD symptoms include distractibility. The most accepted theory about ADHD rests on the fact that about 70% of the brain's function is to regulate input to the other 30%. The cause of ADHD is probably a decreased ability to suppress sensory input both internal and external input. Basically the brain is overwhelmed with to much information come to fast. In ADHD the brain is cluttered with and too aware of all the nuances of a person's daily experience. This phenomenon is caused by a dopamine dysfunction.

Dopamine (a neurotransmitter in the brain) is a key suppressor of stimuli to the brain.It works by a unique mechanism, retrograde inhibition. By the dopamine depolarizing the neuron that just released it, that neuron becomes more difficult to stimulate and the speed and frequency of neural firing in that part of the brain is decreased. Without dopamine, we cannot distinguish or maybe we should say, focus and concentrate on what is important information (a boss giving us important instructions) from not so important information (e.g., daydreams). Persons with ADHD have significant irregularities in their dopamine management systems[1]

In the late 1990's, researchers discovered how Ritalin®, the popular ADHD treatment, works¾it increases dopamine levels[2]. The story would have ended there if Ritalin did not have significant potential to cause permanent brain damage and psychiatric problems[3],[4]. (Bill this seems way too strong) Even non-stimulant ADHD drugs have serious psychiatric problems[5].

Since the endocannabinoid system was discovered, many studies revealed that marijuana also modulates the dopamine system[6] and therefore is a potential ADHD treatment. As recounted in the physicians' stories below, marijuana may a safer, less costly, and more effective treatment than anything available from the pharmaceutical companies.

Doctors Speak Up

Dr. Claudia Jensen, a 49-year old California pediatrician and mother of 2 teenage daughters, says marijuana might be the best treatment for ADHD. In a recent interview with the FOX news network, she said:

"Why would anyone want to give their child an expensive pill...with unacceptable side effects, when he or she could just go into the backyard, pick a few leaves off a plant and make tea...?"

"Cannabinoids are a very viable alternative to treating adolescents with ADD and ADHD...I have a lot of adult patients who swear by it."

In her testimony, before the House Committee on Government Reform on Marijuana (2004) Dr. Jensen discussed the practice of recommending marijuana to patients with ADHD in an 11-page statement. Her testimony summarized hundreds of published scientific articles on the safety/ efficacy of marijuana that have produced strong scientific evidence that marijuana is important medicine.

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Her reasons for looking to marijuana as treatment for ADHD?
"The other legal drugs used to treat ADD are helpful in many patients, but they all have side effects....the other five of the nine drugs used to treat ADD in this country haven't even been scientifically tested...for ADD in children. These are drugs for depression and high blood pressure...Of all the drugs used to treat ADD, cannabis has the least number of serious side effects.

Her explanation for why marijuana is opposed by the pharmaceutical companies:
"The real problem with allowing patients to use Cannabis as a medication is economics...If Cannabis were approved for use in just the ADD/ ADHD market alone, it could significantly impact the $1 Billion a year sales for traditional ADD/ ADHD pharmaceuticals..."

Dr. Tom O'Connell

blog.

Dr. Tom O'Connell, a retired surgeon who works with patients in the Bay Area is studying with patients that self-medicate with marijuana. In O'Shaughnessy's, The Journal of Cannabis in Clinical Practice (Spring 2005) Dr. O'Connell summarized his study of 790 patients:
"...There is universal agreement among applicants who have been diagnosed with and/or treated for ADD that cannabis helps them achieve and retain focus..."

Dr. O'Connell states there is a strong argument for promoting marijuana with ADHD because it is safer than all other medications. In 2004 a statement to Fox news, he said:
"...Although it flies in the face of conventional wisdom, it's nevertheless true that cannabis is far safer and more effective than the prescription agents currently advocated for treatment of ADD-ADHD..."

Is Marijuana Safe?

The preponderance of scientific evidence points to marijuana as being exceptionally safe, for adolescents and adults. Leo Holllister's two papers Health Aspects of Cannabis (1986) and Health aspects of cannabis: revisited (1998) are widely considered the most authoritative compendia on the safety of marijuana. Hollister's opinion is best summarized in his 1986 paper:
"...one is forced to conclude that cannabis is relatively safe...toxicity studies of cannabis and its constituents lead to the inescapable

conclusion that it is one of the safest drugs ever studied in this way."

In the many papers that cite Hollister's work, there is a common theme:
"By any standards, THC must be considered a very safe drug both acutely and on long-term exposure."

"...although there have been many rumors that the long term use of marijuana leads to irreversible damage to higher brain functions the results of numerous scientific studies have failed to confirm this... Based on the results of the three best studies performed (Schwartz, Pope and Block et al.,) residual cognitive effects are seldom observed and if present are mild in nature."

FDA Administrative Law Judge Young couldn't have been clearer in describing the safety of cannabis. He said after a two year FDA rescheduling hearing, that marijuana was one of the safest therapeutic agents known to man. According to ALJ Young marijuana is safer than eating 10 potatoes. His recommendation to reschedule marijuana to schedule II was turned down by President Bushes' political appointee, FDA Director John Lawn

CANNABINOIDS OFFER EXCITING POSSIBILITIES
With the discovery of cannabinoid receptors and a greater understanding of the endocanabinoid system we stand at the dawn of a new era of understanding how the brain works and applying new solutions. This is a time to look at not only creating new agonists and antagonists based on the cannabinoid molecule but also to recognize the medicinal role that cannabis can play not only in treating ADHD but also possibly seizures, Tourette's Syndrome, PTSD,OCD and panic attacks. We in the US need to reevaluate our attitude regarding phytochemicals.

There is a contemporary movement that physicians practice evidence based medicine. That is much easier said then done. As the recent Vioxx ® example demonstrates FDA approval does not guarantee that serious side effects won't be discovered after a studied and FDA approved drug is used by tens of millions, rather than a few thousand test subjects. We need to adopt some of the European respect for the value of hundreds and in some cases (as is the case with cannabis) years of human experience with herbal and natural preparations as some sort of evidence. In some instances these traditional remedies, sometimes ridiculed, have fewer side effects and work as well or better than FDA approved , single chemical drugs.

Let's face it, not all so called research is done by the federal or state government. Most clinical studies are done by drug companies and they are not an unbiased third party. We most rely on the expertise and over site of the FDA to sort the wheat from the chaff. They must pay attention to the science and methodology of the studies submitted. If possible they should have access to the studies which weren't submitted to them since pharmaceutical companies have been known to provide biased research, rooted in financial conflict and preconceived notions. Costly proprietary drugs are heavily promoted but herbal remedies, which cost the patient relatively little, have at least until recently, been given relatively short shrift by the medical

establishment.

Six years ago the CMA, at their annual leadership conference, urged physicians to be aware of their patients' use of complementary and alternative medicines (CAM). Over 60 % of the average doctors patients use CAM, however at least 40% do not share that fact with their physician. One of those herbs [patients use is cannabis and one of the conditions patients have found it's useful for is treating ADHD.

Physicians, judges, and researchers are beginning to acknowledge the medical value of cannabis. In the case of treatment for ADHD cannabis and cannabinoids are often an effective, safer alternative then sympathomimetic prescription drugs. These stimulant drugs have an unacceptable side effect profile.

If you want to talk about marijuana, choose a physician that has experience with marijuana. More and more physicians are becoming aware of cannabis medicinal value. UCSD is headquarters for the California Marijuana Research Center. They administer 18 FDA approved smoked cannabis medical clinical studies.

These studies are showing good results and being presented at medical meetings. Bayer is marketing tincture of cannabis in Canada under the trade name Sativex .The International Cannabis Research Society has annual meetings discussing progress being made in how cannabis works for patients in a way which patients say "almost seems like its magic".

posted by from the office of Dr. David Bearman @ 4:27 PM 0 comments

ADHD & Marijuana

There is a remarkable new story in healthcare: marijuana may be good for attention-deficit/hyperactivity disorder (ADHD or ADD). The trend recently caught the attention of conservative FOX news, which quoted pediatrician Dr. Claudia Jensen "...cannabinoids [marijuana] are a very viable alternative to treating adolescents with ADD and ADHD..." [1]

Marijuana appears to treat ADHD with fewer side effects than traditional treatments, amphetamines (e.g., Ritalin®). With today's skyrocketing heath costs, it makes good sense to look at treatments that do not require pharmacies and health insurance.

ADHD is a costly problem that strains medical/mental health, educational, and legal institutions[2]. The most accepted ADHD statistics place 3-5% of Americans afflicted[3]. It is one of the most common psychiatric disorders of childhood[4] and in adults frequently results in tragic consequences in professional and personal lives[5]. ADHD is characterized by persistent impairments in attention (or concentration) and/or symptoms of hyperactivity and impulsivity[6].

US federal statistics place the number of regular marijuana users at approximately 6% of the population. The preponderance of studies show marijuana use is overwhelmingly prevalent with ADHD sufferers, either as a self-medicament or for recreation[7]. Some doctors now claim this relationship is healthy—marijuana may improve the lives of ADHD sufferers, with less negative side effects of common ADHD treatments.

Given the "spaced out" stereotypes of marijuana users its hard to imagine how marijuana helps ADHD sufferers. To grasp this concept recognize that ADHD sufferers react profoundly different to psychoactive drugs from the average population[8]. This was discovered in 1937 when Dr. Charles Bradley attempted to treat the first recognized cases of ADHD. After trying sedatives his patients became more hyperactive, noting this

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[1] http://www.foxnews.com/story/0,2933,117541,00.html
[2] Diagnosis and Treatment of Attention Deficit Hyperactivity Disorder. NIH Consensus Statement Online 1998 Nov 16-18; 16(2): 1-37.
[3] National Institutes of Health Consensus Development Conference Statement. Diagnosis and treatment of attention-deficit/hyperactivity disorder (ADHD). *Journal of the American Academy of Child and Adolescent Psychiatry*, 2000; 39(2): 182-93.
[4] American Psychiatric Association (2000) *Diagnostic and Statistical Manual of Mental Disorders*, 4th edn. Text Revision), Washington, DC: APA.
[5] Murphy K. Psychosocial Treatments for Adhd in Teens and Adults: a Practice-Friendly Review. J Clin Psychol. 2005; 61(5):607-19.
[6] Kutcher S. et al. International Consensus Statement on Attention-Deficit/Hyperactivity Disorder (Adhd) and Disruptive Behaviour Disorders (Dbds): Clinical Implications and Treatment Practice Suggestions. Eur Neuropsychopharmacol. 2004; 14(1):11-28.
[7] Dodson Ww. Pharmacotherapy of Adult Adhd. J Clin Psychol. 2005; 61(5):589-606.
[8] Dodson Ww. Pharmacotherapy of Adult Adhd. J Clin Psychol. 2005; 61(5):589-606.

paradox he tried amphetamines—which calmed them down. From that point, psychostimulants became the mainstay for treating ADHD. Therefore, it seems, if marijuana makes some people "spaced out" it may give ADHD sufferers the opposite effect—help them focus.

Marijuana Science & the Brain

In the 1940s **tetrahydrocannabinol (THC)** was identified as the major psychoactive in marijuana. The psychoactive effects of marijuana are still mostly attributed to THC[9].

In the 1990s, scientists discovered vast amounts THC receptors in the mammalian (e.g., cows, dolphins, humans) central nervous system. This system is now known as the **endocannabinoid system** and is a major part of the human brain. Its importance was accepted when it was learned that THC receptors exist in greater numbers than receptors for hundreds of drugs used in modern medicine[10]. This initiated widespread medical interest in marijuana; new findings appear daily in the medical literature[11].

The first cannabinoid-receptors in the human brain were named **CB_1** and **CB_2**. Soon afterwards, it was found that the human brain produces over 60 endocannabinoids (i.e., THC-like substances)[12]. Endocannabinoids exert most of their pharmacological actions by activating the CB_1 receptor in the brain[13]. While the brain produces its own cannabinoids, smoking marijuana also stimulates the body's endocannabinoid system.

70% of the brain's job is to inhibit sensory input from the other 30%

Typical ADHD symptoms include distractibility. The most accepted theory about ADHD rests on the fact that about 70% of the brain's function is to regulate input to the other 30% (need a reference here). The cause of ADHD is probably a decreased ability to suppress input (need a reference here), essentially allowing the brain to be too aware of all the nuances of a persons daily experience.

Dopamine (a neurotransmitter in the brain) is a key suppressor of stimuli to the brain. Without dopamine, we cannot distinguish what is important information (a boss giving us important instructions) from not so important information (e.g., daydreams). Persons with ADHD have significant irregularities in their dopamine management systems[14]

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[9] Pertwee Rg. The Central Neuropharmacology of Psychotropic Cannabinoids. Pharmacol Ther. 1988; 36(2-3):189-261.

[10] Herkenham M et. al. Cannabinoid Receptor Localization in Brain [Journal Article]. Proc Natl Acad Sci U S A. 1990; 87(5):1932-6.

[11] De Petrocellis L et. al. The Endocannabinoid System: a General View and Latest Additions [Journal Article]. Br J Pharmacol. 2004; 141(5):765-74.

[12] Murillo-Rodriguez Eric, et. al. Anandamide Modulates Sleep and Memory in Rats. Brain Research. 1998; 812(1-2):270-274.

[13] Pertwee Rg. Pharmacology of Cannabinoid Cb1 and Cb2 Receptors. Pharmacol Ther. 1997; 74(2):129-80.

[14] Oades, et. al., Dev Sci. 2005 Mar;8(2):122-31.

Effet contre tendances suicidaires. 80

In the late 1990's, researchers discovered how Ritalin®, the popular ADHD treatment, works—it increases dopamine levels[15]. The story would have ended there if Ritalin did not have significant potential to cause permanent brain damage and psychiatric problems[16],[17]. Even non-stimulant ADHD drugs have serious psychiatric problems[18].

Since the endocannabinoid system was discovered, many studies revealed that marijuana also modulates the dopamine system[19] and therefore is a potential ADHD treatment. As recounted in the physicians' stories below, marijuana may a safer, less costly, and more effective treatment than anything available from the pharmaceutical companies.

Doctors Speak Up

Dr. Claudia Jensen

Dr. Claudia Jensen, a 49-year old California pediatrician and mother of 2 teenage daughters, says marijuana might be the best treatment for ADHD[20]. In a recent interview with the FOX news network, she said:

> "Why would anyone want to give their child an expensive pill…with unacceptable side effects, when he or she could just go into the backyard, pick a few leaves off a plant and make tea…?"
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> "Cannabinoids are a very viable alternative to treating adolescents with ADD and ADHD…I have a lot of adult patients who swear by it."

In her testimony, for the House Committee on Government Reform on Marijuana (2004) Dr. Jensen discussed the practice of recommending marijuana to patients with ADHD[21] in an 11-page statement. Her testimony summarized *hundreds* of published scientific articles on the safety/ efficacy of marijuana that have produced strong scientific evidence that marijuana is important medicine. Her reasons for looking to marijuana with ADHD?

> "The other legal drugs used to treat ADD are helpful in many patients, but they all have side effects....the other five of the nine drugs used to treat ADD in this country haven't even been scientifically tested...for ADD in children. These are drugs for depression and high blood pressure...Of all the drugs used to treat ADD, cannabis has the least number of serious side effects.

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Dr. Tom O'Connell

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[15] Nora D. Volkow et. al. The Journal of Neuroscience 2001;21:RC121.
[16] Moll GH et. al. J Child Adolesc Psychopharmacol 2001 Spring;11(1):15-24
[17] William Carlezon et. al. Biol Psychiatry. 2003 Dec 15;54(12):1330-7.
[18] Food and Drug Administration. Public Health Advisory Suicidal Thinking in Children and Adolescents Being Treated With Strattera (Atomoxetine). 29 September 2005.
[19] Rodriguez De Fonseca F et. al. Role of the Endogenous Cannabinoid System as a Modulator of Dopamine Transmission: Implications for Parkinson's Disease and Schizophrenia. Neurotox Res. 2001; 3(1):23-35.
[20] http://www.foxnews.com/story/0,2933,117541,00.html
[21] http://reform.house.gov/UploadedFiles/Claudia%20Jensen.pdfx

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> "...one is forced to conclude that cannabis is relatively safe...toxicity studies of cannabis and its constituents lead to the inescapable conclusion that it is one of the safest drugs ever studied in this way."[23]

In the many papers that cite Hollister's work, there is a common theme:

> "By any standards, THC must be considered a very safe drug both acutely and on long-term exposure."[24]

> "...although there have been many rumors that the long term use of marijuana leads to irreversible damage to higher brain functions the results of numerous scientific studies have failed to confirm this...Based on the results of the three best studies performed (Schwartz, Pope and Block et al.,) residual cognitive effects are seldom observed and if present are mild in nature."[25]

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Talking with a Physician

Virtually all information on treating disorders like ADHD is provided by pharmaceutical companies directly to physicians. As the recent Vioxx® example demonstrates pharmaceutical companies provide flawed and deceptive information about treatment

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[22] Mechoulam R and Golan D. Comment on 'health Aspects of Cannabis: Revisited'. Int J Neuropsychopharmcol. 1998; 1(1):83-85.
[23] Hollister Le. Health Aspects of Cannabis. Pharmacol Rev. 1986; 38(1):1-20.
[24] Iversen, Leslie L. The science of marijuana, p. 181. Oxford University Press, 2000.
[25] Iversen, Leslie L. The science of marijuana, p. 97. Oxford University Press, 2000.

options[26]. This problem of biased research is rooted in financial conflict and prejudices, with many prestigious researchers suppressing information or deliberately promoting false conclusions[27]. Proprietary drugs are promoted and herbal remedies, which cost the patient relatively little, are ignored or slandered mercilessly.

Marijuana may proove to me a much safer option for treating ADHD in children and adults. Physcicans, judges, and researchers are beginning to acknowledge that the medical value is a viable soultion to today's unworkable soutions pushed by the pharmaceutical companies. Do not wait for your physician to recommend marijuana. It is up to you to broach the subject as few doctors want to go on record with insurance companies (which pay their bills) as promoting marijuana.

If you want to talk about marijuana, choose a physician that has experience with marijuana. Keep in mind that there is a concerted and well-financed campaign against legalizing marijuana for any purpose and therefore your average physician will be at best intimidated to talk about it.

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[26] John P. A. Ioannidis. Why Most Published Research Findings Are False. PLoS Med. 2005 August; 2(8): e124.

[27] John P. A. Ioannidis, MD. Contradicted and Initially Stronger Effects in Highly Cited Clinical Research. *JAMA.* 2005; 294:218-228.

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Cannabis and cannabinoids effectively treat ADHD with fewer side effects than traditional sympathomimetic treatments(e.g. amphetamines , Ritalin®, Adderal, Strattera). With today's skyrocketing heath costs, here is a treatment that does not require pharmacies and health insurance.

NOTE TO BILL: Given our focus I suggest we may want to lead with something like the next two paragraphs or we can just add them here, or not.

So you say your 15 year old doesn't pay attention in school, he fidgets in class, is sometimes disruptive and is just barely getting by grade wise. But wait. Recently he's been doing a lot better. He's keeping up with his homework and his grades have gone from C minuses to As and Bs. Then all of a sudden he's back to his old tricks, busted at school for marijuana.

Maybe we need to take a closer look and see if there really is something more significant going on. Maybe, just maybe, the scientists who are studying the endocannabinoid system and clinicians treating patients with cannabis, cannabinoids and anandamide blockers are right and he really is treating his ADHD. Your first reaction is to dismiss that idea. But read on and you won't be so quick to be influenced by 90 years of unscientific propaganda.

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[1] http://www.foxnews.com/story/0,2933,117541,00.html

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[3] National Institutes of Health Consensus Development Conference Statement. Diagnosis and treatment of attention-deficit/hyperactivity disorder (ADHD). *Journal of the American Academy of Child and Adolescent Psychiatry*, 2000; 39(2): 182-93.

[4] American Psychiatric Association (2000) *Diagnostic and Statistical Manual of Mental Disorders*, 4th edn. Text Revision), Washington, DC: APA.

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The preponderance of studies show marijuana use is overwhelmingly prevalent with ADHD sufferers, either as a self-medicament or for recreation[7]. While some apply preconceptions that marijuana exacerbates ADHD almost all California cannabinologists believe camnabis and cannabinoids have substantially improved the lives of ADHD sufferers, and with less negative side effects than common stimulant drug ADHD treatments.

As we have come to understand more about the brain and the role of dopamine and the endocannabinoid system we are starting to unravel how cannabis, anandamide and dranabinol act to free up dopamine and decrease the overstimulation of the midbrain. But before we jump to the present we need to recognize that ADHD sufferers react differently to stimulant drugs from the average population[8]. This was first noted in 1937 when Dr. Charles Bradley attempted to treat the first recognized cases of ADHD.(ADHD has gone by the names of Minimal Brain Dysfunction [MBD], hyperkinesis, etc.) After trying sedatives his patients became more hyperactive. Noting this paradox he tried amphetamines—which calmed them down.

From that point, stimulants (sympathomimetic drugs) became the mainstay for treating ADHD. It turns out that they work by tying up dopamine transporter thus freeing up dopamine, previously bound to the dopamine transporter, to engage in retrograde inhibition. Dopamine is essentially acting as a damper on neurotransmission by depolarizing the neuron that just released it. The stimulants main draw back is that they come with a host of unacceptable side effects-jitteriness, anxiety, sleep difficulty, appetite suppression and a propensity to be quick to anger.

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The results in treating ADHD with cannabis are often spectacular .Patients report grades going from Cs and Ds to As and Bs. Dr. David Bearman, a physician practicing in Santa Barbara California, reports patients have said "I graduated from the Maritime Academy

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[5] Murphy K. Psychosocial Treatments for Adhd in Teens and Adults: a Practice-Friendly Review. J Clin Psychol. 2005; 61(5):607-19.
[6] Kutcher S. et al. International Consensus Statement on Attention-Deficit/Hyperactivity Disorder (Adhd) and Disruptive Behaviour Disorders (Dbds): Clinical Implications and Treatment Practice Suggestions. Eur Neuropsychopharmacol. 2004; 14(1):11-28.
[7] Dodson Ww. Pharmacotherapy of Adult Adhd. J Clin Psychol. 2005; 61(5):589-606.
[8] Dodson Ww. Pharmacotherapy of Adult Adhd. J Clin Psychol. 2005; 61(5):589-606.

because I smoked marijuana", and "I got my Ph. D because of smoking marijuana." Almost universally ADHD patients who therapeutically used cannabis reported it helped them pay attention in lecture, focus their attention instead of thinking of several ideas almost at the same time, helped them to stay on task and do their homework.

Marijuana Science & the Brain

In the 1940s **tetrahydrocannabinol (THC)** was identified as the major psychoactive in marijuana.In 1964 Israeli scientist Rapheal Mechulam, isolated the most pharmacologically active of the 483 chemical in cannabis,delta 9 THC. While the psychoactive effects of marijuana are still mostly attributed to THC[9] several cannabinoids, flavinoids and terpenoids also from cannabis, are thought to have therapeutic value.

In the 1990s, scientists discovered vast amounts of THC receptors in the mammalian (e.g., cows, dolphins, humans) central nervous system. This system is now known as the **endocannabinoid system** and is a major part of the human brain. Its importance was accepted when it was learned that THC receptors exist in greater numbers than receptors for hundreds of drugs used in modern medicine[10]. This initiated widespread medical interest in marijuana; new findings appear almost daily in the medical literature[11].

The first cannabinoid-receptors in the human brain were named **CB_1** and **CB_2**. They are located in high concentration in the midbrain in the limbic system and in the forebrain of the cerebral cortex. Soon it was found that the human brain produces over 60 endocannabinoids (i.e., THC-like substances)[12]. Endocannabinoids exert most of their pharmacological actions by activating the CB_1 receptor in the brain[13]. While the brain produces its own cannabinoids, smoking marijuana also stimulates the body's endocannabinoid system.

70% of the brain's job is to inhibit sensory input from the other 30%

Typical ADHD symptoms include distractibility. The most accepted theory about ADHD rests on the fact that about 70% of the brain's function is to regulate input to the other 30% (ADHD page). The cause of ADHD is probably a decreased ability to suppress sensory input both internal and external input (need a reference here).Basically the brain is overwhelmed with to much information come to fast. In ADHD the brain is cluttered with and too aware of all the nuances of a person's daily experience. This phenomenon is caused by a dopamine dysfunction.

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[9] Pertwee Rg. The Central Neuropharmacology of Psychotropic Cannabinoids. Pharmacol Ther. 1988; 36(2-3):189-261.

[10] Herkenham M et. al. Cannabinoid Receptor Localization in Brain [Journal Article]. Proc Natl Acad Sci U S A. 1990; 87(5):1932-6.

[11] De Petrocellis L et. al. The Endocannabinoid System: a General View and Latest Additions [Journal Article]. Br J Pharmacol. 2004; 141(5):765-74.

[12] Murillo-Rodriguez Eric, et. al. Anandamide Modulates Sleep and Memory in Rats. Brain Research. 1998; 812(1-2):270-274.

[13] Pertwee Rg. Pharmacology of Cannabinoid Cb1 and Cb2 Receptors. Pharmacol Ther. 1997; 74(2):129-80.

Dopamine (a neurotransmitter in the brain) is a key suppressor of stimuli to the brain.It works by a unique mechanism, retrograde inhibition. By the dopamine depolarizing the neuron that just released it, that neuron becomes more difficult to stimulate and the speed and frequency of neural firing in that part of the brain is decreased. Without dopamine, we cannot distinguish or maybe we should say, focus and concentrate on what is important information (a boss giving us important instructions) from not so important information (e.g., daydreams). Persons with ADHD have significant irregularities in their dopamine management systems[14]

In the late 1990's, researchers discovered how Ritalin®, the popular ADHD treatment, works—it increases dopamine levels[15]. The story would have ended there if Ritalin did not have significant potential to cause permanent brain damage and psychiatric problems[16],[17]. (Bill this seems way too strong) Even non-stimulant ADHD drugs have serious psychiatric problems[18].

Since the endocannabinoid system was discovered, many studies revealed that marijuana also modulates the dopamine system[19] and therefore is a potential ADHD treatment. As recounted in the physicians' stories below, marijuana may a safer, less costly, and more effective treatment than anything available from the pharmaceutical companies.

Doctors Speak Up

Dr. Claudia Jensen

Dr. Claudia Jensen, a 49-year old California pediatrician and mother of 2 teenage daughters, says marijuana might be the best treatment for ADHD[20]. In a recent interview with the FOX news network, she said:

> "Why would anyone want to give their child an expensive pill... with unacceptable side effects, when he or she could just go into the backyard, pick a few leaves off a plant and make tea... ?"
>
> "Cannabinoids are a very viable alternative to treating adolescents with ADD and ADHD... I have a lot of adult patients who swear by it."

In her testimony, befor the House Committee on Government Reform on Marijuana (2004) Dr. Jensen discussed the practice of recommending marijuana to patients with ADHD[21] in an 11-page statement. Her testimony summarized *hundreds* of published scientific articles on the safety/ efficacy of marijuana that have produced strong scientific evidence that marijuana is important medicine.

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[14] Oades, et. al., Dev Sci. 2005 Mar;8(2):122-31.
[15] Nora D. Volkow et. al. The Journal of Neuroscience 2001;21:RC121.
[16] Moll GH et. al. J Child Adolesc Psychopharmacol 2001 Spring;11(1):15-24
[17] William Carlezon et. al. Biol Psychiatry. 2003 Dec 15;54(12):1330-7.
[18] Food and Drug Administration. Public Health Advisory Suicidal Thinking in Children and Adolescents Being Treated With Strattera (Atomoxetine). 29 September 2005.
[19] Rodriguez De Fonseca F et. al. Role of the Endogenous Cannabinoid System as a Modulator of Dopamine Transmission: Implications for Parkinson's Disease and Schizophrenia. Neurotox Res. 2001; 3(1):23-35.
[20] http://www.foxnews.com/story/0,2933,117541,00.html
[21] http://reform.house.gov/UploadedFiles/Claudia%20Jensen.pdfx

Her reasons for looking to marijuana as treatment for ADHD?

> "The other legal drugs used to treat ADD are helpful in many patients, but they all have side effects....the other five of the nine drugs used to treat ADD in this country haven't even been scientifically tested...for ADD in children. These are drugs for depression and high blood pressure...Of all the drugs used to treat ADD, cannabis has the least number of serious side effects.

Her explanation for why marijuana is opposed by the pharmaceutical companies:

> "The real problem with allowing patients to use Cannabis as a medication is economics...If Cannabis were approved for use in just the ADD/ ADHD market alone, it could significantly impact the $1 Billion a year sales for traditional ADD/ ADHD pharmaceuticals..."

Dr. Tom O'Connell

Dr. Tom O'Connell, a retired surgeon who works with patients in the Bay Area is studying with patients that self-medicate with marijuana. In O'Shaughnessy's, The Journal of Cannabis in Clinical Practice (Spring 2005) Dr. O'Connell summarized his study of 790 patients:

> "...There is universal agreement among applicants who have been diagnosed with and/or treated for ADD that cannabis helps them achieve and retain focus..."

Dr. O'Connell states there is a strong argument for promoting marijuana with ADHD because it is safer than all other medications. In 2004 a statement to Fox news, he said:

> "...Although it flies in the face of conventional wisdom, it's nevertheless true that cannabis is far safer and more effective than the prescription agents currently advocated for treatment of ADD-ADHD..."

Is Marijuana Safe?

The preponderance of scientific evidence points to marijuana as being exceptionally safe, for adolescents and adults. Leo Holllister's two papers *Health Aspects of Cannabis* (1986) and *Health aspects of cannabis: revisited* (1998) are widely considered the most authoritative compendia on the safety of marijuana[22]. Hollister's opinion is best summarized in his 1986 paper:

> "...one is forced to conclude that cannabis is relatively safe...toxicity studies of cannabis and its constituents lead to the inescapable conclusion that it is one of the safest drugs ever studied in this way."[23]

In the many papers that cite Hollister's work, there is a common theme:

> "By any standards, THC must be considered a very safe drug both acutely and on long-term exposure."[24]

> "...although there have been many rumors that the long term use of marijuana leads to irreversible damage to higher brain functions the results of numerous scientific studies have failed to confirm this...Based on the results of the three best studies performed (Schwartz, Pope and Block et al.,) residual cognitive effects are seldom observed and if present are mild in nature."[25]

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[22] Mechoulam R and Golan D. Comment on 'health Aspects of Cannabis: Revisited'. Int J Neuropsychopharmcol. 1998; 1(1):83-85.
[23] Hollister Le. Health Aspects of Cannabis. Pharmacol Rev. 1986; 38(1):1-20.
[24] Iversen, Leslie L. The science of marijuana, p. 181. Oxford University Press, 2000.
[25] Iversen, Leslie L. The science of marijuana, p. 97. Oxford University Press, 2000.

FDA Administrative Law Judge Young couldn't have been clearer in describing the safety of cannabis. He said after a two year FDA rescheduling hearing, that marijuana was one of the safest therapeutic agents known to man. According to ALJ Young marijuana is safer than eating 10 potatoes. His recommendation to reschedule marijuana to schedule II was turned down by President Bushes' political appointee, FDA Director John Lawn

CANNABINOIDS OFFER EXCITING POSSIBILITIES

With the discovery of cannabinoid receptors and a greater understanding of the endocanabinoid system we stand at the dawn of a new era of understanding how the brain works and applying new solutions. This is a time to look at not only creating new agonists and antagonists based on the cannabinoid molecule but also to recognize the medicinal role that cannabis can play not only in treating ADHD but also possibly seizures, Tourette's Syndrome, PTSD,OCD and panic attacks. We in the US need to reevaluate our attitude regarding phytochemicals.

There is a contemporary movement that physicians practice evidence based medicine. That is much easier said then done. As the recent Vioxx ® example demonstrates FDA approval does not guarantee that serious side effects won't be discovered after a studied and FDA approved drug is used by tens of millions, rather than a few thousand test subjects. We need to adopt some of the European respect for the value of hundreds and in some cases (as is the case with cannabis) years of human experience with herbal and natural preparations as some sort of evidence. In some instances these traditional remedies, sometimes ridiculed, have fewer side effects and work as well or better than FDA approved , single chemical drugs.

Let's face it, not all so called research is done by the federal or state government. Most clinical studies are done by drug companies and they are not an unbiased third party. We most rely on the expertise and over site of the FDA to sort the wheat from the chaff. They must pay attention to the science and methodology of the studies submitted. If possible they should have access to the studies which weren't submitted to them since pharmaceutical companies have been known to provide biased research, rooted in financial conflict and preconceived notions. [26]. [27]. Costly proprietary drugs are heavily promoted but herbal remedies, which cost the patient relatively little, have at least until recently, been given relatively short shrift by the medical establishment.

Six years ago the CMA, at their annual leadership conference, urged physicians to be aware of their patients' use of complementary and alternative medicines (CAM). Over 60 % of the average doctors patients use CAM, however at least 40% do not share that fact with their physician. One of those herbs [patients use is cannabis and one of the conditions patients have found it's useful for is treating ADHD.

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[26] John P. A. Ioannidis. Why Most Published Research Findings Are False. PLoS Med. 2005 August; 2(8): e124.

[27] John P. A. Ioannidis, MD. Contradicted and Initially Stronger Effects in Highly Cited Clinical Research. *JAMA*. 2005; 294:218-228.

Physicians, judges, and researchers are beginning to acknowledge the medical value of cannabis. In the case of treatment for ADHD cannabis and cannabinoids are often an effective, safer alternative then sympathomimetic prescription drugs. These stimulant drugs have an unacceptable side effect profile.

If you want to talk about marijuana, choose a physician that has experience with marijuana. More and more physicians are becoming aware of cannabis medicinal value. UCSD is headquarters for the California Marijuana Research Center. They administer 18 FDA approved smoked cannabis medical clinical studies. These studies are showing good results and being presented at medical meetings. Bayer is marketing tincture of cannabis in Canada under the trade name Sativex .The International Cannabis Research Society has annual meetings discussing progress being made in how cannabis works what almost seems like its magic.

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Cannabis as a medical treatment for attention deficit disorder

"Why would anyone want to give their child an expensive pill... with unacceptable side effects, when he or she could just go into the backyard, pick a few leaves off a plant and make tea for him or her instead? Cannabinoids are a very viable alternative to treating adolescents with ADD and ADHD"

WASHINGTON - As a California pediatrician and 49-year-old mother of two teenage daughters, Claudia Jensen says pot might prove to be the preferred medical treatment for attention deficit disorder - even in adolescents.

While some wonder whether Jensen was smoking some wacky weed herself, the clinician for low-income patients and professor to first-year medical students at the University of Southern California said her beliefs are very grounded: The drug helps ease the symptomatic mood swings, lack of focus, anxiety and irritability in people suffering from neuropsychiatric disorders like ADD and attention deficit/hyperactivity disorder.

"Cannabinoids are a very viable alternative to treating adolescents with ADD and ADHD.I have a lot of adult patients who swear by it."

Under California state law, physicians are allowed to recommend to patients the use of cannabis to treat illnesses, although the federal government has maintained that any use of marijuana - medicinal or otherwise - is illegal. The federal courts have ruled that physicians like Jensen cannot be prosecuted for making such recommendations.

Jensen said she regularly writes prescriptions recommending the use of cannabis for patients -particularly those suffering pain and nausea from chronic illnesses, such as AIDS, cancer, glaucoma and arthritis.
She has also worked with one family of a 15-year-old - whose family had tried every drug available to help their son, who by age 13 had become a problem student diagnosed as suffering from ADHD. Under Jensen's supervision, he began cannabis treatment, settling it on in food and candy form, and he has since found equilibrium and regularly attends school.

But not everyone is so high on the idea of pot for students with neurological illnesses. Subcommittee Chairman Mark Souder, R-Ind., who invited Jensen to testify after reading about her ideas in the newspaper, was hardly convinced by her testimony.

"I do believe that Dr. Jensen really wants to help her patients, but I think she is deeply misguided when she recommends cannabis to teenagers with attention deficit disorder or hyperactivity," he told Foxnews.com. "There is no serious scientific basis for using marijuana to treat those conditions, and Dr. Jensen didn't even try to present one."

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Dr. Tom O'Connell, a retired chest surgeon who now works with patients at a Bay Area clinic for patients seeking medical marijuana recommendations, is working on it. He said cannabis not only helps pain, but also can treat psychological disorders. He is currently conducting a study of hundreds of his patients, whom he said he believes have been *self-medicating* with pot and other drugs for years, and he hopes to publish a paper on the subject soon.

"My work with cannabis patients is certainly not definitive at this point, but it strongly suggests that the precepts upon which cannabis prohibition have been based are completely spurious," O'Connell said. Worse yet, he added, the prohibition has successfully kept certain adolescents away from pot who now turn to tobacco and alcohol instead.

Jensen, who said she believes Souder invited her to testify to "humiliate me and incriminate me in some way," suggested that a growing body of evidence is being developed to back medical cannabis chiefly for chronic pain and nausea. She said it is difficult, however, for advocates like herself to get the funding and permission to conduct government-recognized tests on ADD/ADHD patients.

"Unfortunately, no pharmaceutical companies are motivated to spend the money on research, and the United States government has a monopoly on the available cannabis and research permits," she told Congress. Studies done on behalf of the government, including the 1999 Institute of Medicine's "Marijuana and Medicine: Assessing the Science Base," found that cannabis delivers effective THC and other cannabinoids that serve as pain relief and nausea-control agents. But these same studies warn against the dangers of smoking cannabis and suggest other FDA-approved drugs are preferable.

"We know all too well the dangerous health risks that accompany (smoking)," said Rep. Elijah Cummings, D-Md., ranking member on the subcommittee, who like Souder, was not impressed by Jensen's arguments. "It flies in the face of responsible medicine to advocate a drug that had been known to have over 300 carcinogens and has proven to be as damaging to the lungs as cigarette smoking," added Jennifer Devallance, spokeswoman for the White House Office of Drug Control Policy.

The government points to Food and Drug Administration-approved Marinol, a THC-derived pill that acts as a stand-in for marijuana. But many critics say there are nasty side effects, and it's too expensive for the average patient.

On the other hand, Jensen and others say cannabinoids can be made into candy form, baked into food or boiled into tea. They say that despite the FDA blessing, **giving kids amphetamines like Ritalin for ADD and other behavioral disorders might be more dangerous**.

"Ritalin is an amphetamine - we have all of these youngsters running around on speed," said Keith Stroup, spokesman for the National Organization for the Reform of Marijuana Laws.

"Although it flies in the face of conventional wisdom, it's nevertheless true that cannabis is far safer and more effective than the prescription agents currently advocated for treatment of ADD-ADHD," O'Connell said.

Stroup said if Souder's intention was to harangue Jensen, he was unsuccessful in the face of her solid and articulate testimony on April 1."It was a good day for her, and a good day for medical marijuana in Congress," he said.

Nick Coleman, a subcommittee spokesman, said Souder doesn't "try to humiliate people.

"But to promote medical cannabis for teenagers with ADD... he does not feel that is a sound and scientific medical practice," Coleman said. While the issue of treating adolescents with medical marijuana is fairly new, the idea of using pot to treat chronically and terminally ill patients is not. Nine states currently have laws allowing such practices. A number of lawmakers on both sides of the aisle have added that they want the states to decide for themselves whether to pursue medical marijuana laws.

Among those lawmakers are Reps. Ron Paul, R-Texas, a physician; Dana Rohrabacher, R-Calif.; and Barney Frank, D-Mass. "(Rep. Paul) believes there are some legitimate applications," like for pain and nausea, said spokesman Jeff Deist. "But the real issue is that states should decide for themselves."

Kelley Beaucar Vlahos
www.foxnews.com 04/20/2004

Update Wednesday 19 January 2005 13:28, published Wednesday 19 January 2005 13:17

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Cannabis as a medical treatment for attention deficit disorder

After being tossed out by two high schools I took it upon myself to finish my high school degree online, which during this time I also became a regular cannabis user. Smoking cannabis helped me focus entirely on my work and even other aspects of my life where otherwise I would become distracted and lose concentration. The list of ailments cannabis has helped me overcome is virtually unfathomable and no other pharmaceutical proved itself to be even remotely as effective, not to mention the countless side-effects I endured consequently just to medicate myself legally.

Answer to this message

22 09 2008

Cannabis as a medical treatment for attention deficit disorder

I too have Adhd and am a frequent user of marijuana....i started smoking at 15 my freshman year and now 2 years later at 17 have graduated high school a year early ... My whole life i had to deal with the problems and side effects that come along with standard stimulant treament and after smoking marijuana i was able to focus just as good was happier and did not have the come downs of these so called safe drugs ... anyone who thinks marijuana doesn't help adhd either don't have adhd or have never smoked

Answer to this message

5 09 2008 by Auston Arellano

Cannabis as a medical treatment for attention deficit disorder

this sort of things pisses me right off,people dont like pot. so the stupid people say it cant be the cure but i believe it is. i have a brother and another sister all with add and adhd. out of all of us i am the one who smokes pot, and i say to you it works, i failed all throught school. i could never concentrate, as soon as i was out of high school i began smokeing pot and i saw so many changes things actually felt real i could focus and think through things. recently i went back to classes to get my ged, and much to my suprise i understand everything after being told how its down what never stuck before in school works for me now, i dont think this is coincidental. weed is the cure

Answer to this message

4 06 2008 by John

Cannabis as a medical treatment for attention deficit disorder

I am really happy to read your words. I am ADD two and self medicate since 14 years, diagnose since 6 months. Cannabis saved my life. I have been able to graduate a master degree. Since january I am involved in IACM (http://www.acmed.org). I am pushing up to create an interest group in the IACM .... so I recommand, if you want to involve yourself in the cause, to join us (me and another lux patient) .... The more we will be, the more we will be able to make things moving :-) Also I recommand evrerybody who would be interested in ADD/ADHD and cannabis to join IACM, and presenting as interest in ADD/ADHD. You will be welcome :-)I will be reachable by the IACM.
Best to all and peace.
Ludo

Answer to this message

12 07 2008 by Ludozz

Cannabis as a medical treatment for attention deficit disorder

Same here. I am not even a pot-crusader myself. I just try and apprehend issues with an open mind. And I also feel like I've been self-medicating for my vicious ADD for a long time. Weed seems to work... Now, a couple of things I have noticed: Don't smoke weed, but blend it in a juice, or a tea, or a cake or whatever you want, but ingest it. Here's why, I think:

Smoking weed (which usually means 2/3 joints a day to feel the effect long enough) will knock you out, and in time depress you (does that to me anyway). Or it will simply make you unable to continue growing in your life, go places, be tight and all. After a while all those lost opportunities because you were too spaced out to seize them are gonna catch up with you, and you won't be much happier for it.

Smoking it will make for a strong "spike" in effect, but a quick disappearance. Like first you're out of it, and then the buzz is gone, to caricature. Better to ingest some, the release is a lot smoother, softer and lasts all through the day.

Finally I believe that there are a fair few cancerigens produced by the combustion of weed, when you inhale it in your lungs, it is detrimental to your respiratory system. Seems intuitively to be much healthier to ingest it for that too.

I guess we all have different reactions to it, but here is, for what it's worth, what my investigations have yielded...

Also, multi-vitamin (including lots of magnesium which is too often missing in our diet), and vigorous exercise work best for me. I think it's important to treat it by using all means available, at all levels at the same time. It's a whole, holistic issue...

Answer to this message

23 07 2008 by Stephane

Cannabis as a medical treatment for attention deficit disorder

After smoking for most of my collegiate career I have to agree that it has helped me. I can also say that anything is easily abused whether it is the conventional treatments for add or other things.... I am more curious about ingesting this wonderful plant. I read the last post and agree with the affect of smoking except when I smoke there is probably 1-3 hours of "highness" and then I actually feel great and get alot done and am more social... basically all aspects of my life seem to come together at once. With the conventional treatments at least in the stimulant form I definitely got things done but felt the come down and felt like I needed them. I also noticed my social life suffered and found it hard to express myself. Now I am currently trying out strattera (however you spell it) and I get almost all of the side effects (I am still in the 1st month of the drug) I get nauseous, depressed, mood swings, etc. most of which have been treated by smoking. I could not say which is most effective the smoking or the prescription drug, becuase in my state I cannot legally have a prescription for marijuana. However, If I knew a reliable way to ingest I think it would help alot. I have tried brownies but I try to eat healthy and wouldnt consider eating a bunch of butter and sugar everyday. Also the dosage was way out of wack and I was "messed up" for a couple days. Hence I would like to know what im doing before I do that again...
Any help would be appreciated.

Answer to this message

14 09 2008 by duckmn56

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Winter/Spring 2005
O'Shaughnessy's
Journal of the California Cannabis Research Medical Group

Cannabis Use in Adolescence: Self-Medication for Anxiety

Data from the author's practice show that many Californians use cannabis to treat emotional conditions. Government studies obscure this reality and some reformers seem reluctant to acknowledge it.

By Tom O'Connell, M.D.

In response to TV news footage of able-bodied young men buying cannabis in Oakland, city officials voted in 2004 to limit the number of dispensaries. The politicans were exploiting (and re-enforcing) a misconception that Californ-ia's medical marijuana law applies only to those with serious physical illnesses.

Many of my own patients are seemingly able-bodied young men. Their histories reveal problems that are indeed serious (impaired functionality at school and/or work, use of addictive drugs) and that are treated effectively with cannabis.

I began screening Californians seeking a physician's approval to use cannabis in November 2001. Although the reference in Proposition 215 to a doctor's "recommendation" of cannabis implied that some applicants would be seeking to use it medicinally for the first time, the applicants I encountered, almost invariably, had been using it in non-addictive, stable patterns. Use of cannabis typically preceded —often by years— the onset of whatever physical symptoms they were citing to justify their use.

These patients were among those identified

O'Shaughnessy's

O'Shaughnessy's is the journal of the CCRMG/SCC. Our primary goals are the same as the stated goals of any reputable scientific publication: to bring out findings that are accurate, duplicable, and useful to the community at large. But in order to do this, we have to pursue parallel goals such as removing the impediments to clinical research created by Prohibition, and educating our colleagues, co-workers and patients as we educate ourselves about the medical uses of cannabis.

SCC

The Society of Cannabis Clinicians (SCC) was formed in the Autumn of 2004 by the member physicians of CCRMG to aid in the promulgation of voluntary standards for clinicians engaged in the recommendation and approval of cannabis under California law (HSC §11362.5).

As the collaborative effort continues to move closer to issueing guidelines, this site serves as a public venue for airing and discussing these

as criminals and deviants for decades by government propaganda. The idea that they were criminals who belonged in jail or addicts requiring "treatment" simply didn't make sense.
Never in history has such a large collection of admitted illegal drug users been so willing to present themselves for unbiased examination.

guidelines.

Visit the SCC Site for more information.

Developing Research Tools
Although basic demographic data could be obtained by questionnaire, I developed a detailed interview to examine pertinent areas of personal history. Systematic exploration of prior drug use revealed that nearly all had tried alcohol and tobacco aggressively about the same time they tried pot. Many had then tried a variety of other drugs.
My patients' drug-initiation patterns suggested they had been addressing similar needs. Herein, I realized, might be a key to defining the "medical" use of cannabis and perhaps to better understand its appeal as a "recreational" agent. I adapted my interview accordingly, as I learned more.
The discovery that most were using cannabis to treat insomnia suggested self-medication of anxiety or depression —so I expanded that portion of the interview dealing with psychotropic symptoms. Upon learning that many of the younger males had already been labeled with ADD, I sharpened my focus on school and family histories.
The finding that a large percentage had been raised by single mothers and that many biological fathers of intact families were either heavy drinkers or preoccupied with work suggested a common etiology for the symptoms exhibited in adolescence.
By June 2002 I had a standardized list of questions on a form that doubled as a cue sheet and a place to record answers efficiently and inobtrusively.

Study Population
A total of 3,815 patient encounters between mid-November 2001 and December 1, 2004 have been recorded. Of those, 2,799 were evaluated with the

structured interview. An earlier group of 1,016 had been screened with a more traditional history and physical. Approximately two thirds (1,850) of the 2,799 structured interviews were first-timers; the rest were 'renewals' of patients seen at least once previously. The applicants were seen at several different venues in the Bay Area and many had traveled from other parts of the state— sometimes hundreds of miles Virtually all of my original patients had been made aware of my availability through word of mouth spread through the loose network of buyers' clubs, which had —over the first five years of Prop 215— become concentrated in the few Bay Area counties where they were tolerated by local governments. Presumably they knew that I was pro-cannabis, but not that I looked favorably on its use as a treatment for depression and anxiety.
This article relies on detailed data from 790 patients and demographic data from an additional 364 patients.

Age
Only 3.6% (34/937) were older than 60 when first seen.
5.5% were born before 1946.
16.4% born 1946 - 1955
15.4% born 1956 - 1965
28.0% born 1966 - 1975
35.6% born 1976 - 1985

Those who initiated cannabis use in the 1960s are now in their fifties and sixties. Most have been using cannabis on a regular basis for decades, others have resumed after periods of abstinence. The sharp cut-off in the upper age limit of this population is evidence that an illegal mass market for "marijuana" really didn't begin until large numbers of vulnerable adolescents were exposed to it.

Gender
Of 1118 applicants, 236, or 21.1% were female, a 4:1 ratio which has obtained throughout the three years of the study. The same 4:1 ratio of males to females seems to apply to all racial groups.

Race/Ethnicity
Applicants were assigned to four rather arbitrary categories on the basis of race. When there was doubt about which category was most appropriate, they were asked their preference. The only observed areas of significant racial differences were in drug initiation rates. Although the rates at which Black cannabis smokers try illegal drugs other than cannabis are considerably higher than the those reported in annual national surveys, they are considerably lower than among White pot smokers— especially for psychedelics, methamphetamine and heroin (see table at top left, next page).

Patterns of Use
Patients report that in terms of potency (although not variety), the cannabis found "on the street" in Northern California is comparable to that available in clubs.
Although the vast majority were experienced, chronic users, their knowledge of cannabis lore varied widely and seemed mostly to reflect individual differences in curiosity. Some were very knowledgeable about strains and delivery systems, others extremely naive. Very few were using edibles on a regular basis— many had either experienced or heard about the extended cognitive effects that can follow ingestion of innocuous appearing baked goods, and —although not clear on the reasons— preferred to avoid them.
Overwhelmingly, the mode of ingestion favored by applicants was smoking. Knowledge of vaporizers is beginning to spread, thanks to the cannabis clubs that sell them. Younger patients seem more inclined to use them on a regular basis. Some older users express resistance — the best vaporizers are expensive and old habits hard to change. Several complained that taste and aroma were lacking.
Late afternoon and evening are the favored times to use cannabis. Early morning use is favored by those with ADD type symptoms and is discussed more fully under that heading. Almost all patients have fairly consistent

schedules for their use of cannabis; it is generally solitary and private unless trusted friends are around. Most people did not tempt fate by smoking at or near work.
Consumption, measured in ounces per week, varied from as little as 1/16 to well over an ounce, with 70% reporting they use between 1/8 and1/4 ounce. People smoking 1/2 ounce or more were more apt to either grow it themselvesor have access to a friend who did.
My impression is that the extreme variations in amounts consumed are more a reflection of different sensitivities to cannabis than to any greater desire to get "stoned." In fact, the impression one gets from discussing cognitive effects in general is that almost all find excessive effects undesirable and try hard to avoid them (which is the main reason inhalation is favored over oral ingestion).

Alcohol & Tobacco Use
The most obvious relationship between alcohol, tobacco, and cannabis is that nearly all those who try cannabis have either tried the others or will soon do so. That linkage —first noted in the mid-1970s1— was amply confirmed by the present study: 100% of applicants had tried cannabis by attempting to get "high," usually as adolescents (about 30% either failed on their first attempt or weren't sure). 99.3% had also tried alcohol by getting drunk (many were also monumentally sick) and 93.7% had tried tobacco by inhaling at least one cigarette.

Few are teetotalers, but nearly all who still drink do so moderately.

Repeat use of both alcohol and tobacco tended to be aggressive. More than half had binged in high school or as young adults; 35% had experienced alcohol black-outs; and 12.5% had received DUI citations. Yet essentially all who have continued to use cannabis on a regular basis subsequently moderated their alcohol consumption. Few are teetotalers, but nearly all who still drink

do so moderately. Most have reduced alcohol consumption to 20% of their peak levels —or less.
Cannabis also has enabled patients to reduce tobacco use. Although 68.1% of cannabis applicants became daily cigarette smokers for a while, over half (53%) of the smokers have since been able to quit and almost all the rest are trying. Even inveterate tobacco smokers (those unable to remain abstinent) uniformly relate their cigarette consumption to both stress and access to cannabis: when the former is high and the latter is low, they tend to smoke a lot more tobacco.
I can recall only two applicants who said they enjoyed smoking cigarettes and had no intention of quitting.

Initiation of Other Drugs
An individual's first use of a drug is important for the obvious reason that drugs never tried never become problems. However, mere trial of an agent does not signal that repeat use will follow or what its pattern might be if it does. How chronic use of one agent might ultimately affect use of others is largely ignored by conventional research.
While children as young as nine occasionally initiate drugs, the greatest incidence is from 12 on.2 Since most people have tried all the drugs they will ever use by age 25, adolescence and young adulthood are clearly important areas for any drug policy to focus on. At first glance, the high initiation rates for other drugs observed in this population (table at top of next page) would seem to support the hypothesis that cannabis is a "gateway" to use of other drugs.
A more detailed evaluation discloses that relatively few episodes of problem use or "addiction" ensued. Those whose use became problematic were generally able to solve their problems without professional help. Discussing those issues with applicants left a strong impression that continued use of cannabis had played a significant role in helping them control not only alcohol and tobacco, but illegal drugs as well. Their aggressive trials of psyche-delics

can be seen as a manifestation of the same curiosity exhibited for other agents and presumably impelled by the same symptoms which had led them to try alcohol, tobacco and cannabis in the first place. The response of many to being questioned about peyote and mescaline was that they would have tried them had they been able to find them.
The fact that white cannabis users tried psychedelics at more than double the rate of blacks is startling and remains unexplained. Availability in their respective communities is probably a factor.

Paternal Influences
In attempting to determine the origin of the symptoms motivating this population's aggressive adolescent drug sampling, the most obvious place to start was family background. A common element was the absence of their biological fathers from their early lives —either physically, through early death or divorce, or emotionally, through a variety of other mechanisms listed below

Paternal Factors Associated With Adolescent Use of Cannabis

1. Early Death (before age 6)
2. Early Divorce
3. Alcoholic Father
4. Workaholic Father
5. Elderly Father (over 40 when patient born)
6. Invalid Father

The role played by insecurity and low self-esteem during applicants' school careers became increasingly transparent. One or more of the above situations obtained in nearly all patients.

School Careers
Pre-school day care, kindergarten and primary school are the first opportunities for most children to socialize outside the family. Being different for any reason — too short, too tall, unfashionable attire, unusual name, etc.— can quickly become something one is teased about.

Intrinsic shyness and sensitivity to teasing can make the school setting difficult to bear.

Applicants are now asked to rate their experiences in primary, junior high and high school as "happy," "unhappy." or "mixed." After emotional tone is registered, they are asked if they were ever "class clowns" or considered disruptive by their teachers. They are also asked if descriptions of "Attention Deficit Hyperactivity Disorder" apply to them.

ADHD and ADD are diagnostic labels increasingly applied to school children exhibiting behaviors that irritate and frustrate their teachers. The concept that the condition frequently persists throughout life ("Adult ADD") has been endorsed by the medical establishment, and increasing numbers of patients are being treated with Adderall and other long-acting amphetamines.3

Although the behaviors had long been noted among educators and pediatricians, a unifying diagnosis seems to have originated in the late '60s with Paul Wender, a child psychiatrist at the University of Utah.4, 5 Treatment of affected children with stimulants, primarily methylphenidate (Ritalin), began in the 1970s and has become both increasingly common. The ADD/ADHD diagnoses are now codified in the American Psychiatric Association's Diagnostic and Statistical Manual of Mental Disorders.

ADD has been associated from the beginning with dyslexia and several other so-called "learning disorders." Among my male patients, the diagnosis of ADD was either made or suggested for some 10-15% while they were in school. Nearly as many were diagnosed as adults, or the diagnosis was applied informally by family members or close friends.

The ADD diagnosis is associated in conventional literature with both "substance abuse" during adolescence and low self-esteem. The ratio of boys to girls diagnosed with ADD has remained at about 4:1. As the diagnosis is made more frequently in adults, it has been noted that fathers with ADD are more

apt to have sons with the condition (and vice-versa). This is a pattern one might expect in a highly competitive, male dominated society.
The idea that "self-esteem" is both important to a child's early success and strongly influenced by the biologic father is certainly not new. Single mothers, low self-esteem, and a proclivity to try multiple drugs in adolescence have all been reported as common in children diagnosed with ADD.

There is universal agreement among applicants who have been diagnosed with and/or treated for ADD that cannabis helps them achieve and retain focus.

The term "attention deficit disorder" is clearly a misnomer. These individuals are not inattentive; rather, their problem seems to be that they are so aware of other stimuli around them that they have trouble remaining focused on the chore/problem at hand. There is universal agreement among applicants who have been diagnosed with and/or treated for ADD that cannabis helps them achieve and retain focus. They also are the ones most likely to use cannabis early in the day.

Cannabis as Palliative
ADD and other psychiatric conditions are defined by the DSM without reference to the objective external standards which Anatomic and Clinical Pathology readily provide for 'somatic' (physical) diseases.6
Upon closer analysis, modern "mood" and "behavioral" disorders represent various combinations of symptoms either observed in— or reported by— those said to be afflicted. The symptoms include chronic insomnia, dysphoria, depression, anxiety, excessive anger, difficulty in focusing, agoraphobia, and morning appetite inhibition.
These symptoms abound in the chronic cannabis users I have interviewed. They had usually been present since

adolescence and predated whatever somatic symptoms the patient could cite —with varying degrees of credibility— as their reason for seeking an application.

Prop 215, the state initiative that legalized the medical use of marijuana, refers to "seriously ill patients." Why would applicants prefer to cite somatic symptoms instead of emotional ones? Several explanations can be offered:

• Many male adolescents feel that a "macho" image allows for physical injury and pain, but not for emotional impairment.

• Medical marijuana advocates, in seeking to maximize public support for their cause, often invoke "the dying."

• Law-enforcement opponents of medical marijuana, starting with former state attorney general Dan Lungren, have sought to trivialize mood disorders and assert that they are not properly treated by cannabis. Former Drug Czar Barry McCaffrey, in his first public response to California's new law, ridiculed the inclusion of chronic insomnia on a list of conditions treatable by cannabis .

• There is general agreement by all but the most doctrinaire opponents of medical use of cannabis that it effectively palliates a wide variety of symptoms produced by an even wider variety of named diseases. The most common symptoms are chronic pain both of neuritic and musculo-skeletal origin.

The effectiveness of cannabis in treating two "functional" disorders, migraine and asthma — which are classically exacerbated by but not thought to be caused by emotions— was well established before the Marijuana Tax Act of 1937. Cannabis also helps control chronic diarrhea produced by Crohn's Disease, Ulcerative Colitis, or Irritable Bowel Syndrome. Its effectiveness in controlling the tenesmus and cramping of the latter condition also suggests a spasmolytic mechanism is involved.

In a context where most of the somatic conditions were clearly additive in that the applicants had aleady been using cannabis to manage emotional

symptoms, the expenditure of scarce assets to "confirm" what amounted to a somatic excuse for their pot use did not seem reasonable; particularly when the underlying psychotropic reasons for its use were deemed adequate and a detailed history had shown they fit the "profile." There is also a relatively small subset in whom more sporadic and casual use of pot had become far more regular after the patient developed a new somatic condtion.

The Gateway Hypothesis

Drugs are initiated in sequence. Prior to the late 1960s, alcohol and tobacco were primary agents tried by adolescents. When researchers began studying the phenomenon of youthful cannabis initiation they reported that nearly all their subjects had already tried both alcohol and tobacco— and that many had subsequently tried several other agents. Their assumption that cannabis was a "gateway" from legal to illegal drugs became the prevailing explanation.7

The presumption that all drug use is both hedonistic and harmful added conviction to that interpretation. Data showing that most heroin addicts had used cannabis before heroin bolstered the gateway theory, and it seems to have gone unchallenged for 30 years even though it never met a basic theoretical test of "causality."

Evidence that cannabis is capable of benignly and effectively palliating the psychotropic symptom complexes so often encountered in juveniles and young adults was clearly beyond the scope of any research funded— or even permitted— by NIDA. That such symptoms tend to persist into mid-life for many who suffer from them is now endorsed in psychiatric literature and has spurred development of a host of pharmaceuticals intended to treat them. Yet most of applicants for whom these pharmaceuticals were prescribed report that cannabis provides more effective and durable relief.

A little-noticed 2002 paper by Morral et al demonstrated that a theoretical "common factor" could provide a better

explanation than "gateway" for the initiation patterns observed.8 My data suggest that the common factor is adolescent angst.

The previously unrecognized role of cannabis as effective self-medication for symptoms experienced by adolescents also explains why so many adults have continued to use it despite potential social and legal penalties.

Summary

Proposition 215 encouraged many individuals who had been considered "recreational" users of cannabis to apply for "medical" status. Interviews placing their cannabis use in broader context showed that it is frequently an alternative to the use of alcohol, tobacco, and "harder" drugs.

The federal government, by imposing a Prohibition based on biased, inadequate studies, is depriving the American people of a safe and effective medicine. Beyond that concern, the increasing enthusiasm for drug testing and punishing those who test positive for cannabis wth either criminal or social sanctions is destructive to the large — but at this writing unknown —number of Americans treating emotional symptoms with what may be, for them, the best agent available.

References

1. Kandel, DB, Editor. Examining the Gateway Hypothesis; Stages and Pathways of Drug Involvement. Cambridge University Press 2002.

2. Guo, JieHill, Karl G.Hawkins, J. David Catalano, Richard F. Abbott, Robert D. Journall of the American Academy of Child and Adolescent Psychiatry, July, 2002

3. Pary R, Lewis S, Matuschka PR, Rudzinskiy P, Safi M, Lippmann S. Attention deficit disorder in adults. Ann Clin Psychiatry. 2002 Jun;14(2):105-11

4. Wender, PH Minimal Brain Dysfunction in Children. Wiley New York1971.

5.Wender, PH ADHD; Attention-Deficit Disorder in Children and Adults Oxford, 2000 University Press.

6. Kirk, SA & Kutchins, H. The Selling of DSM; the rhetoric of science in

Psychiatry. Aldyne De Gruyter New York 1992
7. Kandel, DB, Logan, JA. Patterns of Drug Use from Adolescence to Young Adulthood: I. Periods of Risk for Initiation, Continued Use, and Discontinuation AJPH 74 (7) 660
8. Morral AR, McCaffrey DF, Paddock SM. Reassessing the marijuana Gateway Effect Addiction. 2002, 97 1499

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Marijuana and ADD

by Kort E Patterson

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Therapeutic uses of Medical Marijuana in the treatment of Attention Deficit Disorder

Note: The following research was part of a petition filed in 1999 to add Attention Deficit Disorder to the Oregon Medical Marijuana Act. The Oregon Health Division continues to refuse to add mental conditions such as ADD to the list of OMMA "covered conditions". The Health Division claims that medical marjuana isn't effective for mental conditions even though the primary justification for marijuana prohibition is its alleged psycho-active effectiveness.

At first glance it might seem counter-intuitive to use a medication that has a public perception of decreasing attention to treat a condition whose primary symptom is a deficit of attention. But just as taking stimulants often calms those with hyperactivity, medical marijuana improves the ability to concentrate in some types of ADD.

Categorizing The Condition

Attention Deficit Disorder (ADD) is a very broad category of conditions that share some symptoms but appear to result from different underlying causes. Most seem to involve, at least in part, imbalances in neural transmitter levels and functions. Some experts in the field expect that the broad category of ADD will be refined in the future, with many conditions that are now diagnosed as ADD being recognized as separate disorders.

The particular type of ADD under consideration for treatment with medical marijuana might better be termed "Racing Brain Syndrome" (RBS). A useful analogy for this mental condition is that of a centrifugal pump that is being over-driven. As the pump speed increases, cavitation sets in and the pump's output decreases. The faster the pump is driven the greater the cavitation until a point is reached

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where large amounts of energy are being input but nothing is being output. Without medication there is a sensation that thoughts flash through the brain too fast to "think" them. Medical marijuana slows the brain down sufficiently to achieve impressive improvements in functionality.

This syndrome probably only afflicts a small minority of all those diagnosed with ADD. The condition doesn't respond to the standard ADD medications, indicating that it results from different underlying processes than other forms of ADD. Individuals with types of ADD that do respond to the standard ADD medications also tend to have a far different reaction to medical marijuana than those with RBS. At this point in our limited understanding of the condition, it appears that RBS would make a good candidate to be redefined as a separate condition outside of the general diagnosis of ADD.

Treating ADD/RBS With Medical Marijuana

There is some evidence available that medical marijuana has been found to be an effective medication for some types of ADD by other researchers in the field.(1) Unfortunately, ADD encompasses such a variety of conditions that the limited amount of research in the field leaves many of the effective therapeutic mechanisms under-investigated. Considering the regulatory difficulties in researching the effects of medical marijuana, it isn't surprising that the information regarding medical marijuana and ADD is largely anecdotal(2).

Individuals with RBS tend to have a very low tolerance for most stimulants, and report even caffeine aggravates their disorder. The one exception appears to be low doses of Dextrostat. While Dextrostat does have a calming effect, it fails to address the higher level mental functions needed for complex intellectual demands. Larger doses of Dextrostat tend to produce undesirable mental and physical stimulation, greatly limiting the level of medication that can be tolerated.

Medical marijuana remains the only single medication that provides an adequate solution for RBS, and remains a necessary component in a multi-drug approach.

Dextrostat does appear to reduce the amount of medical marijuana needed by individuals with RBS to achieve a functional mental state. This

reduction probably justifies continuing with Dextrostat as a means of reducing the quantity of medical marijuana that must consumed, as well as allow those with RBS to gain the maximum benefit possible within the quantity limitations of the OMMA.

The green leaves of certain strains of medical marijuana appear to provide the best therapeutic effects for RBS. Experiments with Marinol seem to indicate that THC is involved, but is not the primary therapeutic agent. The therapeutic agent(s) most useful in treating RBS appear to be present in relatively low concentrations in medical marijuana. As such those with this condition must consume a larger quantity of medical marijuana in order to ingest a sufficient dosage of the target agent(s). This would explain why dried low-THC green leaves appear to be the most effective treatment. The patient can consume enough of this low-THC marijuana to acquire the levels of the needed active agent (s) necessary to treat the condition without in the process consuming sufficient THC to become intoxicated.

Underlying Cause of RBS

It has long been suspected that RBS involved a deficit of one or more neural transmitters. It was observed as long ago as the 1970's that high levels of adrenaline had a residual therapeutic effect in those with RBS. The effect was first noted in those engaged in such activities as skydiving. Individuals with RBS reported that their mental functions were improved in the days following skydiving. It was first assumed that adrenaline stimulated the production of all neural transmitters - including those that were in deficit. It's now thought that while adrenaline initially acts as a stimulant of neural transmitter production, it has a secondary effect of depleting neural transmitters. The limited effectiveness of Dextrostat, as well as additional information about the secondary effects of adrenaline, suggests the possibility that at least part of the underlying cause of RBS may also be a surplus of one or more neural transmitters.

The partial solution offered by Dextrostat also suggests that at least some part of the condition results from those neural transmitters and/or hormones that are influenced by both Dextrostat and medical marijuana. The failure of Dextrostat to provide a complete solution suggests two possible alternatives: (1) that the effects of Dextrostat and medical marijuana are

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additive - with both influencing the same neural transmitters and/or hormones, and together delivering the required level of therapeutic effect; or (2) that the condition is the result of multiple imbalances, some of which are unaffected by Dextrostat, but all of which appear to be affected by medical marijuana.

Potential Beneficial Therapeutic Effects

The research that has been done on the therapeutic effects of medical marijuana on other conditions provides a number of potential mechanisms that may be involved in RBS. The following are documented effects of medical marijuana that appear to have some potential for involvement.

Perhaps the most obvious possibility is suggested by the fact that both Dextrostat and medical marijuana influence the release and/or functions of serotonin(3)(4). Since both Dextrostat and medical marijuana appear to decrease the apparent availability and effectiveness of serotonin, it would appear possible that a surplus of serotonin is involved in some way.

There are over 60 cannabinoids and cannabidiols present in medical marijuana. The effect of most of these substances is at present largely unknown.(5)

The discovery of a previously unknown system of cannabinoid neural transmitters is profound. (6) The different cannabinoid receptor types found in the body appear to play different roles in normal human physiology.(5) An endogenous cannabinoid, arachidonylethanolamide, named anandamide, has been found in the human brain. This ligand inhibits cyclic AMP in its target cells, which are widespread throughout the brain, but demonstrate a predilection for areas involved with nociception. The exact physiological role of anandamide is unclear, but preliminary tests of its behavioral effects reveal actions similar to those of THC.(7)

Cannabinoid receptors appear to be very dense in the globus pallidus, substantia nigra pars reticulata (SNr), the molecular layers of the cerebellum and hippocampal dentate gyrus, the cerebral cortex, other parts of the hippocampal formation, and striatum - with the highest density being in the SNr. The Neocortex has moderate receptor density, with peaks in superficial and deep layers. Very low and

homogeneous density was found in the thalamus and most of the brainstem, including all of the monoamine containing cell groups, reticular formation, primary sensory, visceromotor and cranial motor nuclei, and the area postrema. The hypothalamus, basal amygdala, central gray, nucleus of the solitary tract, and laminae I-III and X of the spinal cord showed slightly higher but still sparse receptor density.(5)

While there are cannabinoid receptors in the ventromedial striatum and basal ganglia, which are areas associated with dopamine production, no cannabinoid receptors have been found in dopamine-producing neurons. According to the congressional Office of Technology Assessment, research over the last 10 years has proved that marijuana has no effect on dopamine-related brain systems.(6) However, cannabidiol has been shown to exert anticonvulsant and antianxiety properties, and is suspected by some to exert antidyskinetic effects through modulation of striatal dopaminergic activity.(3)

It's been suggested that the cannabinoid receptors in the human brain play a role in the limbic system, which in turn plays a central role in the mechanisms which govern behavior and emotions. The limbic system coordinates activities between the visceral base-brain and the rest of the nervous system. Cannabis acts on memory by way of the receptors in the limbic system's hippocampus, which "gates" information during memory consolidation.(6)

In addition, some effects of cannabinoids appear to be independent of cannabinoid receptors. The variety of mechanisms through which cannabinoids can influence human physiology underlies the variety of potential therapeutic uses for medical marijuana.(8)

When the effects of cannabis on a "normal" brain are tracked on an electroencephalogram (EEG), there is an initial speeding up of brain wave activity and a reactive slowing as the drug effects wear off. The higher the dosage, the more intense the effects and longer the experience. There is an increase in mean-square alpha energy levels and a slight slowing of alpha frequency.(5) There is also an increase of beta waves reflecting increased cognitive activity. The distortion of time resulting from the "speeding up of thoughts" causes a subjective perception that there is a slowing of time.(9)

As the cannabis effects wear off, stimulation gives way to sedation. The cognitive activity of the beta state gives way to alpha and theta frequencies. Theta waves are commonly associated with visual imagery. These images interact with thinking and disrupt the train of thought. Thinking can be distracted by these intrusions, with thought contents being modified to some extent depending on dose, expectations, setting, and personality.(9)

Cannabis decreases emotional reactivity and intensity of affect while increasing introspection as evidenced by the slowing of the EEG after initial stimulation. Obsessive and pressured thinking is replaced by introspective free associations. Emotional reactivity is moderated and worries become less pressing.(10)

Cannabis causes a general increase in cerebral blood flow (CBF). This increase in blood circulation is due to decreased peripheral resistance, which is in turn due to the dilation of the capillaries in the cerebral cortex. Changes in CBF affect the mental processes of the brain, with increases stimulating cognition, while decreases accompany sedation.(9)

Relative Safety of Medical Marijuana

"Marijuana is the safest therapeutically active substance known to man... safer than many foods we commonly consume."
DEA Judge Francis L. Young, Sept. 6, 1988

"After carefully monitoring the literature for more than two decades, we have concluded that the only well-confirmed deleterious physical effect of marihuana is harm to the pulmonary system."
Grinspoon M.D., James B. Bakalar,

Medical Marijuana has been in use for thousands of years, and in spite of substantial efforts to find adverse effects, it remains the safest medication available for RBS. There has never been a single known case of lethal overdose. The ratio of lethal to effective dose for medical marijuana is estimated to be as 40,000 to 1. By comparison, the ratio is 3-50 to 1 for secobarbital and 4-10 to 1 for alcohol.(11)

During the 1890s the Indian Hemp Drugs Commission interviewed some eight hundred people and produced a report of more than 3000 pages. The report concluded that "there was no evidence that moderate use of cannabis drugs

produced any disease or mental or moral damage, or that it tended to lead to excess any more than the moderate use of whiskey."(12)

The Mayor's Committee on Marihuana examined chronic users in New York City who had averaged seven marihuana cigarettes a day for eight years and "showed no mental or physical decline."(13) Several later controlled studies of chronic heavy use failed to establish any pharmacologically induced harm.(14) A subsequent government sponsored review of cannabis conducted by the Institute of Medicine, a branch of the National Academy of Sciences, also found little evidence of its alleged harmfulness.(15) Several studies in the United States found that fairly heavy marihuana use had no effects on learning, perception, or motivation over periods as long as a year.(16)

Studies of very heavy smokers in Jamaica, Costa Rica, and Greece "found no evidence of intellectual or neurological damage, no changes in personality, and no loss of the will to work or participate in society."(17) The Costa Rican study showed no difference between heavy users (seven or more marihuana cigarettes a day) and lighter users (six or fewer cigarettes a day).(18) In addition, none of the studies involving prolonged and heavy use of medical marijuana have shown any effects on mental abilities suggestive of impairment of brain or cerebral function and cognition.(2)

The inhalation of the combustion products of burning plant material is the cause of the only well-confirmed deleterious physical effects of medical marijuana. These adverse effects can be eliminated by using one of the non-combustion means of ingesting the mediation. Marijuana can be eaten in foods or inhaled using a vaporizer. The therapeutic agents in medical marijuana vaporize at around 190 degrees centigrade, while it takes the heat of combustion of around 560 degrees centigrade to generate the harmful components of marijuana smoke. A vaporizer heats the medical marijuana to the point where the therapeutic agents are released and can be inhaled, without getting the plant material hot enough to burn.(19)

Cannabis And Blood Pressure Research Proposal

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1. Possible Therapeutic Cannabis Applications for Psychiatric Disorders, Tod H. Mikuriya, M.D.

2. Marihuana, The Forbidden Medicine, Lester Grinspoon M.D., James B. Bakalar, Yale University Press, 1997

3. MARIJUANA AND TOURETTE'S SYNDROME, Journal of Clinical Psychopharmacology, Vol. 8/No. 6, Dec 1988

4. CANNABINOIDS BLOCK RELEASE OF SEROTONIN FROM PLATELETS INDUCED BY PLASMA FROM MIGRAINE PATIENTS, Int J Clin Pharm. Res V (4) 243-246 (1985), Volfe Z., Dvilansky A., Nathan I. Blood Research, Faculty of Health Sciences, Soroka Medical Center, Ben-Gurion University of the Negev, P.O. Box 151, Beer-Sheva 84101, Israel.

5. Nelson, P. L. (1993). A critical review of the research literature concerning some biological and psychological effects of cannabis. In Advisory Committee on Illicit Drugs (Eds.), Cannabis and the law in Queensland: A discussion paper (pp. 113-152). Brisbane: Criminal Justice Commission of Queensland.

6. Marijuana And the Brain, by John Gettman, High Times, March, 1995

7. Cannabis for Migraine Treatment: The Once and Future Prescription?: An Historical and Scientific Review; Ethan B. Russo, M.D.

8. Marijuana and Medicine, Assessing the Science Base, Janet E. Joy, Stanley J. Watson, Jr., and John A. Benson, Jr., Editors Division of Neuroscience and Behavioral Health, INSTITUTE OF MEDICINE

9. Marijuana Medical Handbook, by Tod Mikuriya, M.D.

10. Medicinal Uses of Cannabis, Tod H. Mikuriya, M.D. (c)1998

11. Marihuana as Medicine: A Plea for Reconsideration; Lester Grinspoon M.D., James B. Bakalar; Journal of the American Medical Association (JAMA); June 1995

12. Report of the Indian Hemp Drugs Commission, 1893-1894, 7 vols. (Simla: Government Central Printing Office, 1894); D. Solomon, ed., The Marihuana Papers

(Indianapolis: Bobbs-Merrill, 1966).

13. Mayor's Committee on Marihuana, The Marihuana Problem in the City of New York (Lancaster, Pa.: Jacques Cattell, 1944).

14. M. H. Beaubrun and F Knight, "Psychiatric Assessment of Thirty Chronic Users of Cannabis and Thirty Matched Controls," American journal of Psychiatry 130 (1973): 309; M. C. Braude and S. Szara, eds., The Pharmacology of Marihuana, 2 vols. (New York: Raven, 1976); R. L. Dombush, A. M. Freedman, and M. Fink, eds., "Chronic Cannabis Use," Annals of New Yorh Academy of Sciences 282 (1976); J. S. Hochman and N. Q. Brill, "Chronic Marijuana Use and Psychosocial Adaptation," American journal of Psychiatry 130 (1973):132; Rubin and Comitas, Ganja in Jamaica.

15. Institute of Medicine, Marijuana and Health (Washington, D.C.: National Academy of Sciences, 1982).

16. C. M. Culver and F W King, "Neurophysiological Assessment of Undergraduate Marihuana and LSD Users," Archives of General Psychiatry 31 (1974): 707-711; P.J. Lessin and S. Thomas, "Assessment of the Chronic Effects of Marijuana on Motivation and Achievement: A Preliminary Report," in Pharmacology of Marihuana, ed. Braude and Szara, 2:681-684.

17. Cognition and Long-Term Use of Ganja (Cannabis), Reprint Series, 24 July 1981, Volume 213, pp. 465-466 SCIENCE, Jeffrey Schaeffer, Therese Andrysiak, and J. Thomas Ungerleider Copyright 1981 by the American Association for the Advancement of Science

18. Rubin and Comitas, Ganja in Jamaica; W E. Carter, ed., Cannabis in Costa Rica: A Study of Chronic Marihuana Use (Philadelphia: Institute for the Study of Human Issues, 1980); C. Stefariis, J. Boulougouris, and A. I-iakos, "Clinical and Psychophysiological Effects of Cannabis in Long-term Users," in Pharmacology of Marihuana, ed. Braude and Szara, 2:659-666; P Satz, J. M. Fletcher, and L. S. Sutker, "Neurophysiologic, Intellectual, and Personality Correlates of Chronic Marihuana Use in Native Costa Ricans," Annals of the New York Academy of Sciences 282 (1976): 266-306.

19. Is Marijuana The Right Medicine For You?; Bill Zimmerman Ph.D., Rick Bayer M.D., and Nancy Crumpacker M.D.; (1998): pp. 125; Keats Publishing Inc.

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USE OF MEDICINAL CANNABIS IN CALIFORNIA

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Pursuant to the enactment of Proposition 215 in November, 1996, California has an extensive population of patients legally using cannabis as medicine. All such patients must obtain authorization from a licensed physician; however, there exists no statewide patient or physician registration system. Reluctance on the part of many physicians to authorize cannabis use has resulted in the emergence of de facto specialists whose practice is mainly devoted to cannabis medicine. In the absence of a statewide database, they comprise the best source of information on the current practice of cannabis medicine in the state.

Methods: Interviews were conducted in the fall of 2006 with 21 cannabis specialists, 14 of whom also completed an e-mailed survey. The survey posed 11 questions concerning medical conditions treated, patient demographics, dosage, efficacy, adverse reactions, cannabis substitution for alcohol and other drugs, and unusual indications.

Results: The surveyed physicians reported issuing cannabis approvals to some 160,000 patients, 95 -99% of whom had prior experience with cannabis. Most common indications were chronic pain (40%-85%), depression, anxiety and related mood disorders (15%-30%), gastrointestinal disorders (~15%), headache, including migraines (~10%), arthritis (~20%), insomnia (up to 20%-30%), neurological disorders/MS (~10%), nausea (10%-17%), alcoholism and other drug dependence (<10%) et al. ADD/ADHD, PTSD and bipolar disorder were frequently mentioned by physicians treating mood disorders. Cancer and HIV/AIDS patients were relatively few; presumably, most get recommendations from their primary care physicians. Numerous unusual medical problems were reported to be alleviated by cannabis. Physicians consistently reported that cannabis helped patients reduce or discontinue intake of other drugs, including opioids, sedatives, non-steroidal anti-inflammatories, muscle relaxants, hypnotics, etc.

Conclusions: Over the past decade, some 200,000 to 350,000 Californians have received physician approval to use cannabis in treating a wide range of conditions. In many cases efficacy can be confirmed by reduced use of other medications. No deaths or major adverse events have been attributed to cannabis (although the suicide of an adolescent who was also taking an SSRI antidepressant and an atypical antipsychotic must be noted). Many of the most common uses of cannabis reported by practitioners remain to be investigated in controlled clinical studies, among them: intractable chronic pain (e.g. from arthritis and injuries), migraines, gastrointestinal diseases, depression, anxiety, insomnia, PTSD, ADHD and bipolar disorder.

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What Have California Doctors Learned About Cannabis?

Oct 23rd 2006 An Interview with Jeffrey Hergenrather, MD By FRED GARDNER

It has been **10 years** since California voters enacted Proposition 215, making it legal to grow and use cannabis, with a doctor's approval, for medical purposes. Prop 215 didn't create a record-keeping system because the authors didn't trust the government and didn't want to generate a master list of cannabis users. So, over the course of the past decade, a vast public health experiment has been conducted in California but no state agency has been tracking doctors who approve cannabis use or patients who medicate with it.

To assess the results in the absence of government-garnered data, I surveyed doctors associated with the Society of Cannabis Clinicians. The SCC was founded by Tod Mikuriya, MD, in 2000 so that doctors monitoring their patients' use of cannabis could share data for research purposes (and, alas, respond to threats from federal and state authorities). More than 20 doctors have attended SCC meetings, which are held quarterly. Philip A. Denney, MD, is the current president.

Some responses to the survey have not yet been received, but it appears that the specialists have approved cannabis use by more than 140,000 patients. "Approve," not "recommend," is the apt term, since more than 95 percent of the patients consulting specialists had been self-medicating previously.

The specialists account for approximately 40% of the letters of authorization on file with an agency that issues ID cards on behalf of cannabis dispensaries (to spare them having to confirm the validity of each customer's letter of approval). Extrapolating from this ratio, I estimate the number of Californians who have used and/or provided medicinal cannabis legally under Prop 215 to be about 350,000.

The complete survey will appear in the Fall issue of **O'Shaughnessy**'s, a **journal** I produce for the SCC.

What follows are **excerpts from the response of Jeffrey Hergenrather, MD, a former family practitioner who has been conducting cannabis consultations in Sebastopol since 1999:**

Q. How many patients will have received your approval to use cannabis through October 2006?

A. 1,430

Q. What percentage had been self-medicating with cannabis prior to consulting you?

A. 99%

Q. With what medical conditions have they presented? List top five and approximate percentage (total can exceed 100%).

Chronic pain (62%), Depression and other mental disorders (30%), Intestinal disorders (12%), Harmful dependence (10%), Migraine (9%) are the most common conditions being treated.

Q. **What results do patients report? How does cannabis appear to work in treating their symptoms?**

A cannabis specialist soon becomes aware of two remarkable facts. The range of conditions that patients are treating successfully with cannabis is extremely wide; and patients get relief with the use of cannabis that they cannot achieve with any other pharmaceuticals.

The **testimonies that I hear** on a daily basis from people with serious medical conditions **are moving and illuminating**. From many people with cancer and AIDS come reports that **cannabis has saved their lives** by giving them an appetite, the ability to keep down their medications, and mental ease. **No other drug works like cannabis** to reduce or eliminate pain without significant adverse effects. It evidently works on parts of the brain involving short-term memory and pain centers, enabling the patient to stop dwelling on pain. Cannabis helps with muscle relaxation, and it has an anti-inflammatory action. Patients with rheumatoid arthritis stabilize with fewer and less destructive flare-ups with the regular use of cannabis.

Other rheumatic diseases similarly show remissions. Spasticity cannot be treated any more quickly or efficiently than with cannabis, and, again, without significant adverse effects.

Patients who suffer from migraines can reduce or omit conventional medications as their headaches become less frequent and less severe.

About half of the patients with mood disorders find that they are adequately treated with cannabis alone while others reduce their need for other pharmaceuticals. **In my opinion, there is no better drug for the treatment of anxiety disorders, brain trauma and post concussion syndrome, ADD and ADHD, obsessive compulsive disorder, and post-traumatic stress disorder**. Patients with Crohn's disease and ulcerative colitis are stabilized, usually with comfort and weight gain, while most are able to avoid use of steroids and other potent immunomodulator drugs.

People who were formerly dependent on alcohol, opiates, amphetamines and other addictive drugs have had their lives changed when substituting with cannabis. Patients with end-stage renal disease on dialysis and those with transplanted kidneys show mental ease, comfort, and lack of significant graft-versus-host incompatibility reactions in my small series.

Diabetics report slightly lower and easier-to-control blood sugar levels, yet to be studied and explained.

Sleep patterns are typically improved, with longer and deeper sleep without any hangover or significant adverse effects.

Many **patients with multiple sclerosis report that their condition has not worsened for many years** while they have been using cannabis regularly. MS and other neurodegenerative diseases share the common benefits of reduced pain and muscle spasms, improved appetite, improved mood and fewer incontinence problems. Many patients with epilepsy are adequately treated with or without the use of other anticonvulsants.

Patients with skin conditions associated with systemic disease such as psoriasis, lupus, dermatitis herpetiformis, and eczema **all report easement and less itching when using cannabis regularly**.

Airway diseases such as asthma, sleep apnea, COPD, and chronic sinusitis deserve special mention because I encourage the use of cannabis vapor or ingested forms rather

than smoking to reduce airway irritation. Finally, most obese and morbidly obese patients respond with weight loss and improved self esteem. **I believe that cannabis and psychotherapy work well together in fostering behavioral changes.**

Q. Have you compiled demographic data or can you estimate the breakdown with respect to your patients' age, gender, race, economic status?

Gender: 62% male, 38% female. Ages range from 14 to 86 years old. The male mean age is 45.9 years with a median age of 46. The female mean age is 47.4 with a median age of 48 years. The graphs of the age and gender distribution are similar with the exception that there is a bump in the leading edge of my male patient population as compared to the females, which I account for by young men's work injuries, sports injuries, motor vehicle accidents, and problems stemming from military service, including injuries and post-traumatic stress disorder. The vast majority of patients in my practice are of Caucasian / Indo-European descent, with only about 1% African-American, 2% Native American, 1% Pacific Islanders, and 2% Asian.

Q. Have you **observed or had reports of adverse effects from cannabis?** If so, please describe.

Is there a downside to the use of cannabis? The sense of intoxication rarely lasts longer than an hour and tends to be more troubling to the novice than to the experienced user. For some people cannabis can induce dry mouth, red eyes, unsteady gait, mild in-coordination, and short-term memory loss, all of which are transient. These **effects are reportedly trivial compared to those brought on by pharmaceutical alternatives**.

Cannabis use is steadily finding acceptance in society. Still, for many it remains awkward if not totally impractical in the workplace. People whose jobs require multi-tasking such as pilots, drivers, dispatchers, switchboard operators, and many professionals find the intoxicating effects of cannabis inappropriate in the workplace, and therefore reserve their use for after work. Strains

Q. What have you learned re strains and dosage?

Cannabis is a complex, un-patentable plant with vast pharmacologic potential. Different strains contain different mixes of cannabinoids and terpenes that give them distinct qualities. Some strains energize you; others put you to sleep. **Many patients, when they find a strain that suits their needs, try to obtain it on a regular basis**. Unless they are growing their own from cuttings, however, they have to rely on growers and distributors to reproduce and make available the preferred strain from year to year.

Due to Prohibition, California growers have been denied the tools of analytical chemistry to test the cannabinoid contents of their plants. This has impeded the development of strains aimed at treating various conditions. Nevertheless, **patients continue to educate themselves about cannabis as medicine and how best to use it**. Over the years that I have specialized in cannabis therapeutics, **health benefits reported by patients have been substantiated and explained by findings from research centers around the world**.

Vaya Con Dios

The great Freddy Fender died last week -lung cancer at age 69. From his Associated

Press obit: "his career was put on hold [in 1960] when he and his bass player ended up spending almost three years in prison in Angola, LA., for marijuana possession." He was born Baldemar Huerta and took the name Fender in honor of his electric guitar when he signed with Imperial Records in 1959. He took "Freddy" because he thought it sounded good with Fender. I never stopped giving my vinyl "Best of Freddy Fender" a spin.

Look at how America treats its artists, its national treasures! Clifford Antone, founder of Austin's famous blues club, died in May of this year. He served two prison terms, according to his obit in the New York Times, "one in the 1980's for possessing marijuana and another from 2000 to 2002 for dealing more than 9,000 pounds of the drug and laundering money Mr. Antone was known for his generosity to musicians. He organized a series of benefits for victims of Hurricane Katrina and recently he helped arrange an apartment and nursing care for the 92-year-old pianist Pinetop Perkins."

Paul Armentano of NORML has analyzed a new U.S. Department of Justice report for 2004 and concludes that U.S. taxpayers are now spending more than a billion dollars annually to incarcerate citizens for pot. Armentano estimates that 33,655 state inmates and 10,785 federal inmates are locked up on marijuana charges. The DOJ report didn't provide stats for county jails.

Thousands behind bars cannot see the stars Shining o'er the land of the free They could be at home if they could grow their own Or get it from the local pharmacy.

Fred Gardner can be reached at fred@plebesite.com

Ed note: .

Please also read: **Long Term Exposure To Cannabis**

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Medical Cannabis and Brain Disorders

An AAMC Poll Summary

Jay R. Cavanaugh, PhD
November 2002

While conceding that Internet website polls are hardly scientific, they can provide first party experience about medical cannabis, its applications, and effects.

In our latest poll we looked at whether or not patients utilized adjunctive therapy with medical cannabis for a variety of brain disorders. The general public commonly believes (wrongly) that the primary use for medical cannabis is for cancer or AIDS. While useful in these disorders we've seen substantial first party evidence of cannabis use for a host of disorders including this latest reporting on brain disorders.

As a neuroprotective, anti-inflammatory, antioxidant, and mood stabilization drug, cannabis is routinely used by many patients with brain disorders. Often this use is not recognized or sanctioned by patients' physicians who remain uneducated about the use of cannabis in clinical practice. Our "flash" poll up on our AAMC website just three weeks shows that many patients find cannabis useful in the management of their condition. We hope physicians and researchers take notice.

Due to the limited number of choices available on a poll of this type (a maximum of ten) we deliberately left out a number of brain disorders for which cannabis has shown promise including epilepsy, Alzheimer's disease, Huntington's disease, and others. The largest reporting group on the poll (104 patients or 26%) were in this category described as "other".

The leading disorder on the poll was bipolar disease (81 patients or 20%). Bipolar disease is widely recognized as a psychiatric disorder whose origins have to do with the anatomy, physiology, and function of the brain. Bipolar disease can be notoriously difficult to treat and medications often involve drugs far more toxic than cannabis including Lithium, Depakote, Neurontin, and various anti-psychotics.

Second in numbers was ADHD/ADD with 53 patients (13%) reporting cannabis use for this common brain disorder usually treated with powerful stimulant drugs such as Adderol or Ritalin. Clinic evaluations from a major cannabis clinic in the Bay Area by Dr. Tom O'Connell (soon to be published) indicates that ADHD/ADD is found as a contributing factor in hundreds of patient in astonishing frequency. Much of youthful cannabis experimentation may, in fact, be related to the self medication of this disorder.

Nerve disorders such as multiple sclerosis (32 patients- 8%) and neuropathy (35 patients- 9%) came in third and fourth. Cannabis is apparently highly effective for these patients to treat the pain, tingling, and spasticity of these disorders.

Of extraordinary interest is the finding in the poll that 30 patients (7%) were using cannabis for post-traumatic stress disorder. Recent research findings that demonstrate that cannabis helps extinguish "frightful" or negative memories may explain cannabis use in this population.

Another extraordinary finding is that a number of patients (24 or 6%) use cannabis for the treatment of obsessive compulsive disorder. The etiology of OCD remains unknown but its effects can be serious including major reductions in functionality and increased stress.

While relatively unknown, cannabis can be an effective treatment for Tourette 's syndrome, considered to be a type of seizure disorder. Seventeen patients or 4% of our respondents use cannabis to control symptoms of Tourette's including vocal tic.

Only recently, research reports have begun suggesting a possible role for cannabis in the treatment of both the progression and symptoms of Parkinson's disease. Fifteen or 4% of the respondents use medical cannabis for treating their Parkinson's. The primary medication for Parkinson's is levo-dopa or dopamine mimetic drugs which often cause dyskinesthias, rigidity, and involuntary muscle movements. Cannabis attenuates these side effects while providing neuroprotective effects to the

dopaminergic cells at risk in Parkinson's.

Finally, we note an astonishing ten patients (2%) using cannabis for the treatment of autism. It is unknown how cannabis can help stabilize behavior in autistic individuals but we are now receiving credible first and second party reports of amazing results.

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USE OF MEDICINAL CANNABIS IN CALIFORNIA

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Pursuant to the enactment of Proposition 215 in November, 1996, California has an extensive population of patients legally using cannabis as medicine. All such patients must obtain authorization from a licensed physician; however, there exists no statewide patient or physician registration system. Reluctance on the part of many physicians to authorize cannabis use has resulted in the emergence of de facto specialists whose practice is mainly devoted to cannabis medicine. In the absence of a statewide database, they comprise the best source of information on the current practice of cannabis medicine in the state.

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Results: The surveyed physicians reported issuing cannabis approvals to some 160,000 patients, 95 -99% of whom had prior experience with cannabis. Most common indications were chronic pain (40%-85%), depression, anxiety and related mood disorders (15%-30%), gastrointestinal disorders (~15%), headache, including migraines (~10%), arthritis (~20%), insomnia (up to 20%-30%), neurological disorders/MS (~10%), nausea (10%-17%), alcoholism and other drug dependence (<10%) et al. ADD/ADHD, PTSD and bipolar disorder were frequently mentioned by physicians treating mood disorders. Cancer and HIV/AIDS patients were relatively few; presumably, most get recommendations from their primary care physicians. Numerous unusual medical problems were reported to be alleviated by cannabis. Physicians consistently reported that cannabis helped patients reduce or discontinue intake of other drugs, including opioids, sedatives, non-steroidal anti-inflammatories, muscle relaxants, hypnotics, etc.

Conclusions: Over the past decade, some 200,000 to 350,000 Californians have received physician approval to use cannabis in treating a wide range of conditions. In many cases efficacy can be confirmed by reduced use of other medications. No deaths or major adverse events have been attributed to cannabis (although the suicide of an adolescent who was also taking an SSRI antidepressant and an atypical antipsychotic must be noted). Many of the most common uses of cannabis reported by practitioners remain to be investigated in controlled clinical studies, among them: intractable chronic pain (e.g. from arthritis and injuries), migraines, gastrointestinal diseases, depression, anxiety, insomnia, PTSD, ADHD and bipolar disorder.

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10 Years of Legalized Medical Marijuana in California

by Fred Gardner, CounterPunch
November 4th, 2006

Tod Mikuriya, MD (Berkeley), was the first California doctor to monitor patients' cannabis systematically. In the early 1990s his interviews with members of the San Francisco Cannabis Buyers Club documented Dennis Peron's observation that peo self-medicating for an extremely wide range of problems.

The broad range of applications confirmed what Mikuriya had learned from his the pre-prohibition medical literature on cannabis, and so when Prop 215 was drafted, he urged that it apply not only to people with a list of named conditio those treating " ... any other illness for which marijuana provides relief."

No sooner had Prop 215 passed than top California law enforcement agents co with Clinton Administration officials and Prohibitionist strategists from the priv sector to plan its disimplementation. On Dec. 30, 1996, Drug Czar Barry McCa Attorney General Janet Reno, Health & Human Services Secretary Donna Shal the director of the National Institute of Drug Abuse, Alan Leshner, held a press conference to threaten California doctors with loss of their licenses, i.e., their livelihoods, if they approved marijuana use by their patients. McCaffrey stood alongside a large chart headed "Dr. Tod Mikuriya's, (215 Medical Advisor) Med of Marijuana." Twenty-six conditions were listed in two columns. ("Migranes" w misspelled.) "This isn't medicine, this is a Cheech and Chong show," he said. F prosecutors would focus on doctors who were "egregious" in approving mariju by patients.

Dr. Mikuriya watched the press conference on CNN at his home in the Berkeley "As doctors become more fearful," he says. "I'll obviously get more and more who are using cannabis or are considering it. Will that make it seem that there something 'egregious' about my practice? You bet it will!"

>From the Attorney General's office in Sacramento a memo went out from Se Deputy AG John Gordnier to district attorneys in all 58 counties asking them t any cases involving Mikuriya. In due course, on the basis of complaints from s cops, and DAs, Mikuriya was investigated by the medical board and found to h committed "extreme departures from standard practice." He was placed on pr and ordered to pay $75,000 for his own prosecution.

Over the years the number of cannabis specialists among California doctors ha slowly but steadily. In 2000 Mikuriya organized a group, now known as the So Cannabis Clinicians, to share data for research purposes. More than 20 doctors become involved with the SCC. Collectively they have approved cannabis use f estimated 350,000 patients. This summer, with the 10th anniversary of Prop 2 passage approaching, I surveyed the SCC doctors get their basic findings. Her Mikuriya's responses to the survey he inspired.

Approvals issued to date: 8,684. Previously self-medicating: >99% Category c Analgesic/immunomodulator 41% Antispasmodic/anticonvulsant 29% Antidepresssant/Anxiolytic 27% Harm reduction substitute: 4%

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Results reported are dependent on the conditions and symptoms being treatec primary benefit is control without toxicity for chronic pain and a wide array of conditions. Control represents freedom from fear and oppression. Control -or l thereof- is a major element in self-esteem.

With exertion of control, with freedom from fear of incapacity, quality of life is improved. The ability to abort an incapacitating attack of migraine, asthma, anxiety depression empowers.

Relief from the burden of criminality through medical protection enhances a sa self-perception.

Alteration in the perception of and reaction to pain and muscle spasticity is a u property of cannabis therapy.

Patient reports are diverse yet contain common elements. 100% report that ca is safe and effective. Return for follow-up and renewal of recommendation anc approval confirms safety and efficacy.

Cannabis seems to work by promoting homeostasis in various systems of the l salient effects are multiple and concurrent. They include- o Restoration of norr functioning of the gastrointestinal tract with normalization of peristalsis and re of appetite. o Normalizing circadian rhythm, which relieves insomnia. Sleep is therapeutic in itself and synergistically helps with pain control. o Easement of depression, and anxiety. Cannabis as an anxiolytic and antidepressant modula emotional reactivity and is especially useful in treating post-traumatic stress d

≃ 1% .

Patients treated for ADHD: 92 Patients using cannabis as a substitute for alcohol : 683
The slow poisoning by alcohol with its sickening effects on the body, psyche, a family can be relieved by cannabis.

Medications no longer needed? Opioids, sedatives, NSAIDS (non-steroidal anti inflammatories), and SSRI anti-depressants are commonly used in smaller am discontinued. These are all drugs with serious adverse effects. Opioids and sec produce depression, demotivation, and diminished mobility. Weight gain and diminished functionality are common effects. Cognitive and emotional impairm depression are comorbid conditions. Opioids adversely effect vegetative functi with constipation, dyspepsia, and gastric irritation. Pruritus is also an issue for Circadian rhythms are disrupted with sleep disorders and chronic sedation cau these agents. Dependence and withdrawal symptoms are more serious than w sedatives.

Opioids are undoubtedly the analgesic of choice in treating acute pain. For chr pain, however, I recommend the protocol proposed by a doctor named Fronmı the Ohio Medical Society in 1859: primary use of cannabis, resorting to opiate episodic worsening of the condition. Efficacy is maximized, tolerance and adve effects are minimized. (Neither cannabis nor human physiology has changed s 1859.)

NSAIDs can be particularly insidious for those who do not immediately react w gastric irritation and discontinue the drug. Chronic irritation with bleeding may serious morbidity. Most often, the dyspepsia produced is suppressed with anta other medications. Many patients tolerate acute intermittent use but not chror SSRIs, if tolerated, coexist without adverse interaction with cannabis. Some S say cannabis is synergistic in that it treats side effects of jitteriness or gastroir problems.

Many patients report pressure exerted by the Veterans Administration, HMOs Kaiser Permanente, and workers' compensation program contractors to remair pharmaceutical regimens. A significant number describe their prescribed drugs ineffectual and having undesirable effects. "Mainstream" doctors frequently re

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reports of adverse effects by prescribing additional drugs. Instead of negating problem, they often complicate it. Prevailing practice standards encourage polypharmacy -the use of multiple drugs, usually five or more.

Out of the ordinary conditions? While all pain reflects localized immunologic ac secondary to trauma or injury, the following atraumatic autoimmune disorders comprise a group of interest: Crohn's disease Atrophie blanche, Melorheostosi Porphyria, Thallasemia, Sickle cell anemia, Amyloidosis Mastocytosis, Lupus, Scleroderma, Eosinophilia myalgia syndrome. These are all clearly of autoimm etiology, difficult to treat. Specific metabolic errors such as amyloidosis and ce anemias warrant further study and may elucidate the underlying mechanisms illnesses and the therapeutic effects of cannabis. Multiple sclerosis with its ran severity varies in therapeutic response to cannabis.

Demographics: male patients, 72; female, 28%. Women are more likely than use cannabis for psychotherapeutic purposes (32% to 18%). Men are more lik use for harm reduction (4% to 1%). A roughly bell-shaped curve describes the my patients. 0-18 years, 1%; 19-30, 19%; 31-45, 36%; 45-60, 37%; older th 7%.

Additional Observations:

Proactive structuralism works. Meaning: people can create something and by c set a precedent.

Medical cannabis users are typically treating chronic illnesses -not rapidly debi acute illnesses.

The cash economy works better than the bureaucratic alternative. Word of mo builds a movement.

The private sector is handling marijuana distribution because the government defaulted.

Cannabis was once on the market and regulated, then it was removed from th and nearly forgotten.

Not all that we've learned in the past 10 years is new.

Once upon a time the California Compassionate Use Act of 1996 became the la state. We had the mistaken belief that civil servants, sworn to uphold the law, set about implementing the new section of the Health & Safety Code. Hardly... California doctors have been investigated by the Medical Board for approving c use by their patients. Limited immunity from prosecution for physicians was ei proclaimed invalid or, more commonly, evaded by the Board and the Attorney They dissimulate, pretending that it is not the physician's approval of marijuar issue, but his or her standard of practice. They then hold cannabis consultants standard that most HMO doctors violate constantly.

The fix is in. The state criminal justice entities share information and operate i with the DEA. There has been a total end run around the injunctive protection Conant ruling. [In Conant, a federal court enjoined the government from threa doctors who discuss cannabis as a treatment option with patients.] General m indifference enables this RICO under color of authority and the continuing defi the will of Californians who spoke ten years ago.

This is counterbalanced by the rewards of helping patients with serious chronic aliments who have adverse experience utilizing so-called main stream medicin

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Long term marijuana users seeking medical cannabis in California (2001–2007): demographics, social characteristics, patterns of cannabis and other drug use of 4117 applicants

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Abstract

Background: Cannabis (marijuana) had been used for medicinal purposes for millennia. Cannabinoid agonists are now attracting growing interest and there is also evidence that botanical cannabis is being used as self-medication for stress and anxiety as well as adjunctive therapy by the seriously ill and by patients with terminal illnesses. California became the first state to authorize medicinal use of cannabis in 1996, and it was recently estimated that between 250,000 and 350,000 Californians may now possess the physician's recommendation required to use it medically. More limited medical use has also been approved in 12 additional states and new initiatives are being considered in others. Despite that evidence of increasing public acceptance of "medical" use, a definitional problem remains and all use for any purpose is still prohibited by federal law.

Results: California's 1996 initiative allowed cannabis to be recommended, not only for serious illnesses, but also "for any other illness for which marijuana provides relief," thus maximally broadening the range of allowable indications. In effect, the range of conditions now being treated with federally illegal cannabis, the modes in which it is being used, and the demographics of the population using it became potentially discoverable through the required screening of applicants. This report examines the demographic profiles and other selected characteristics of 4117 California marijuana users (62% from the Greater Bay Area) who applied for medical recommendations between late 2001 and mid 2007.

Conclusion: This study yielded a somewhat unexpected profile of a hitherto hidden population of users of America's most popular illegal drug. It also raises questions about some of the basic assumptions held by both proponents and opponents of current policy.

Methods

Development of standardized interview

The early discovery that nearly all applicants had tried (initiated) cannabis, alcohol, and tobacco during adolescence eventually led to selection of a standardized clinical interview (SCI) as the optimum way to obtain the basic information required to assess their past use of cannabis.

Data gathered using a prototype of the SCI to screen 622 consecutive new applicants between July 1 and December 31, 2002 were analyzed in a simple relational database.

Results were later reported at a May 2004 meeting and eventually published in 2005 [1]. Meanwhile, the original questions, in somewhat modified form, have been used to screen all new applicants including those seeking annual "renewals," from January 2003 on. Thus 199 of 951 (21%) of those originally screened with less searching examinations while the SCI was being developed, eventually served as their own controls. Their responses confirmed that they shared the same general characteristics as the others and also that the sensitive information sought would be provided only if specifically requested. In late 2005 a more sophisticated relational database was created and later customized with drop-down menus to allow responses to be entered directly into a laptop computer in real time, thus incorporating the database as an intrinsic part of the medical record.

Selection of areas of interest

Once the linkage between cannabis, alcohol, and tobacco had been appreciated, questions focusing on initiation and subsequent use of all three drugs were asked of several hundred consecutive applicants. The further discovery, that many had tried other "drugs of abuse" was explored by adding questions requiring yes-no responses about their initiations of 8 specific illegal agents. When patterns in personal histories suggested that family relationships and school experiences had also played a significant role in their adolescent drug initiations, the inquiry was broadened to include those areas. A prototype of the standardized clinical interview (SCI) became ready for clinical use by July 1, 2002.

Results

Demographics

4117 individual applicants were seen on as many as four occasions between November 2001 and June 30, 2007. All were seeking a physicians' approval of their use of cannabis; 3187 (77.4%), were male, ranging in age from 16 to 91 when first seen (median age 31). 930 (22.6%) were female, ranging in age from 16 to 89, with a median age of 36. The median age of the entire population was 32, reflecting both the smaller number of females and their somewhat greater age when first seen.

Table 1: Race/ethnicity of entire population (N = 3515). As subsequently shown by a more searching analysis, the composition of the applicant population has been changing steadily.

Caucasian	68.8%
African American	16.2%
Hispanic	8.1%
Asian	5.1%
Other	1.7%

Table 1 shows race/ethnicity for the entire population. Analysis by year-of-birth (Table 2) reveals more Asians and Hispanics among the younger applicants, reflecting the two groups that have been immigrating to California in the greatest numbers in recent years. Analysis by both age and race also revealed other differences.

Tables 3 and 4 summarize educational and occupational histories; Table 5 provides data on applicants who were unemployed when first seen. Overall, this population exhibited lower High School drop out rates and higher percentage of graduates than national averages. The percentages earning Bachelors' degrees and Doctorates are nearly identical to the national average, but only about one half as many had earned Masters' degrees.

Their occupations resembled US averages in some employment areas and were quite different in others (Table 4); in terms of non-occupational divisions (Table 5), a much smaller percentage are retirees, a finding that reflects both their relative youth and the paucity of applicants born before 1946.

Although the extremes of applicant age ranged from 16 to 91, only 3 were under 18 when first seen. The great majority (84.16%) were between 21 and 60, a finding further emphasized when the population is examined by year of birth (Table 6), a perspective that also discloses how few (4.53%) had been born before 1946. The overall male female ratio was nearly four to one (Table 7); however when examined as year of birth cohorts, it varies from over 5:1 for the youngest applicants to almost 3:1 for the oldest. Nearly 70% were Caucasians and 16% were Black, with sizable numbers of Hispanics and Asians (Table 1).

Table 2: Cohort analysis of race/ethnicity (N = 3185). Analysis of racial composition by year of birth cohorts also shows that the applicant population has reflected immigration trends.

	1936–1945	1946–1955	1956–1965	1966–1975	1976–1985
Caucasian	68.3%	76.6%	74.0%	65.4%	66.3%
African American	24.2%	17.5%	16.9%	19.0%	12.7%
Hispanic	5.0%	3.5%	4.8%	7.6%	11.6%
Asian	0.8%	1.0%	3.0%	5.5%	8.0%
Other	1.7%	1.4%	1.3%	2.6%	1.4%

Table 3: Highest Education Attainment over 25, Applicants compared to US Population (N = 936). In general, cannabis applicants compared favourably with national averages.

	Patient	US
Drop Out	11.1%	14.1%
Diploma	61.3%	49.0%
Associate	5.3%	8.8%
Bachelors	18.3%	18.4%
Master/Prof	2.7%	8.4%
Doctorate	1.3%	1.3%

Initiation and use of cannabis

An overwhelming majority (87.9%) of 3038 applicants queried about the details of their cannabis initiation had tried it before the age of 19, usually in the company of older siblings, cousins or peers. After subtracting those born before 1946, the percentage of applicants who had tried marijuana before the age of twenty went up to 90%. Some became regular users almost immediately, while others remained sporadic users for years (that interval was estimated by asking them when they first began to "buy their own").

Amounts and patterns of cannabis use

Essentially all applicants queried about their current use were consuming inhaled cannabis on a regular basis in amounts that varied considerably, but tended to remain stable over time. The range is from less than one sixteenth ounce per week to over one ounce, with about 70% estimating they consume between 1/8 and 1/4 oz./week. Almost 90% acknowledge daily, or near daily ("six days a week") use, with about 10% insisting their use is far less frequent, in the range of two to five days/week.

Table 4: Occupational divisions for employment for applicants and US population (N = 2092). The two groups are quite similar with the exception of Construction and Extraction, Office and Administrative Support, which are gender specific professions.

Occupational Divisions	Patient	US
Management	4.59%	4.57%
Business and Financial Operations	3.25%	4.15%
Computer and Mathematical	3.59%	2.27%
Architecture and Engineering	1.72%	1.83%
Life, Physical, and Social Science	.53%	.91%
Community and Social Service	1.67%	1.3%
Legal	.76%	.76%
Education, Training and Library	3.15%	.62%
Arts, Design, Entertainment Sports and Media	7.46%	1.29%
Healthcare Practitioner and Technical	1.58%	5.02%
Healthcare Support	2.82%	2.58%
Protective Service	1.34%	2.35%
Food Preparation and Service Related	6.98%	8.29%
Building and Grounds Cleaning and Maintenance	2.63%	3.33%
Personal Care and Service	2.15%	2.45%
Sales and Related	9.03%	10.69%
Office and Administrative Support	3.35%	17.49%
Farming, Fishing and Forestry	.72%	.34%
Construction and Extraction	18.36%	4.89%
Installation, Maintenance And Repair	8.56%	4.07%
Production	10.9%	7.87%
Transportation and Materials Moving	4.88%	7.36%

Table 5: Non-occupational divisions for applicants and US population (N = 494) The two groups are quite similar except for the relative scarcity of retirees in the applicant population.

Non-Occupational Divisions	Patient	US
Student	8.62%	8.86%
Disabled	3.56%	4.1%
Retired	3.44%	16.92%
Unemployed	3.48%	3.33%

Mode of cannabis use

There was a decided preference for inhaled cannabis. Most had not tried edibles until their own recommendation, or that of a friend, gave them access to edibles from a club or dispensary. Only 50 of 830 (6%) questioned about edibles were using them on a regular basis. The reasons given were that edible effects were more difficult to control and more likely to be undesirable and/or prolonged.

Initiation and use of tobacco and alcohol

One of the more significant patterns revealed by comparing average initiation ages for cannabis, alcohol and tobacco within the context of birth cohorts was that the oldest Baby Boomers had tried cannabis at a considerably later age than their younger successors. By 1975, less than ten years after the "Summer of Love," in 1967, cannabis was being initiated by over half of all American adolescents at close to the same average ages they also were trying alcohol and tobacco (Table 8, Figure 1).

Essentially all applicants also admitted to trying alcohol. Nearly two thirds (64.3%) of the 1226 specifically queried about alcohol blackouts had experienced at least one and 6.26% admitted to four or more. Of 1214 applicants asked to compare their current alcohol consumption with their previous lifetime peak, 130 (10.7%) claimed to be

Table 6: Distribution by year of birth cohorts (N = 3946). This further emphasizes that one's birth cohort determines what drugs one can try during adolescence.

Before 1945	4.5%
1946–1955	14.6%
1956–1965	17.3%
1966–1975	25.9%
1976–1985	35.1%
After 1986	2.6%

Table 7: Birth cohorts and gender (N = 3906). Although women were outnumbered by men in each cohort, there were significant differences noted with age.

	Male	Female
1936–1945	73.4%	26.6%
1946–1955	68.6%	31.4%
1956–1965	72.5%	27.5%
1966–1975	80.1%	19.9%
1976–1985	82.5%	17.5%
? 1986	82.3%	17.7%

abstinent, 341 (28%) said they were drinking less than 5% of their lifetime peaks, and an overwhelming 1058 (87%) claimed to be drinking less than half as much. Most of those who noted little change from their lifetime peaks had been moderate drinkers to begin with. This is evidence that once cannabis was established as their drug of choice, this population's subsequent alcohol consumption diminished; both collectively, and as individuals, a finding that clearly deserves further evaluation.

A history of cigarette initiation, later followed by chronic use, was prevalent in this population. 2559 of 2741 (96.4%) applicants, when asked if they had ever tried inhaling a cigarette, had done so; of 1324 who were specifically queried about their lifetime cigarette use, 872 (65.8%) had become daily smokers for some length of time. Although all but four of those still smoking claim they want to quit, only 316 (36.2%) of all smokers (23.9% of respondents) had been able to do so by the time of the interview. Most who are still smoking have reduced their daily cigarette consumption; a majority relate temporary increases in their daily cigarette use to "stress." Thus the impact of daily cannabis use on cigarette consumption, although less impressive than is the case with alcohol, also seems significant and worthy of further exploration.

Table 8: Average initiation ages for entry level agents (N = 2498). This table is depicted by Figure 1 and emphasizes the rapid fall in age at initiation of cannabis after it first became available in high schools.

	Tobacco	Alcohol	Cannabis
1936–1940	16.07	16.43	26.39
1941–1945	15.86	15.89	21.12
1946–1950	14.98	16.18	18.64
1951–1955	14.88	15.79	16.58
1956–1960	14.8	15.25	15.87
1961–1965	14.74	14.71	15.66
1966–1970	15.28	14.84	14.92
1971–1975	15.08	15.04	15.68
1976–1980	14.99	15.22	15.15
1981–1985	14.29	14.66	14.32

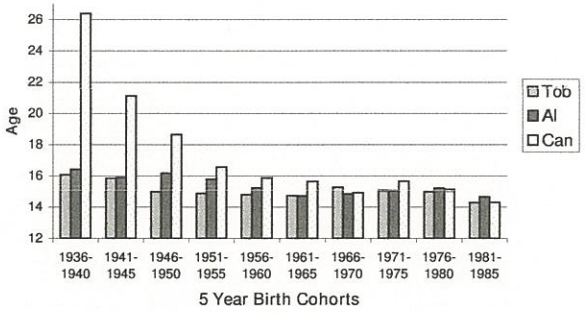

Figure 1
Average initiation age tobacco, alcohol and cannabis. Those born before 1940 were fewest in number; they had also tried cannabis at the oldest average age. Baby Boomers born after 1946 were the first large cohort, and their successors were still younger when they tried cannabis. The 61–65 cohort initiated cannabis, alcohol, and tobacco at essentially the same average age.

Other drug initiations

When examined from the standpoint of both year of birth (YOB) cohorts and admitted initiations of other illegal agents (Table 9, Figure 2) noticeable and consistent differences are revealed: whites in every age cohort had consistently tried all other illegal agents more frequently than other racial groups (Table 10).

Further cohort analysis of this population's adolescent interest in other illegal drugs, plus its nearly universal initiation of alcohol and tobacco, suggest that while race (Table 10), and generation (Table 9) exert significant influences, gender merely parallels ethnicity (Table 11).

Despite such differences (Tables 9 &10), all cohorts and racial groups have shown steady downward trends in their initiation of all other illegal drugs, with the interesting exception of psychedelic mushrooms (psilocybin) and, perhaps, ecstasy (MDMA).

Discussion

It has long been recognized that users of illegal drugs may be difficult to identify, let alone recruit into a study [2]. That chronic users of cannabis would seek medical evaluations and be so willing to share sensitive personal information within the context of their required evaluations was the unanticipated benefit of Proposition 215 that made this study possible.

Birth cohort analysis of the average ages at which applicants reported first trying alcohol, tobacco and cannabis (Table 8, Figure 1) demonstrates that a surge in youthful marijuana use began in the US in the mid Sixties. How-

Table 9: Initiation rates for other illegal drugs by YOB cohorts (N = 2364). With the exception of "magic mushrooms," and ecstasy (a psychedelic made illegal in 1988), initiation rates for all Schedule One drugs have declined since 1975.

	1936–45	1946–55	1956–65	1966–75	1976–85
Psilocybin	61.36%	74.52%	71.06%	71.07%	74.58%
LSD	67.05%	79.56%	63.79%	61.68%	50.60%
P/M	60.92%	65.75%	40.89%	22.27%	15.09%
Cocaine	81.82%	87.60%	81.50%	63.59%	54.40%
Meth	44.83%	60.44%	57.21%	48.28%	31.56%
MDMA	16.28%	16.57%	20.79%	51.49%	55.89%
Heroin	25.29%	33.06%	16.67%	13.02%	7.69%

ever, that event was not documented until publication of the first Monitoring the Future (MTF) data in 1975 demonstrated that over half of American adolescents were trying marijuana while still in High School [3].

Close questioning of applicants suggests that the majority had been motivated by a mix of physical and emotional symptoms which had been experienced at varying times in their lives. Further, that a majority had become initiates, and later chronic users of cannabis under circumstances that suggest that it was for relief of emotional symptoms in most instances. Their discovery (usually later), that cannabis also relieved physical symptoms, was most frequently made within a context of established chronic use. That notion is further supported by recent literature indicating that phytocannabinoids, newly discovered endocannabinoids, and synthetic cannabinoid agonists all seem to manifest anxiolytic effects in both humans and animals [4-8].

More than 85% of applicants had tried other illegal drugs, principally lysergic acid diethylamide (LSD), psilocybin, cocaine, and/or MDMA. The majority of those doing so hadn't remained chronic users of any except cannabis. While a majority have continued to use alcohol occasionally, the volumes consumed and the occurrence of events related to alcohol excess have sharply diminished.

A "gateway" hypothesis had developed from observations [9] that most marijuana users studied in the early Seventies were adolescents and young adults who had first tried alcohol and tobacco; also that many had tried marijuana before later trying heroin. However, subsequent efforts to establish a definitive causal link between marijuana and "harder" drugs have been largely unsuccessful [10]. More recently, a theoretical alternative was shown to provide an explanation for accumulated MTF data that is at least as coherent [11].

A significant percentage of male applicants under 30 had been treated or evaluated for treatment with Ritalin or other stimulants for attention deficit hyperactivity disorder (ADHD) as children and their histories of a preference for morning use of minimal amounts strongly suggest that inhaled cannabis enhances their ability to concentrate. The statement of one, a construction company estimator, was revealing: "after two hits (of marijuana), and my morning coffee I'm the best estimator in the company." Another, a dental technician, stated that, when I first look at my workbench, I think I'll never finish, but after a couple of tokes (of marijuana), I'm through (with work) by two o'clock." Thus, reduction of work related anxiety seems a major factor in deciding to apply for legalized use of cannabis.

Conclusion

Analysis of the demographic and social characteristics of a large sample of applicants seeking approval to use marijuana medically in California supports an interpretation of long term non problematic use by many who had first

Table 10: Initiations of other illegal drugs by race (N=2400). Although race seems related to initiation rates throughout, this shows that drug initiations by all aces trying cannabis have been falling proportionately as the adolescent market matured.

	Caucasian	African American	Hispanic	Asian
Psilocybin	82.33%	41.08%	53.50%	65.21%
LSD	69.56%	28.13%	43.71%	42.47%
P/M	35.19%	21.42%	15.34%	19.82%
Coke	74.21%	49.76%	55.55%	51.30%
Meth	52.59%	20.28%	34.67%	30.08%
MDMA	43.43%	28.88%	31.21%	64.65%
Heroin	17.54%	10.50%	7.10%	7.75%

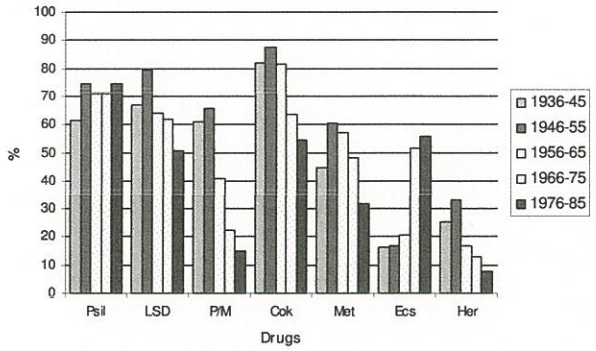

Figure 2
Other illegal drugs tried by 10 year cohort analysis. Interestingly, while all cohorts sampled other illegal drugs aggressively during adolescence, the rates at which they've done so have fallen progressively. Note also the striking generational differences in peyote/mescaline initiations by older cohorts and ecstasy by younger ones.

tried it as adolescents, and then either continued to use it or later resumed its use as adults. In general, they have used it at modest levels and in consistent patterns which-anecdotally- often assisted their educational achievement, employment performance, and establishment of a more stable life-style. These data suggest that rather than acting as a gateway to other drugs, (which many had also tried), cannabis has been exerting a beneficial influence on most.

Anecdotal evidence from repeated clinical contacts, and other data gathered incidentally over five years of experience with this population suggests that, except for very modest alcohol consumption and obligatory (addictive) use of tobacco by those trying to quit, cannabis is the only drug used past the age of twenty-five by most. Indeed, their total drug use histories suggest that by competing successfully with other, potentially more harmful agents, cannabis may have actually been protective. Evidence from federal agencies confirms that, since 1970, there has been a gradual decrease in consumption of both tobacco and alcohol (with correlated improvements in health outcomes) even as cannabis initiation by adolescents has remained at significant levels and overall chronic use by adults has been rising steadily.

Table 11: Initiations of Other Illegal Drugs by Gender (N=2464). Similarly, although women consistently tried all agents somewhat less often than men, the close parallels and internal consistency suggests the data are reliable.

	Male	Female
Psilocybin	74.31%	62.41%
LSD	60.06%	57.54%
P/M	30.93%	28.17%
Coke	67.32%	65.85%
Meth	44.82%	44.01%
MDMA	41.61%	37.57%
Heroin	15.86%	12.32%

While this is a self-selected sample (which restricts the generalizations that can be made from the observations reported), its large size, the consistency of the patterns uncovered, as well as their alcohol and tobacco outcomes, seem significant. For the majority, cannabis can be seen as an effective anxiolytic/antidepressant, performing as well or better than many currently available pharmaceutical agents prescribed for the same symptoms. This finding lends important support to the concept of allowing cannabis to be used medically by all those who have been chronic users and found it beneficial.

Abbreviations

Attention deficit hyperactivity disorder (ADHD)

Cannabis (Marijuana)

Cocaine (Coke)

Ecstasy (MDMA)

Lysergic acid diethylamide (LSD)

Monitoring the Future (MTF)

Peyote/mescaline (P/M)

Psychedelic mushrooms (Psilocybin)

Standardized clinical interview (SCI)

Tokes (Marijuana)

Year of birth (YOB)

Authors' contributions

TJO conceived the study, designed it, conducted all the clinical interviews, and wrote the report.

CBB designed the relational data-base for data analysis and later modified it to serve as medical record since December 2005. Conducted statistical analysis of data and contributed several other valuable suggestions and helped write and edit the report.

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Mike Gray: Coordinated the project team, funding and editing.

References

1. O'Connell TJ: **Cannabis use in adolescence: self-medication for anxiety.** *O'Shaughnessy's* 2005 [http://ccrmg.org/journal/05spr/anxiety.html]. Winter/Spring
2. Weibel WW: **Identifying and gaining access to hidden populations.** *The Collection and Interpretation of Data from HiddenPopulations NIDA Research Monograph. 98 1990 Lambert, EY Ed* [http://www.nida.nih.gov/pdf/monographs/98.pdf].
3. Johnston LD, O'Malley PM, Bachman JG, Schulenberg JE: **Monitoring the Future national survey results on drug use, 1975–2006. Volume I: Secondary school students.** :699 [http://www.monitoringthefuture.org/pubs/monographs/vol1_2006.pdf]. Bethesda, MD: National Institute on Drug Abuse (NIH Publication No. 07-6205)
4. Zuardi AW, Crippa JA, Hallak JE, Moreira FA, Guimarães FS: **Cannabidiol, a cannabis sativa constituent, as an antipsychotic drug.** *Braz J Med Biol Res* 2006, **4**:421?429.
5. Ashton CH, Moore PB, Gallagher P, Young AH: **Cannabinoids in bipolar affective disorder: a review and discussion of their therapeutic potential.** *J Psychopharmacol* 2005, **19**:293?300.
6. Müller-Vahl KR: **Cannabinoids reduce symptoms of Tourette's syndrome.** *Expert Opin Pharmacother* 2003, **4**:1717?1725.
7. Di Marzo V, Bifulco M, De Petrocellis L: **The endocannabinoid system and its therapeutic exploitation.** *Nat Rev Drug Discov* 2004, **9**:771?784.
8. Witkin JM, Tzavara ET, Nomikos GG: **A role for cannabinoid CB1 receptors in mood and anxiety disorders.** *Behav Pharmacol* 2005, **16**:315.
9. Kandel DB: **Stages and pathways of drug involvement.** In *Stages and pathways of drug involvement, Examining the gateway hypothesis* Edited by: Kandel DB. New York: Cambridge University Press; 2002:3?19.
10. Kandel DB, Jessor R: **The gateway hypothesis revisited.** In *Stages and Pathways of Drug Involvement, Examining the gateway hypothesis* Edited by: Kandel DB. New York: Cambridge University Press; 2002:365?392.
11. Morral AR, McCaffrey DF, Paddock SM: **Reassessing the marijuana gateway effect.** *Addiction* 2002, **12**:1493?1504.

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Cannabis Eases Post Traumatic Stress

By Tod Mikuriya, MD

William Woodward, MD, of the American Medical Association, testifying before Congress in 1937 against the Prohibition of cannabis, paraphrased a French author (F. Pascal, 1934) to the effect that "Indian hemp has remarkable properties in revealing the subconscious." A Congressman asked, "Are there any substitutes for that latter psychological use?" Woodward replied, "I know of none. That use, by the way, was recognized by John Stuart Mill in his work on psychology, where he referred to the ability of Cannabis or Indian hemp to revive old memories —and psychoanalysis depends on revivivification of hidden memories." For including that reference to Mill (1867) in the list I have been compiling of conditions amenable to treatment by cannabis, I was ridiculed by Drug Czar Barry McCaffrey in 1996. I stand by its inclusion, of course, and in the 10 years since California physicians have been approving cannabis use by patients, I have found myself appreciating and confirming Mill's insight with every report that cannabis has eased symptoms of post-traumatic stress disorder.

PTSD As a Dissociative Disorder

PTSD—a chronic condition involving horrific memories that cannot be erased—is a dissociative identity disorder. The victims's psyche is fragmented in response to contradictory inputs that cannot be resolved.

O'Shaughnessy's

O'Shaughnessy's is the journal of the CCRMG/SCC. Our primary goals are the same as the stated goals of any reputable scientific publication: to bring out findings that are accurate, duplicable, and useful to the community at large. But in order to do this, we have to pursue parallel goals such as removing the impediments to clinical research created by Prohibition, and educating our colleagues, co-workers and patients as we educate ourselves about the medical uses of cannabis.

SCC

The Society of Cannabis Clinicians (SCC) was formed in the Autumn of 2004 by the member physicians of CCRMG to aid in the promulgation of voluntary standards for clinicians engaged in the recommendation and approval of cannabis under California law (HSC §11362.5).

As the collaborative effort continues to move closer to issueing guidelines, this site serves as a public venue for airing and discussing these

Dissociative identity disorders are expressed in bizarre or inappropriate behaviors with intense sadness, fear, and anger. Repression or "forgetting" of the experiences may develop as a coping mechanism.

When traumatic or abusive experiences cannot be integrated into normal consciousness —as in the case of the Jekyl-Hyde behaviors of abusive parents or caregivers— creation of separate personalities or identities may occur.

For example, the woman who was molested by a family member may have both superfically-compliant and repressed-raging identities. The persona that's presented to the world can be swept away when a stimulus calls forth the overwhelming rage.

Such fragmenting of the individual personality causes tremendous stress. The psyche is incomplete because of repression and denial. The person tries to appear normal and logical but in fact is in turmoil, angry and depressed. The inability to deal directly with emotional issues results in ongoing splitting and compartmentalization of the personality —and in extreme cases, multiple personalities, hysterical fugue (a separate state of consciousness that the individual may not recall), blindness, paralysis, and other functional disruptions.

In 1994 the term "Multiple Personality Disorder" was replaced with the more widely applicable "Dissociative Identity Disorder." As an article (by Foote et al) and editorial (Spiegel) in the April 2006 American Journal of Psychiatry attest, it is only relatively recently that PTSD has been characterized as a dissociative disorder. [continued below]

guidelines.

Visit the SCC Site for more information.

Démonisation

Case Report:

A 52-year-old retired executive secretary brought her 20-year-old daughter along to her follow-up interview two years after starting cannabis therapy. During her initial visit she had not disclosed fully the causality of her chronic depression with symptoms of PTSD (nightmares, chronic insomnia, dissociative episodes, rage).

She was experiencing loss of emotional control with crisis psychiatric interventions.

Hypervigilance characterized her presentation; she described herself as being "all clenched up."

On follow-up she reported being able to recover and process repressed memories of sexual abuse from age five to 15 by her father (a preacher) and having been beaten by her enraged mother. She reported the diminution and cessation of dissociative reactions to the painful memories. This permitted her to process and resolve —or come to

an accord with— these unthinkable memories. Her continuing psychotherapy focused on these issues. She no longer experienced episodes of loss of control. She was able to relax her hypervigilance. Her self-esteem was significantly improved and she seemed happy and optimistic
Her daughter confirmed that her mother was less irritable and more emotionally available since starting cannabis therapy. Both described improvement in their relationship.

Case Report:
A 55-year-old disabled male veteran had been a naval air crewman on patrol during the Vietnam war. A P2V turbo-prop engine failed to reverse properly on landing. A propeller broke loose, pierced the fuselage, and instantly killed his crew mate who was two feet away. He brought a large binder of documentation of the incident.
His PTSD was expressed primarily through a haunting, recurrent flashback nightmares that replayed the traumatic event. Attendant were the feelings of being emotionally overwhelmed. Sleep deficit was a salient aggravating factor for increasing vulnerability. Cannabis restored sleep and controlled nightmares. Depression and irritability had been eased.

Easement by Cannabis
Approximately eight percent of the >9,000 Californians whose cannabis use I have monitored presented with PTSD (309.81) as a primary diagnosis. Many of them are Vietnam veterans whose chronic depression, insomnia, and accompanying irritability cannot be relieved by conventional psychotherapeutics and is worsened by alcohol. For many of these veterans, chronic pain from old physical injury compounds problems with narcotic dependence and side effects of opioids. Survivors of childhood abuse and other traumatic experiences form a second group manifesting the same symptoms —loss of control and recurrent episodes of anxiety, depression, panic attacks and mood swings, chronic sleep deficit and nightmares. The brief case reports in the box at the right of this page, unique though the subjects may be, typify two different forms that PTSD takes, both of which are eased by cannabis. The recurrent nightmares from the vet's traumatic episode took on a life of their own, causing nocturnal turmoil and dread. The repressed memories of the sexually abused and beaten woman were symptoms of a fragmented, dissociative response to the disorder.
Easement by cannabis helped both —the vet by

toning down his reaction to the nightmares and restoration of his sleep, the woman by modulating her emotional reactivity and permitting her to process and integrate the experience and give up the fragmented, dissociative defense mechanisms, which in due course she no longer needed.

Repression and suppression are defense mechanisms that break down when the victim is fatigued and/or hurting and subjected to triggering stimuli. With cannabis, vegetative functions necessary for recovery, growth and repair are normalized.

Cannabis relieves pain, enables sleep, normalizes gastrointestinal function and restores peristalsis. Fortified by improved digestion and adequate rest, the patient can resist being overwhelmed by triggering stimuli. There is no other psychotherapeutic drug with these synergistic and complementary effects.

Practical Treatment Goals

In treating PTSD, psychotherapy should focus on improving how the patient deals with resurgent symptoms rather than revisitation of the events. Decreasing vulnerability to symptoms and restoring control to the individual take priority over insight as treatment goals. Revisiting the traumatic events without closure and support is not useful but prolongs and exacerbates pain and fear of loss of control. To repeat: cathartic revisiting of the traumatic experience(s) without support and closure is anti-therapeutic and can exacerbate symptoms.

Physical pain, fatigue, and sleep deficit are symptoms that can be ameliorated. Restorative exercise and diet are requisite components of treatment of PTSD and depression. Cannabis does not leave the patient too immobile to exercise, as do some analgesics, sedatives biodi-azapenes, etc. Regular aerobic exercise (where injury does not interfere) relieves tension and restores control through kinesthetic involvement. Exercise also internalizes the locus of control and diminishes drug-seeking to manage emotional response.

The importance of sound sleep

PTSD often involves irritability and inability to concentrate, which is aggravated by sleep deficit. Cannabis use enhances the quality of sleep through modulation of emotional reactivity. It eases the triggered flashbacks and accompanying emotional reactions, including nightmares.

The importance of restoring circadian rhythm of sleep cannot be overestimated in the management of PTSD. Avoidance of alcohol is important in large part because of the adverse effects on sleep. The short-lived relaxation and relief provided by alcohol are replaced by withdrawal symptoms at night, causing anxiety and the worsening of musculoskeletal pain.

Evening oral cannabis may be a useful substitute for alcohol. With proper dosage, the quality and length of sleep can be improved without morning dullness or hangover. For naïve patients, use of oral cannabis should be gradually titrated upward in a supportive setting; this is the key to avoiding unwanted mental side effects.

I recommend the protocol J. Russell Reynolds M.D., commended to Queen Victoria: "The dose should be given in minimum quantity, repeated in not less than four to six hours, and gradually increased by one drop every third or fourth day, until either relief is obtained, or the drug is proved, in such case to be useless. With these precautions I have never met with any toxic effects, and have rarely failed to find, after a comparatively short time, either the value or the uselessness of the drug."

The advantage of oral over inhaled cannabis for sleep is duration of effect; a disadvantage is the time of onset (45-60 minutes). When there is severe recurrent insomnia with frequent awakening it is possible to medicate with inhaled cannabis and return to sleep. An unfortunate result of cannabis prohibition is that researchers and plant breeders have not been able to develop strains in which sedative components of the plant predominate.

Modulation, Not Extinction

Although it is now widely accepted that cannabinoids help extinguish painful memories, my clinical experience suggests that "extinguish" is a misnomer.
Cannabis modulates emotional reactivity, enabling people to integrate painful memories —to look at them and begin to deal with them, instead of suppressing them until a stimulus calls them forth with overwhelming force.
The modulation of emotional response relieves the flooding of negative affect.
The skeletal and smooth muscle relaxation decreases the release of corticosteroids and escalating "fight-or-flight" agitation. The modulation of mood prevents or significantly decreases the symptoms of anxiety attacks, mood swings, and insomnia.
While decreasing the intensity of affectual response, cannabis increases introspection as evidenced by the slowing of the EEG after initial stimulation. Unique anti-depressive effects are experienced immediately with an alteration in cognition.
Obsessive and pressured thinking give way to introspective free associations (given relaxed circumstances).
Emotional reactivity is calmed, worries become less pressing.
Used on a continuing basis, cannabis can hold depressive symptoms at bay.
Agitated depression appears to respond to the anxiolytic component of the drug.
Social withdrawal and emotional shutting down are reversed.
The short-term memory loss induced by cannabis that may be undesirable in other contexts is therapeutic in controlling obsessive ideation, amplified anxiety and fear of loss of control ignited by the triggering stimuli.

Easement Effects of Cannabis
In treating PTSD, cannabis provides control and amelioration of chronic stressors without adverse side effects.
Mainstream medicine treats PTSD symptoms such as hyperalertness, insomnia, and nightmares with an array of SSRI and tricyclic anti-depressants, sedatives, analgesics, muscle relaxants, etc., all of which provide inadequate

relief and have side effects that soon become problematic. Sedatives, both prescribed and over-the-counter, when used chronically, commonly cause hangovers, dullness, sedation, constipation, weight gain, and depression. See chart at right.
Cannabis is a unique psychotropic immunomodulator which can best be categorized as an "easement." Modulating the overwhelming flood of negative affect in PTSD is analogous to the release of specific tension, a process of "unclenching" or release. As when a physical spasm is relieved, there is a perception of "wholeness" or integration of the afflicted system with the self. For some, this perceptual perspective is changed in other ways such as distancing (separating the reaction from the stimulus, which can involve either lessening the reaction, as with modulation, or repressing/suppressing the memory; walling it off; forgetting). The modulation of emotional response relieves the flooding of negative affect. The skeletal and smooth muscle relaxation decreases the sympathetic nervous reactivity and kindling component of agitation. Fight/flight responses and anger symptoms are significantly ameliorated. The fear of loss of control diminishes as episodes of agitation and feeling overwhelmed are lessened. Experiences of control then come to prevail. Thinking is freed from attachment to the past and permitted to fix on the present and future. Instead of being transfixed by nightmares, the sufferer is freed to realize dreams.

Based on both safety and efficacy, cannabis should be considered first in the treatment of post-traumatic stress disorder. As part of a restorative program with exercise, diet, and psychotherapy, it should be substituted for "mainstream" anti-depressants, sedatives, muscle relaxants, tricyclics, etc.

The Toxic Alternatives

Commonly prescribed medications for PTSD as listed in "Postraumatic Stress Disorder Among Military Returnees From Afghanistan and Iraq," by Matthew J. Friedman, MD, PhD, in the April 2006 American Journal of Psychiatry:

SSRIs

Paroxetine, Sertraline, Pluoxetine, Citalopram, Fluvoxamine
May produce insomnia, restlessness, nausea, decreased appetite, daytime sedation, nervousness, and anxiety, sexual dysfunction, decreased libido, delayed orgasm or anorgasmia. Clincically significant interactions for people prescribed monoamine oxidase inhibitors (MAOIs). Significant interactions with hepatic enzymes produce other drug interactions. Concern about increased suicide risk in children and adolescents.

Other second-generation antidepressants:

Trazadone may be too sedating, may produce rare priapism. **Velafaxine** may exacerbate hypertension. **Buproprion** may exacerbate seizure disoder. **Mirtrazepine** may cause sedation.

MAOIs

Phenetzine
Risk of hypertensive crisis; patients required to follow a strict dietary regime. Contraindicated in combination with most other antidepressants, CNS stimulants, and decongestants. Contraindicated in patients with alcohol/substance abuse/dependence. May produce insomnia, hypotension, anticholinergic side effects, and liver toxicity.

Tricyclic Antidepressants

Imipramine, Amitriptyline, Desipramine
Anticholinergic side effects (dry mouth, rapid pulse, blurred vision, constipation). May produce ventricular arrhythmias. May produce orthostatic hypotension, sedation, or arousal.

Antiadrenergic Agents

Prazosin, Propranolol, Conidine, Guanfacine

May produce hypotension, brachycardia (slow heartbeat), depressive symptoms, psychotomor slowing or bronchospasm.

Anticonvulsants

Carbamazepine may cause neurological symptoms, ataxia, drowsiness, low sodium level, leukopenia. Valproate may cause gastrointestinal problems, sedation, tremor and thrombocytopenia (low platelet levels in blood). It is teratogenic (induces mutations, should not be used during pregnancy). **Gabapentin** may cause sedation and ataxia (difficulty forming sentences). **Lamotrigine** may cause Stevens-Johnson syndrome, rash, fatigue. **Toprimate** may cause glaucoma, sedation, dizziness, and ataxia.

Atypical Antipsychotics

Risperidone, Olanzapine, Quetiapine

May cause weight gain. Risk of type 2 diabetes with olanzapine

Cannabis as a treatment for PTSD provides effective control and relief of chronic stressors. Its side-effect profile seems especially benign when contrasted with those of the prevailing mainstream treatments.--T.H.M.

California Cannabis Research Medical Group

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Spring 2006
O'Shaughnessy's
Journal of the California Cannabis Research Medical Group

Patients Out-of Time Perspectives

PTSD and Cannabis: A Clinician Ponders Mechanism of Action

By David Bearman, MD

One often intractable problem for which cannabis provides relief is post-traumatic stress disorder (PTSD). I have more than 100 patients with PTSD.

Among those reporting that cannabis alleviates their PTSD symptoms are veterans of the war in Vietnam, the first Gulf War, and the current occupation of Iraq. Similar benefit is reported by victims of family violence, rape and other traumatic events, and children raised in dysfunctional families.

Post-Traumatic Stress Disorder

Post-Traumatic Stress Disorder —once referred to as "shell shock" or "battle fatigue" — is a debilitating condition that follows exposure to ongoing emotional trauma or in some instances a single terrifying event. Many of those exposed to such experiences suffer from PTSD. The symptoms of PTSD include persistent frightening thoughts with memories of the ordeal. PTSD patients have frightening nightmares and often feel anger and an emotional isolation.

Sadly, PTSD is a common problem. Each year millions of people around the world are affected by serious emotional trauma. In more than 100 countries there is recurring violence based on ethnicity, culture, religion or political orientation.

Men, women and children suffer from hidden sexual and physical abuse. The

O'Shaughnessy's

O'Shaughnessy's is the journal of the CCRMG/SCC. Our primary goals are the same as the stated goals of any reputable scientific publication: to bring out findings that are accurate, duplicable, and useful to the community at large. But in order to do this, we have to pursue parallel goals such as removing the impediments to clinical research created by Prohibition, and educating our colleagues, co-workers and patients as we educate ourselves about the medical uses of cannabis.

SCC

The Society of Cannabis Clinicians (SCC) was formed in the Autumn of 2004 by the member physicians of CCRMG to aid in the promulgation of voluntary standards for clinicians engaged in the recommendation and approval of cannabis under California law (HSC §11362.5).

As the collaborative effort continues to move closer to issueing guidelines, this site serves as a public venue for airing and discussing these

trauma of molestation can cause PTSD. So can rape, kidnapping, serious accidents such as car or train wrecks, natural disasters such as floods or earthquakes, violent attacks such as mugging, torture, or being held captive. The event that triggers PTSD may be something that threatened the person's life or jeopardized someone close to him or her. Or it could simply be witnessing acts of violence, such as a mass destruction or massacre. PTSD can affect survivors, witnesses and relief workers.

Symptoms

Whatever the source of the problem, PTSD patients continually relive the traumatic experience in the form of nightmares and disturbing recollections. They are hyper-alert. They may experience sleep problems, depression, feelings of emotional detachment or numbness, and may be be easily aroused or startled. They may lose interest in things they used to enjoy and have trouble feeling affectionate. They may feel irritable, be violent, or be more aggressive than before the traumatic exposure.

Triggers

Seeing things that remind them of the incident(s) may be very distressing, which could lead them to avoid certain places or situations that bring back those memories. Anniversaries of a traumatic event are often difficult.

Ordinary events can serve as reminders of the trauma and trigger flashbacks or intrusive images. Movies about war or TV footage of the Iraqi war can be triggers. People with PTSD may respond disproportionately to more or less normal stimuli —a car backfiring, a person walking behind them. A flashback may make the person lose touch with reality and re-enact the event for a period of seconds, hours or, very rarely, days. A person having a flashback in the form of images, sounds, smells, or feelings experiences the emotions of the traumatic event. They relive it, in a sense.

Symptoms may be mild or severe — people may become easily irritated or have violent outbursts. In severe cases

guidelines.

Visit the SCC Site for more information.

victims may have trouble working or socializing. Symptoms can include:
• Problems in affect regulation —for instance persistent depressive symptoms, explosion of suppressed anger and aggression alternating with blockade and loss of sexual potency;
• Disturbance of conscious experience, such as amnesia, dissociation of experience, emotions, and feelings;
• Depersonalization (feeling strange about oneself), rumination;
• Distorted self-perception —for instance, feeling of helplessness, shame, guilt, blaming oneself, self-punishment, stigmatization, and loneliness;
• Alterations in perception of the perpetrator —for instance, adopting distorted beliefs, paradoxical thankfulness, idealization of perpetrator and adoption of his system of values and beliefs;
• Distorted relationship to others, for instance, isolation, retreat, inability to trust, destruction of relations with family members, inability to protect oneself against becoming a victim again;
• Alterations in systems of meaning, for instance, loss of hope, trust and previously sustaining beliefs, feelings of hopelessness;
• Despair, suicidal thoughts and preoccupation;
• Somatization —for instance persistent problems in the digestive system, chronic pain, cardiopulmonary symptoms (shortness of breath, chest pain, dizziness, palpitations).

• **Cannabis**

Ample anecdotal evidence suggests that cannabis enhances ability to cope with PTSD. Many combat veterans suffering from PTSD rely on cannabis to control their anger, nightmares and even violent rage. Recent research sheds light on how cannabis may work in this regard. Neuronal and molecular mechanisms underlying fearful memories are often studied in animals by using "fear conditioning." A neutral or conditioned stimulus, which is typically a tone or a light, is paired with an aversive (unconditioned) stimulus, typically a small electric shock to the foot. After the two stimuli are paired a few times, the

conditioned stimulus alone evokes the stereotypical features of the fearful response to the unconditioned stimulus, including changes in heart rate and blood pressure and freezing of ongoing movements. Repeated presentation of the conditioned stimulus alone leads to extinction of the fearful response as the animal learns that it need no longer fear a shock from the tone or light.

• **Fear Extinction**

Emotions and memory formation are regulated by the limbic system, which includes the hypothalamus, the hippocampus, the amygdala, and several other structures in the brain that are particularly rich in CB1 receptors.

The amygdala, a small, almond-shaped region lying below the cerebrum, is crucial in acquiring and, possibly, storing the memory of conditioned fear.

It is thought that at the cellular and molecular level, learned behavior — including fear— involves neurons in the baso-lateral part of the amygdala, and changes in the strength of their connection with other neurons ("synaptic plasticity").

CB1 receptors are among the most abundant neuroreceptors in the central nervous system. They are found in high levels in the cerebellum and basal ganglia, as well as the limbic system.

The classical behavioral effects of exogenous cannabinoids such as sedation and memory changes have been correlated with the presence of CB1 receptors in the limbic system and striatum.

In 2003 Giovanni Marsicano of the Max Planck Institute of Psychiatry in Munich and his co-workers showed that mice lacking normal CB1 readily learn to fear the shock-related sound, but in contrast to animals with intact CB1, they fail to lose their fear of the sound when it stops being coupled with the shock.

The results indicate that endocannabinoids are important in extinguishing the bad feelings and pain triggered by reminders of past experiences. The discoveries raise the possibility that abnormally low levels of cannabinoid receptors or the faulty release of endogenous cannabinoids are involved

in post-traumatic stress syndrome, phobias, and certain forms of chronic pain. + ADD/ADHD .
This suggestion is supported by our observation that many people smoke marijuana to decrease their anxiety and many veterans use marijuana to decrease their PTSD symptoms. It is also conceivable, though far from proved, that chemical mimics of these natural substances could allow us to put the past behind us when signals that we have learned to associate with certain dangers no longer have meaning in the real world.

What is the Mechanism of Action?

Many medical marijuana users are aware of a signaling system within the body that their doctors learned nothing about in medical school: the endocannabinoid system. As Nicoll and Alger wrote in "The Brain's Own Marijuana" (Scientific American, December 2004):

" Researchers have exposed an entirely new signaling system in the brain: a way that nerve cells communicate that no one anticipated even 15 years ago. Fully understanding this signaling system could have far-reaching implications. The details appear to hold a key to devising treatments for anxiety, pain, nausea, obesity, brain injury and many other medical problems."

idem ADD

As a clinician, I find the concept of retrograde signaling extremely useful. It helps me explain to myself and my patients why so many people with PTSD get relief from cannabis.
We are taught in medical school that 70% of the brain is there to turn off the other 30%. Basically our brain is designed to modulate and limit both internal and external sensory input.
The neurotransmitter dopamine is one of the brain's off switches.The endocannabinoid system is known to play a role in increasing the availability of dopamine. I hypothesize that it does this by freeing up dopamine that has been bound to a transporter, thus leaving dopamine free to act by retrograde inhibition.
By release of dopamine from dopamine transporter, cannabis can decrease the

sensory input stimulation to the limbic system and it can decrease the impact of over-stimulation of the amygdala.
I postulate that exposure to the PTSD-inducing trauma causes an increase in production of dopamine transporter. The dopamine transporter ties up much of the free dopamine. With the brain having lower-than-normal free dopamine levels, there are too many neural channels open, the mid-brain is overwhelmed with stimuli and so too is the cerebral cortex. Hard-pressed to react to this stimuli overload in a rational manner, a person responds with anger, rage, sadness and/or fear.
With the use of cannabis or an increase in the natural cannabinoids (anandamide and 2-AG), there is competition with dopamine for binding with the dopamine transporter and the cannabinoids win, making a more normal level of free dopamine available to act as a retrograde inhibitor.
This leads to increased inhibition of neural input and decreased negative stimuli to the midbrain and the cerebral cortex. Since the cerebral cortex is no longer overrun with stimuli from the midbrain, the cerebral cortex can assign a more rational meaning and context to the fearful memories.
I have numerous patients with PTSD who say "marijuana saved my life," or "marijuana allows me to interact with people," or "it controls my anger," or "when I smoke cannabis I almost never have nightmares." Some say that without marijuana they would kill or maim themselves or others. I have no doubt that cannabis is a uniquely useful treatment. What remains is for the chemists to determine the precise mechanism of action.

Oregon in Denial Over Cannabis as an Antidepressant

By Ed Glick
I've been working as a nurse for 25 years, about half of that in acute care

mental health nursing at Good Samaritan Regional Medical Center in Corvallis, Oregon. Eight years ago the Oregon Medical Marijuana Act pass-ed by the initiative process and a state program began registering patients.

It wasn't long before I started meeting patients coming into the regional mental health unit who reported that they were using cannabis to self-medicate for a variety of mental-health symptoms. It wasn't long after that that I started volunteering at the Compassion Center, a volunteer medical facility that helps assist patients with education, support and registration into the medical marijuana program.

Pretty soon I started seeing the same patients who were having psychiatric emergencies coming to the Compassion Center to see me for cannabis recommendations, which I can't provide and which, actually, they couldn't get because there is no allowance in Oregon for psychiatric treatments. All the "debilitating conditions" are physical with the exception of Alzheimer's agitation.

In Corvallis, a very progressive community, there is virtually no doctor who will recommend cannabis for cancer pain or for severe nausea or AIDS. The whole medical system of Corvallis said "No, you're locked out."

So then I go down to the Compassion Center and all these people from the medical system that I'm employed in say, "My doctor won't do it, he's afraid he'll lose his license."

So we assist these people by trying to find a physical correlation to their psychiatric symptom. For example, if they're having PTSD symptoms they might be sick and have physical symptoms.

How high a percentage of these people were treating psychiatric symptoms? I put together a very simple survey to find out. I reviewed 172 charts. The average patient age was 43. All the patients were registered in OMMA; 95% were registered for pain. A very large percentage of Oregon registrants are pain patients.

Some 40% had multiple qualifying

conditions (not including psychiatric) — physical pain and nausea, for example. Pain and with spasticity —they often go together.

The results: 64% of the patients in the survey showed some kind of significant psychiatric benefit; 39% reported insomnia relief; 5% reported PTSD symptom relief, many of them veterans who go to the VA hospital in Roseburg and are denied. The VA doctors tell them "No, I can't. I'll lose my DEA license." They just don't want to stand up to it —although they're beginning to refer patients to us, which is kind of interesting.

Anxiety, 11%; depressive symptoms, 11%; 15% of the cohort reported that they were using cannabis to decrease the side effects of medications; 56% reported reduced use of medications.

What these patients report to me is that they're sick and tired of Vioxx and they're sick and tired of Flexeril, Vicodin —people are literally sick of these drugs. They can't sleep, they can't function, they're drugged up, they don't have any enjoyment of life.

When they start using cannabis they leave off the Vioxx and they leave off the Vicodin. Vicodin has a place, but for long-term pain management it is really poor.

Appetite stimulation —tremendously important for people who are in pain all the time— was 20%.

I put the survey together as a request to the Oregon Department of Human Services to reconvene the Debilitating Conditions Advisory Panel, which I was a member of in 2000. At that time nine patients had submitted requests to include psychiatric conditions to the list. The state health officer did a fairly good job of bringing together the panel, but the whole thing was skewed from the outset by political manipulation by the governor's office and by the head of the Department of Health Services. The information that they would allow us to consider had to be filtered through rules stating that if it's not a double-blind, peer-reviewed clinical trial, it doesn't get a lot of evidentiary weight.

We were not allowed to give much

weight to patients' reports. And of course there was no relevant double-blind, peer-reviewed clinical trial. So the panel was set up to fail.

A few patients came in and gave very compelling testimonials. And then out of nowhere came a whole bunch of medical experts —psychiatrists from Oregon Health Sciences University and the National Alliance for the Mentally Ill— and they just had fits. "This is quackery," they said.

The only person who even differentiated between affective depressive-type disorders and schizophrenic thought disorders was one of the patients. None of the doctors even made any differentiation between these two completely different sets of medical problems.

After a long, protacted time we all wrote our comments out, and there was a vote, and we voted to add affective disorders —severe agitation and depressive symptoms. Didn't happen. They finally did add Alzheimer's agitation.

So, five years later I brought in the study I'd done with OMMP registrants and asked them to reconvene the Debilitating Conditions Panel based on this new evidence showing that indeed there is some psychiatric effect that people are getting from their cannabis use. And they rejected the request with a "summary denial."

Then Lee Berger, an attorney in Portland, asked if I'd be willing to sue the Department of Human Services' OMMP and I said yes. We filed our petition for judicial review in February —a formal request "to Add Clinical Depression, Depressive Symptoms, Post-Traumatic Stress Disorder (PTSD), Severe Anxiety, Agitation and Insomnia, to Those Diseases and Conditions Which Qualify as 'Debilitating Medical Conditions' under the Oregon Medical Marijuana Act." And it worked! I can't believe it!

We got word last week that, because the OMMP doesn't really want to go to court, they've decided to kind of sue for peace. All we're asking is that they reconvene a panel to evaluate these conditions. So, we're in the process of

negotiatng with them to get this thing back on track.
We want to close some of the loopholes that allow them to skew the evidence base. It's pretty clear that there are a lot of patients who are using cannabis for insomnia, for mood stabilizing, and for peace. Just for a a very simple, elemental peace, especially with chronic diseases like severe chronic pain. Cannabis is actually a miracle drug for pain, in my opinion.
There's no question the last thing the pharmacy industry wants is millions and millions of Americans growing and using their own medicine that covers such a wide array of diseases.

Rodney Dangerfield's Lifelong Romance With Marijuana

By Joan Dangerfield
The comedian's widow gave this talk at the Patients Out of Time conference on cannabis therapeutics in Santa Barbara April 7.
If Rodney were here today he would say something brilliant. He would probably open with a marijuana joke. He'd say, "I tell ya, that marijuana really has an effect on you. The other day I smoked a half a joint and I got so hungry, I ate the other half."
Rodney had a fantastically unique mind. Few people knew he was a mathematical genius, but everyone knew he was hilarious. His humor was a razor thrust into social hypocrisy and the little injustices of life. He wrote "killers" and made the world laugh.
Another thing that was not widely known about Rodney is that he endured quite a bit of personal suffering in his life. He was heartbreakingly neglected as a child. We've all heard the expression "the tears of a clown," and in many ways Rodney embodied that experience. Like most geniuses, the special chemistry that created his remarkable mind also created certain psychological challenges. Acute anxiety and manic depression were congenital

issues that plagued Rodney's life.
To give you an idea of how his anxiety would manifest itself, Rodney couldn't sit still. In Caddyshack, his character, Al Cervic, is constantly fidgeting like he's about to burst out of his skin. The truth is, this was no act. Rodney was under duress. He felt Chevy Chase was talking too slowly and it got on his nerves. Rodney's impatience would come out through his body. The pace of the whole world was too slow for him until he found marijuana.
Rodney first lit up back in 1942 when he was 21. He was hanging out with a comic named Bobby Byron and his friend Joe E. Ross —some of you might remember Joe E. Ross from Car 54. They went to the Belvedere Hotel in New York where Bobby lived. The night would prove to have such an impact on Rodney's life that he even remembered the room number they were in —1411.
Although he was supposed to be enjoying himself with friends, Rodney was characteristically agitated and anxiety ridden. It's how he felt every day of his life to that point. But when Rodney got high, he couldn't believe it. For the first time in his life, he left relaxed and peaceful, and had a sense of well-being. That night marijuana became a new friend that would be in Rodney's life for the next 62 years.
I met Rodney in 1983, and after a 10-year courtship, Rodney and I enjoyed 11 years of marriage. I must admit that when I became a part of Rodney's life, I did not approve of his marijuana use. My Mormon background hadn't given me experience with any illegal substances and I was always afraid Rodney would get arrested.
Rodney was concerned about my feelings and agreed to look for legal alternatives to treat his ailments. Over the years we consulted the best experts we could find in search of legal anti-anxiety and pain medications and even tried Marinol. But nothing worked for him the way real marijuana did.
A couple of years ago Rodney was in the process of writing his autobiography, in which he wanted to be very candid

about everything in his life. He even wanted to title the book "My Lifelong Romance with Marijuana."
I was sure then that Rodney would be arrested. So I looked for, and found, Dr. David Bearman here in Santa Barbara. Dr. Bearman examined Rodney and obtained records from Rodney's other doctors for review. In addition to his anxiety and depression, at the time Rodney's medical conditions included constant pain from the congenital fusion of his spine, an inoperable dislocated shoulder and rotator-cuff tear and arthritis. Rodney wasn't able to take traditional pain medications because of their interactions with his blood-thinning medication, Coumadin.

We were elated a few days after that initial visit with Dr. Bearman when Rodney's medicinal use was approved. Rodney showed the approval letter to everyone and carried miniature versions in his pockets. Ever the worried wife, I included a copy of the letter in the memory box of his casket in case the feds were waiting for him at the Pearly Gates.
Even though Rodney endured numerous health challenges over the years, including aneurysms, heart surgeries and a brain bypass, he remained active and vital during his last incredible year. He swam regularly, went on a multi-city press tour to promote his best-selling book (the publisher made him change the title to "It's Not Easy Bein' Me"), recorded an album of love songs called "Romeo Rodney," and wrote countless new jokes.
After all those years of pot smoking, his memory and his joke-writing ability did not suffer and his lungs were okay. He was as sharp as ever.
Even moments after brain surgery Rodney didn't miss a beat. Rodney's doctor came to his bedside after he was taken off the respirator. He said, "Rodney, are you coughing up much?" And Rodney said, "Last week, five-hundred for a hooker."
Some of you may be aware that 4:20 is a symbolic time of day for many marijuana enthusiasts. About a year after

Rodney's brain surgery, he had heart surgery and due to complications his life ended... Coincidentally, or perhaps meaningfully, at 4:20 p.m. EST.

Guidelines for the Community-Based Distribution of Medical Cannabis in Canada

May 2006

Rielle Capler
British Columbia Compassion Club Society

Philippe Lucas
Vancouver Island Compassion Society

II. ACCESS TO DISPENSARIES

1. Eligibility Requirements

a. Age/Parental Permission

Community-based dispensaries should only distribute cannabis to those 18 years old and over, unless applicants have written consent from a parent or legal guardian.[5] This age-based restriction reflects the legal age of adulthood, while also recognizing that some people under the age of 18 may also need access to a safe source of medical cannabis. In recognition that the legal status and stigma of cannabis use may pose particular difficulties for those under 18 in accessing medical cannabis, dispensaries will continue to monitor the political and legal climate regarding this requirement.

b. Healthcare Practitioner Support

Clients of compassion clubs must have the support of an appropriately licenced healthcare practitioner to verify their medical condition and the therapeutic nature of their cannabis use. Medical cannabis use generally refers to applications that alleviate the suffering of specific symptoms and medical conditions, and to improve the overall sense of well-being. [6]

Despite resistance from their provincial and federal regulatory bodies, an increasing number of physicians support the medical use of cannabis by their patients, and are the main source of patient recommendations for access to medical cannabis.

Given that cannabis is an herbal medicine, recommendations for its use may also be permitted from doctors of Traditional Chinese Medicine and Naturopaths. These health care practitioners are experienced with herbal medicine and have licensing bodies and governing associations necessary for legal recognition and to ensure a certain quality of care and expertise.

c. Recommendations and Confirmation of Diagnosis

Obtaining support from healthcare practitioners for therapeutic cannabis use can be problematic, particularly in rural areas of the country. Many health practitioners refuse to recommend the use of cannabis, even if they believe that it may be therapeutically beneficial to their patients. Although some refusals are due to potential medical concerns, many are the result of the illegal status and social stigma of cannabis, pressure from professional associations and colleges, fear of liability and pressure from insurers, a lack of awareness of the latest clinical research, and general discomfort with the prescription of herbal medicines.

In recognition of this problematic political/legal/regulatory situation, many dispensaries have found it necessary to accept a simple proof of condition for certain ailments rather than requiring an actual recommendation for the use of cannabis. This can help balance both the dispensary's and the local community's need to ensure the legitimacy of the patient's medical claim, while also addressing the patient's need for safe and timely access to medical cannabis.

5. Compassion clubs may chose to require a higher age of entry in recognition of provincial or community norms.

6. It should be noted that while many legitimate medical cannabis users choose to use cannabis after hearing of and/or experiencing its therapeutic benefits, self-referral is not sufficient for access to compassion clubs in the current legal climate.

Therefore, in order to not unduly restrict availability of cannabis to persons who may receive health benefits from its use, a confirmation of diagnosis from an approved health care practitioner is the base requirement for access to a compassion club for those suffering from the following conditions:

HIV/AIDS, ADHD, Arthritis, Brain/Head Injury, Cancer, Colitis, Chemotherapy, Crohn's Disease, Epilepsy, Fibromyalgia, Glaucoma, Hepatitis C, Irritable Bowel Syndrome, Migraines, Multiple Sclerosis, Muscular Dystrophy, Nausea (chronic and debilitating), Pain (chronic), Paraplegia/Quadriplegia, Parkinson's Disease, Radiation Therapy, Seizure disorders, Sleep Disorders, Substance Addiction and Withdrawal.

The above list of conditions is not comprehensive and should be reviewed and modified periodically in light of emerging research or changing social/legal conditions. Any other condition requires an actual recommendation for the use of cannabis from a healthcare practitioner.

It should be noted that some health care practitioners refuse to even confirm their patient's diagnosis, highlighting the necessity for legal reform and professional education. In the meantime, dispensaries must facilitate this process a much as possible to assist their clients in getting the care that they require.

d. Documentation

Ideally, each compassion club will have a form for health care practitioners to fill out. The form will provide health care practitioners the opportunity to both confirm the diagnosis and recommend the use of cannabis. It will also allow them to indicate if they do not recommend the use of cannabis and to state their reasons.

Since experience suggests that some health care practitioners will not feel comfortable filling out these forms, the conditions that require a diagnosis only (see above section c) may be written on prescription pads or practitioner letterhead. In some cases, other government forms that indicate a medical diagnosis supported by a practitioner signature (i.e. disability forms) may be acceptable to confirm an applicant's condition. Prospective clients can also sign release of information forms, requesting that their practitioner release relevant medical information to the compassion club for the confirmation of a health condition.

To ensure the legitimacy of medical documentation, all forms must be faxed to the dispensary directly from the health care practitioner's office, and the dispensary must confirm the origin of the fax. Additionally, the legitimacy of health care practitioners must be verified with their respective licensing bodies.

e. Special Consideration: Mental Health Conditions

Mental health conditions may be the primary or secondary medical reason for the use of cannabis. Some compassion club clients have recommendations for the use of cannabis for mental health conditions such as bi-polar, schizophrenia,

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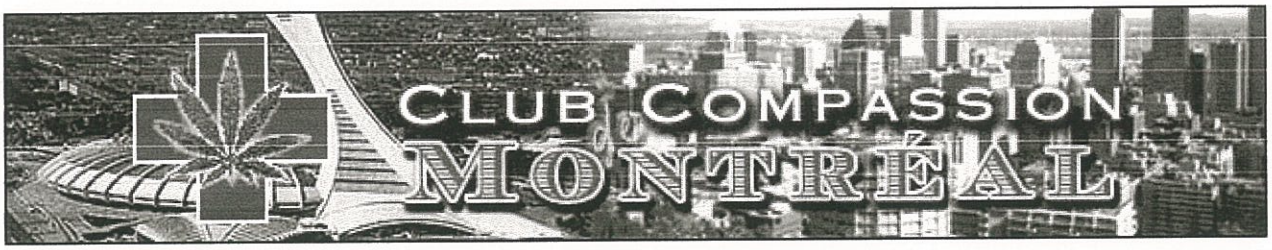

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Informations médicales

Symptômes traitables avec le cannabis

Nausée	Perte d'appétit
Douleur chronique	Perte de poids
Spasmes musculaires	

Conditions chroniques traitables avec le cannabis

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VIH/SIDA	Trouble déficitaire d'Attention/ Hyperactivité (TDAH)
Arthrite	Traumatisme crânien/à la tête
Cancer	Colite
Maladie de Crohn	Épilepsie
Chimiothérapie	Fribromyalgie
Glaucome	Hépatite C
Colon Irritable	Migraines
Sclérose en plaques	Dystrophie musculaire
Nausées	Douleurs chroniques
Paraplégie/Quadraplégie	Radiothérapie
Maladie de Parkinson	Affection neurologique
Problème de sommeil	Calme les effets de manque et aide à la désintoxication

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grassroots, action-oriented patient rights

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Cannabis Research

In response to the spurious and misleading claims by Health Canada suggesting that the safety and efficacy of cannabis has never been examined anywhere in the world, Canadians for Safe Access has collected some of the most relevant international research into cannabinoids and the therapeutic use of cannabis. This research includes peer-reviewed and published studies, as well as some of the most extensive government examinations into cannabis safety and its potential as a therapeutic agent.

"There have been no studies anywhere in the world that have been able to confirm medicinal benefit."
-- Anne McLellan, The Globe and Mail, July 10, 2003

This research can be divided into several categories;

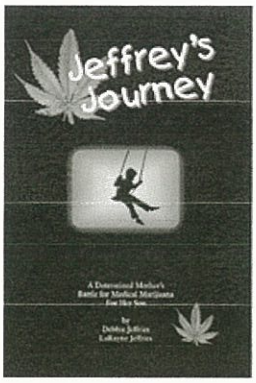
- Safety
- Efficacy
- Potency
- Standards

Symptoms/Conditions (updated: 11/08/07)

- AIDS-HIV
- Alzheimer's Disease
- Appetite Stimulation
- Attention Deficit Disorder (ADD)
- Bi-polar Disorder
- Cancer
- Cognition and Memory
- Diabetes
- Depression
- Hepatitis-C and Liver Disease
- Inflammation
- Multiple Sclerosis
- Osteoporosis
- Pain
- Parkinson's Disease
- Pregnancy
- Schizophrenia
- Sleep

Miscellaneous

- Recent Research on Medical Marijuana
- Marijuana-like compounds in body block pain



Jeffery's Journey
A determined mother's battle for Medical Marijuana for her son

by Philippe Lucas

"Jeffrey's Journey" is the very real and harrowing story of a young boy named Jeffrey and his inner battle with severe emotional and behavioural problems. Written by Debbie and LaRayne Jeffries - the boy's mother and grandmother - Jeffrey's tale takes him from the depths of prescription drug despair, to the high of successful cannabis-based treatment. Before Jeffrey even reached adolescence, he had been diagnosed with multiple emotional and behavioural conditions: ADHD (Attention Deficit Hyperactive Disorder), PTSD (Post Traumatic Stress Disorder), OCD(Obsessive-Compulsive Disorder), ODD (Oppositional Defiant Disorder), IED (Intermittent Explosive Disorder), and Bi-Polar Disorder... to name but a few. Along with these diagnoses came a plethora of pharmaceutical treatments: from Adderall to Zoloft and Zyprexa, Jeffrey was prescribed over a dozen anti-anxiety, anti-depressant drugs, many of which have never even been tested or approved for use by children. After seeing that most of these either had no effect or worsened Jeffrey's condition,

Debbie began to explore the use of medicinal cannabis. This was a rapid and significant transition for the Jeffries family, who describe themselves as conservative Christians. Debbie admits that when California's Proposition 215 (which led to the legalization of medicinal cannabis in California) appeared on the state ballot, she voted against it. However, after contacting WAMM (Wo/Men Alliance for Medical Marijuana) and speaking with founder/director Valerie Corral and speaking with an informed physician, she decided to try this untested therapy. Debbie recounts the morning of Jeffrey's introduction to marijuana therapy through cannabis-laced muffins: "Within 1/2 hour of ingesting the first piece of muffin, I had a new child. We were driving to school, and as I merged into a new lane of highway traffic, Jeff looked over at me and smiled, "Mommy, I feel happy, not mad, and my head doesn't feel so noisy!" This was the beginning of a successful treatment regimen that soon led to Jeffrey being able to make friends and have an 8th birthday party with other kids at the local Chuck E. Cheese's, something that would have been previously unthinkable for the Jeffries.

Sadly, there have been some setbacks. Last year's federal bust of the WAMM cannabis garden led to a break in Jeffrey's line of medicine, which led to a decline of his emotional/behavioural state. This was only restored once the Jeffries were able to once again access the particular strain that helped calm Jeffrey's mind and resultant behaviour. "Jeffrey's Journey" is the tale of a family's sorrow and desperation, and the hope that finally came from an unlikely source: cannabis. Although therapeutic cannabis is by no means a cure-all, it has been able to give the Jeffries happiness where there was once only fear and frustration. As I finished Jeffrey's Journey, I had to wonder how many more families might be struggling with similar problems, and how many severely emotionally handicapped children might benefit from the information in this brave book.

*MAP posted-by: Richard Lake, Pubdate: Fri, 02 May 2003, Source: DrugSense Weekly, Website: http://www.drugsense.org/current.htm. **Details:** http://www. mapinc.org/media/2899*
***Note:** Philippe Lucas is Director of Communications for DrugSense. He is also the founder and director of the Vancouver Island Compassion Society, a medicinal cannabis organization based in Victoria, B.C., http://thevics.com/ **URL:** http://www.mapinc.org/drugnews/v03.n638.a03.html*

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Iain Sumner

maryjane@skinup.co.uk

Clinical Depression and ADHD

Wednesday 05 Dec 2001

From the ages of 11-16 I was prescribed Ritalin. From the ages of 14-16 I was prescribed Cipramil. Due to the large doses of Ritalin I was consuming, I was taking Respiridone (sedative) to knock me to sleep. I knew the medication was bad for me but when I tried to take myself off it my family threatened to throw me out because they thought I would be some kind of uncontrollable monster without it. After being thrown out anyway after I turned 16 I took myself off everything at once. I was in bed for a week and a half (not the best way to spend the first week and whatever that you're living on your own). I went without medication for a long time but things still weren't right. I now smoke cannabis moderately and steadily throughout the day with very little issue.

If any of you are on the medication I was on or suffer from similar issues I suggest you try small and regular doses of cannabis rather than medication. It's more expensive but my life is so much better since I started doing so.

-Iain

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Matt

Lawtino85@aol.com

Attention Deficit Hyperactivity Disorder - ADHD

Thursday 31 Oct 2002

I have Adult Executive corporate ADHD and have found that use of moderate amounts of cannabis is very helpful. I consider the effects of cannabis to be more desirable in most instances, than the medications I have been prescribed.

I am currently on 25-mg capsules of Adorol extended Release. Adorol worked great the first six months during the "Honey moon effect" as my doctor called it. The result during the first six months I felt outweighed the side effects, which included anxiety, nausea, appetite depression, and trouble sleeping even hours after the medication wore off. During the effective period of Aderol I was able to focus, concentrate, listen to conversation, and retain information much easier then ever before. The results were amazing. Soon after that time period I started noticing that the Adorol seemed to be working less and less while the side effects remained constant.

When I went to the doctor I told her that the medication was not working that well anymore and I informed her of the side effects. She decided to move me up from 20 mg to 30 mg Adorol. The first day on the 30-mg capsule was horrid the side effects were much worse and I could not focus or concentrate at all. I told myself that maybe it was just a bad day so the next day I took another and it was another bad experience. I called my doctor and then was put on Conserta which had almost identical side effects but much worse anxiety and no positive results.

Since then I have tried other medications and dosages with no luck until I found that when smoking cannabis I am able to relax and not be so hyper. It also allows me to listen to other people for extended periods of time without becoming uninterested and bored as fast. Mild dosage allows me to get in the mood to spend more time completing many tasks including work and really increases my mood and how I feel.

I have noticed no side effects with cannabis use besides a small increase in appetite, which is not a concern, as I am healthy and fit. I am angered that the US government is denying its citizens this extremely safe and effective medicine. As a result of this it is a rare occasion I get a chance to use this plant in medical application because I have little opportunity in obtaining it. I don't want to deal with shady drug dealers and I don't want to risk prosecution for a drug that is less harmful then many proscription drugs out there.

Hopefully people will see through the misinformation and deception because if I can benefit from cannabis many other people probably can too.

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Kevin

ADHD

Thursday 06 Dec 2007

I use medical cannabis to treat my ADD and it is far better than the stimulant medications, far safer too...I can say with no exaggeration cannabis has saved my life !!!!! the vast majority of people now know the truth so please decriminalize it NOW and focus on hard drugs that are actually doing harm, stop this reefer madness lies.

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Attention Deficit Disorder (ADD) and Marihuana

by
Michael Knutson

Thank you for responding to my letter about how marihuana has been useful in the treatment of my ADD/ADHD [attention deficit hyperactivity disorder]. I'm happy to participate in your efforts to share information with other individuals about how marihuana is useful as a medicine, so I am sending you this account to explain how marihuana has been beneficial to me.

I've experienced problems with ADD/ADHD as far back in childhood as I can remember. My disorder was first noticed in elementary school when I began to show continuous disruptive, boisterous, and hyperactive behavior. I remember having trouble sitting still and concentrating in class. My first visit to see a pediatrician about this abnormal and disruptive behavior was at the age of seven. Until then my behavior was an enigma to my teachers and parents.

My diagnosis by Dr. Pat McGuire at the Marshfield Clinic in Chippewa Falls was ADD/ADHD. Dr. McGuire prescribed Ritalin for the first time.

The Ritalin seemed to drastically change my personality into a whole different child, but for the worse. Even though I had trouble sitting still and concentrating in school, I was never really a serious threat to other children and adults. So was it necessary to prescribe for me a man-made, mind-altering substance that was going to give me the side effects like dizziness, paranoia, weight loss, insomnia, multiple personalities, etc.? Was it really acceptable to exchange my disruptive behavior and having trouble sitting in my chair for all of these ill side effects?

My doctors, physiologists, and pediatricians prescribed Ritalin, Lithium, Prozac, Zanax, Busbar, Proxil, Valium and Dexedrine. Their diagnoses were ADD/ADHD, manic-depression, and even anxiety disorder. These medications and diagnoses were given to me at the ages of seven through sixteen. But none of the medications helped me with my hyperactivity, lack of concentration, disruptive behavior, and depression. Any every different doctor I saw seemed to have a different diagnosis. Which one of them was right? Who knows, ha!! So finally, at the age of seventeen, I gave up on all the doctors and started to self-medicate myself with alcohol. I eventually became an alcoholic. It seemed to be the only thing at the time to help me escape from my problems and feel good. But the alcohol and my disorder did not mix very well. When I

drank I ended up in trouble with the law quite regularly. I felt there was no hope for me, and I was even suicidal. I spent many lonely days fighting the battles within myself, but I was simply going nowhere. That is, until the day I was introduced to the wonderful natural herb that God's green earth produced for me to use as a medicine (marihuana). What a prodigy! What a beautiful organic plant! After that day, I began to use marihuana consistently in acceptable doses to medicate myself. It has been the most therapeutically active drug for me. I've been accomplishing things I could never have done before like: keep a job, relax, have long conversations, feel good about myself, find inner peace, etc. I could go on and on about all the positive energy that marihuana gives me, as well as the absolute inner calm that I know is necessary to my well being. I only have one side effect, a dry mouth and throat sometimes. That ain't nothing that a nice cold glass of water can't take care of. I'm still not sure what my real diagnosis is. But I sure know what the cure is! That's all that matters.

Back to Shared Stories Index

Attention Deficit Disorder (ADD) by L. D. K.

Today, at 32, I am a married computer whiz, master's degree candidate, and proudly employed as an administrator at one of the most prestigious colleges in America. Before I smoked Marijuana no one thought that I would ever go to college. As a child I was diagnosed with dyslexia and learning disabilities in the subjects of Math and English. I spent my childhood plagued by the frustrations and desperation of teachers, my parents and tutors who couldn't accept a straight C average from a bright student with an above average I.Q. I can still hear the choruses of "Try harder!" and "Concentrate harder!" that used to daily echo through my young ears heightening my anxiety level and pounding down my fragile self-esteem. I was always trying as hard as humanly possible, but no one believed me.

By the grace of God I graduated from high school and was in the process of failing my first year of college when I first smoked marijuana. After spending almost every minute of my Fall semester studying in my room or at the tutoring center I still managed to earn a pathetic 1.75 GPA. My mother agreed to let me go back to school spring semester with the understanding that my grades would have to improve dramatically if I was ever to see my Sophomore year.

I was hopeless going into my spring semester. I was already doing my best and failing, since leaving college seemed inevitable I decided to have fun while I was still there. I started studying less and spending time with older students who smoked a lot of marijuana.

In the beginning I started smoking marijuana with them every once in a while. Noticing that smoking made me feel calm, relaxed, and confident, I started smoking more often. By the middle of the semester I was smoking everyday and going to my classes high. When I was high I could concentrate better and not only thoroughly understand the classes and comprehend the textbooks, but for once I could recall the material during tests and exams.

For the first time in my life my papers were being read out loud in class, and my peers looked to me for answers. By the end of this miraculous semester I was on the Dean's list with a GPA of 3.45. (If I started smoking daily at the beginning of the semester I would have earned a straight 4.0.) That semester was my first signal that something was medically wrong with my body chemistry. I continued to smoke marijuana through out college to help me study but not on a daily basis. After college it took me 2 years to identify Attention Deficit Disorder as the source of my academic difficulties and 10 years for a doctor to finally diagnose and treat me (There is still an alarming number of doctors out there who continue to deny that ADD affects women). For the past few years I have exchanged marijuana for more socially acceptable Ritalin and have been thriving in every aspect of my life. I still use marijuana for relief of severe menstrual cramps, and to relieve occasional muscle pain.

Treatment of Cancer, ADD, and PTSD by Patty Allen

I am a 41-year-old woman, mother of three, with breast cancer, currently undergoing chemotherapy. Marihuana is definitely helpful in easing nausea associated with chemotherapy. It also helps with the pain I am experiencing with Taxol chemotherapy. In both instances it takes the edge off -- meaning it has an effect, but doesn't totally eliminate the symptoms. Like a painkiller, it doesn't take away the pain; it just makes it more bearable. So, whether the effect is mental or physical, or both, if there is any question whether it works...yes, it does something.

I also believe in its use for ADD and other neurological disorders, including depression. I am much more able to focus (although I admit it impairs my memory) on a book or movie when I have smoked.

I also suffer from PTSD. I believe its best use is in "calming" the periods of intense anger I experience when memories are triggered. Perspective returns and I am able to "cope" with the issues.

I live in Massachusetts, and at my age find that it is getting more and more difficult to locate quality marihuana for a reasonable price. It isn't that people my age aren't smoking it; it is that they don't talk about it. My teenager has a better chance of finding it than I do. For this reason I support legalization for medicinal use.

If you would like more information, I would be willing to discuss this with you. I have three more months of chemotherapy left and would be happy to be a guinea pig for someone's science project and/or an advocate for medicinal use for pain and nausea relief during chemotherapy.

In return, I'd like to become more involved in your work.

ADHD by Ryan P

Marijuana helps me alot.

Many memories of my life (and current circumstances) bring a great deal of stress especially when I get mad (I think of something bad which leads to more and more bad thoughts that wont stop and it just pisses me off more). I was taken from my parents at age 3 for an uncertain reasons and placed into foster home after foster home because I was a 'bad child', then after about 6 or so homes and 5 years of very bad stuff, I was put into a residential center. Neither place was fun and was making my childhood a worse memory. I was adopted at age 8 and put directly into the special education program at the local grade school, which I think was a bad idea because I could not get the grasp of how the real world/people worked, and being in a special-ed class with kids having the same problems as me was not teaching me good things. I kicked a teacher while being restrained one day (in one of MANY violent tantrums) (about 4 months after starting school), and was expelled into a private alternative school where I seemed to survive and was started on Ritalin. When Ritalin turned me into a zombie, I think adderall, imipromine (might have been to help me sleep), and some others were tried as well.

I was diagnosed with ADHD around age 8 and was placed on many different drugs such as methylphenidate (Ritalin), and probably others that I do not remember. I stopped taking the medications because they were not helping my anger, I barely ate, and things were going to hell. After that, I barely made it out of the private alternative school I was kicked into, finally making back into the public school system. I had about 4 months of 8th grade at a real school to get ready for public high school. I was still having problems but was being heavily monitored and was in after-school therapy 3 times a school-week for about 4 years. Once high school started, things were very different from what I was used to, and I was expected to keep up with everyone (not that I didn't understand the class work or couldn't do it, but things were very out of order and confusing because I was not in-sync with the public school system or anyone in it).

First smoked 3/4 into freshman year. I first tried marijuana not knowing it would help alleviate my stress, depression, and ADHD/RBS while making me finally feel good about myself as a person, but to 'be cool and get high'. When I went to class 'high', I did nothing but pay attention to the teacher (not being able to clearly see the board/TV would usually cause me to not write anything because I couldn't see what to write, or the examples/notes. Being hassled about this was not easy because I did not like to blame it on my lazy eye, or the fact I was too nervous wondering what everyone else was thinking about me (which is why I wouldn't sit up front or closer, usually in the back corner), and this is still a problem. Unfortunately, this was the last class of the day so I could not test "wow I can actually concentrate on what's going on and follow through with what needs to be done without getting confused and lost" for the

whole school day. I tried getting high after 2nd period to see how I could do in my English class, I could concentrate on what was being read without thinking "oh crap she is looking at me" (or something similar) over and over, I was too into the book/movie. I could actually write stories and things I would normally not be able to do (I'd start something else and then forget about my ideas, and would get very mad because this was getting me into trouble). I could calculate math better because I could concentrate on the problems without being distracted and forgetting what I was thinking. I could think more logically, and things came to me way easier. I was also a more social person and could carry on a decent conversation while 'high', it made me more 'loosened up' I guess.

After a short period of 'experimenting' I got caught up in 'fixing myself' and being able to enjoy life, that I started using more and more to level things out and keep being depressed/angry all the time on a down-low. This only caused problems (mostly because its illegal thus most peoples opinions are evil and uneducated), and I got into trouble with my dad for using drugs, got kicked out of public school (back into the previous alternative school) because I was using on school premises and was caught 3 times with a pipe in my possession. I found it nearly unbearable to live without being 'high' (I think being high is a side-effect of using cannabis as a medication) because things were so much easier to comprehend and my brain and body went much smoother, and I rarely got angry. I lost all my dealers by getting kicked out of public school and resorted to some kids around the block. They just dragged me down into much heavier (social, getting wasted) use along with drinking occasionally. When medicating, I never smoked to get high, just little bit every 3 or 4 hours to get mellow and level things out. At parties/friends' house was different. I could not even have friends until I started using because I was too worried about things and would always 'do my own thing'.

I have resorted to some pretty stupid things to attain marijuana. I pretty much feel that life is a pile of sh** when I'm not 'high' because I know things can be SO much better, but the little stupid things (like the law) are keeping me from sustaining a mentality that life is going to be okay. But as soon as the marijuana wears off, I am angry, cant sleep, depressed (I guess because I'm tired of living with all this crap when I know it can all be helped (for a while at least)), and things are again back to the way they have been my whole life, also knowing that it will be very hard to keep medicating with what I know works because people will not recognize it DOES help some people, and can be used in GOOD ways instead of all the hyped-up evil.

The stress factors of the very irritating things I have been through stir up memories and emotions that I must get rid of somehow or another or I feel like I'm going to kill somebody (these feelings can be suppressed while cannabis helps me focus my brain for more productive things like computers (my gift), and my life). I found that it is not the marijuana wearing off that aids in area of stress and depression, but the fact that going back to being screwed up

knowing I could make things all better and function properly with just a little help, but that help is out of my reach (legally, and financially) which causes more stress and depression. This is not necessary.

I do not like smoking, or like smoke period. I think digesting (cooking/eating) cannabis would be substantially better for my health than smoking it (unless things get too hairy and need immediate relief); I have not been able to experience the difference between eating and smoking because I never have had enough, and barely ever have enough to keep my sanity. Like, when I get mad (and I have been known to get mad at the dumbest things), I get extremely angry because after thinking a bit, I know all this stuff, and thoughts go from one to another, yet I can't make things just work out the way they should, and it's not right. I usually try and understand both sides of the problem, but can only really follow through with my thoughts if I am medicated (with cannabis, which seems to be my all-in-one potion).

I am aware there are other man-made medications for ADHD, RBS, depression, anxiety, and whatever else I may have, but I highly doubt they provide the same sense of well-being (relief of depression and clearing the head) while simultaneously treating a persons abnormal brain chemistry that drives them up the wall so to speak. Works on all my aches and pains as well. I have not tried dronabinol (Marinol) because from what I have read, I feel herbal cannabis (and its 60+ cannabinoids) has a much more positive effect on my problems than pure synthesized-THC (which still does not do all the same things as naturally occurring THC). Besides, I do not like the idea of taking any form of coke or speed (that can cause me not to eat (which is already a problem, I get so stressed that I get sick and throw up, and then have to smoke to relieve the stress and be able to put something in my stomach) and may cause a stimulant dependency) when a plant from the ground when eaten, can fix most if not all of my problems allowing me to continue life knowing things will be alright for a while. Why prohibit those who can benefit from natural herbs legally do so? People are dependant on a LOT of things to keep themselves well.

That is just my say in what marijuana does in part of ADD and other psychological areas. I know plenty of other people who can say pretty much say the same.

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Endocannabinoid System Overview

At A Glance

- What is the Endocannabinoid System ?
- Cellular Biology
- Normal Function of the Endocannabinoid System
- Abnormal Function of the Endocannabinoid System
- Interaction with other pathways

← Select →

	Energy Balance and Metabolic Regulation	Dyslipidemia	Glucose Homeostasis
Adipocyte		●	●
CNS	●		
Gut	●		
Liver	●	●	●
Pancreas	●		●
Skeletal Muscle			●

Mission

The mission of the Endocannabinoid System Network (ECSN) is to serve as a multifaceted educational resource that will help scientists and clinicians understand and communicate the mechanisms and functions of the endocannabinoid system (ECS) – integrating knowledge of the cellular/molecular basis with the neural and systemic effects.

Breaking News

ICRS - Carolina Cannabinoid Collaborative
Wrightsville Beach, NC
Oct 12-14, 2007
Click here for more meeting information

ICRS - 2007 Symposium
June 26 - July 1, 2007

What's New on the Site

The 17th Annual International Cannabinoid Research Society Interviews

Endocannabinoid System Animation

Interactive Historical Timeline

ECSN Reports

ICRS - 2007 Symposium
June 26 - July 1, 2007
St-Sauveur, (Québec) Canada

Handbook

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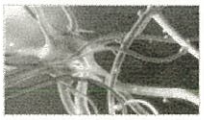
Online Slide Library

The online slide library is a repository of slide presentations and individual slides pertaining to the endocannabinoid system. The entire slide library is searchable by topic and keywords, and the slides can be downloaded.

Video Animations

Endocannabinoid System Animation



Online Presentations

Introduction to the Endocannabinoid System

James L. Early, MD
Clinical Associate Professor, Department of Internal Medicine. University of Kansas School of Medicine-Wichita, Wichita, Kansas

Working Group

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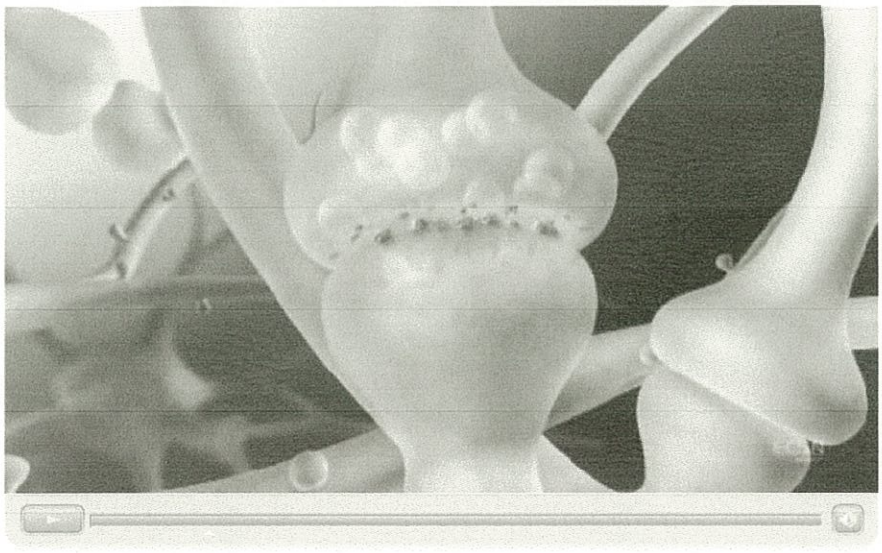
Video Animation



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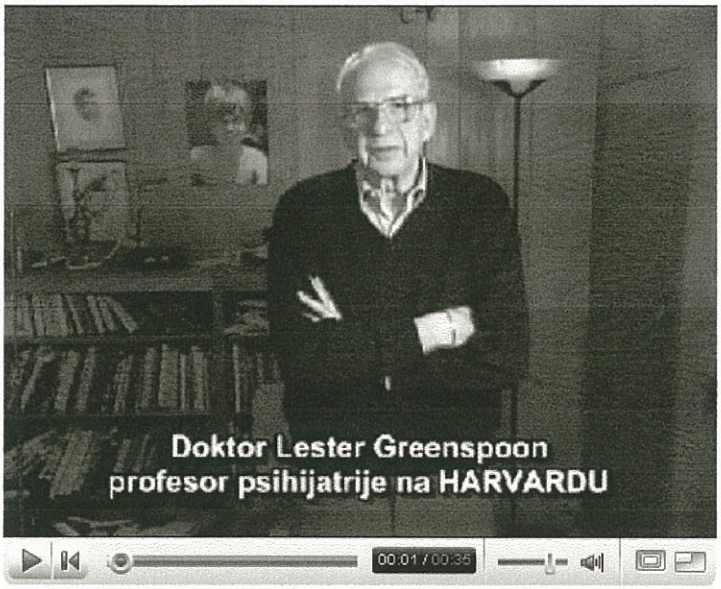

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00:21
OPRAH


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Dr. Lester Grinspoon

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Comments & Responses

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thefakeyeti (2 months ago) Reply 0

You know theres something wrong when the punishment for a drug, is far worse then using the drug itself...

Rick02115 (5 months ago) Reply 0

his books marijuana reconsidered and psychedelic drugs reconsidered are probably the most credible, scientifically sound, and completely readable books on their subjects in print. if not, please let me know what else is.

lequebecfume (7 months ago) Reply +1

To bad there is not more of this video, two minutes does not do justice to the man nor his ideas.

LEQ

thanks for posting it :)

DalFornification (8 months ago) Reply 0

He's very smart and insightful I feel like. It's all about "anecdotal evidence" and "double-blind" scientific studies as far as the medical debate is concerned. If i remember correctly. I may have been too lifted. HAHAHA.

666theCRETIN666 (9 months ago) Reply 0

u fucking rule mate. grinspoon grinspoon grinspoon

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XXX

Medical marijuana for ADD and its good for kids!

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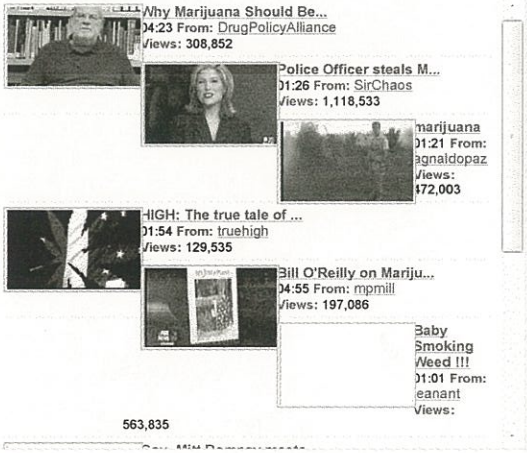

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Text Comments (1837)

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rickrocker (2 days ago) Reply 0

Hey, have you seen Keith Olberman's ratings? Oh, that's right, he doesn't have any because no one watches him.

rickrocker (2 days ago) Show Reply -6

whatsup14666 (1 day ago) Reply -1

lmao your ignorance is very amusing.

bg50287 (1 day ago) Reply 0

druggies dude are you serious, honestly history is doomed to repeat itself, alcohol was illegal once upon a time, any one who consumed or even touched alcohol was considered a criminal, I dont even smoke, but I would love to see it legalized, so that we can tax it and strengthen our economy instead of sending all of our money out to drug lords

Justletmeinyoursite (4 days ago) Reply +2

Just shut the fuck up and legalize it already.

SpaceDrummer16 (4 days ago) Reply 0

your a fucking idiot :)>

hockeyplayer168 (5 days ago) Reply 0

I support this movement, but global warming prevention is clearly a bit of a stretch.

DIRTDIRTDIRTNASTY (5 days ago) Reply -2

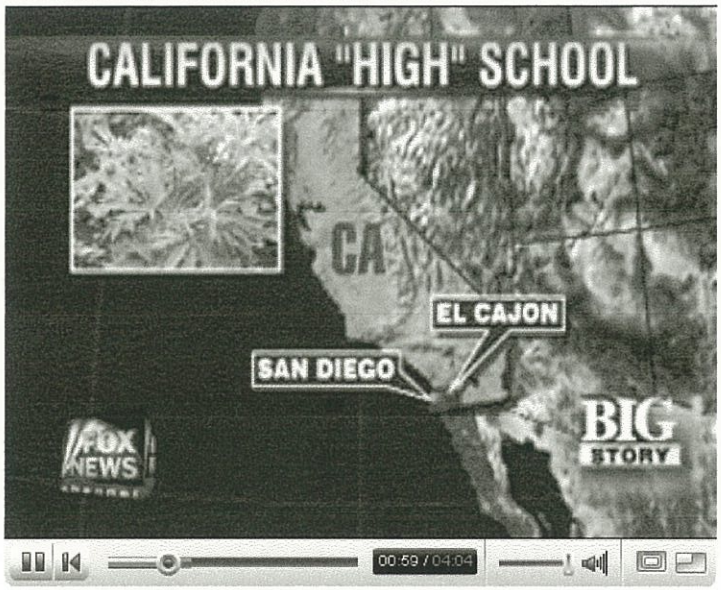
i have an abnormally large penis do they give medicinal marijuana for that?

bg50287 (1 day ago) Reply -1

Avocate

145

Medical Marijuana in California High School



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Text Comments (295)

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CBPrider (1 day ago) Reply 0

hell yea i live in da bay area i just nevr realized thy didnt do dis in othr states but they hav cannabus clubs here everywher u just need 2 be 18 nd tel the doctor ur fake pains dat u hav nd u can buy one for like a hun

CBPrider (1 day ago) Reply 0

but then again thers also lots off ppl dat really do hav serious problems nd need the medicine...

Chet74 (2 days ago) Reply 0

People are starting to see whats been in front of them the whole time. Marijuana is a natural plant that can be used for many, many, many good purposes. You feel pain or can't eat? just smoke some indica.

zakslayya (6 days ago) Reply 0

shgit i smoke this all the time and never even knew how good it was for me!

feedmattnow (1 week ago) Reply 0

mobbin out to cali!!!

pandaonweed (1 week ago) Reply 0

yeah fuck people its all the goverment's fault because its not made from america let hope our new president is a wise guy

Grolsch09 (1 week ago) Reply 0

GO TO LOS ANGELES

swishersweets2008 (1 week ago) Reply +2

im glad people are fighting to end this war on marijuana.

From: MPPstaff
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FOX News presents a surprisingly balanced repor...
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Added: March 12, 2007

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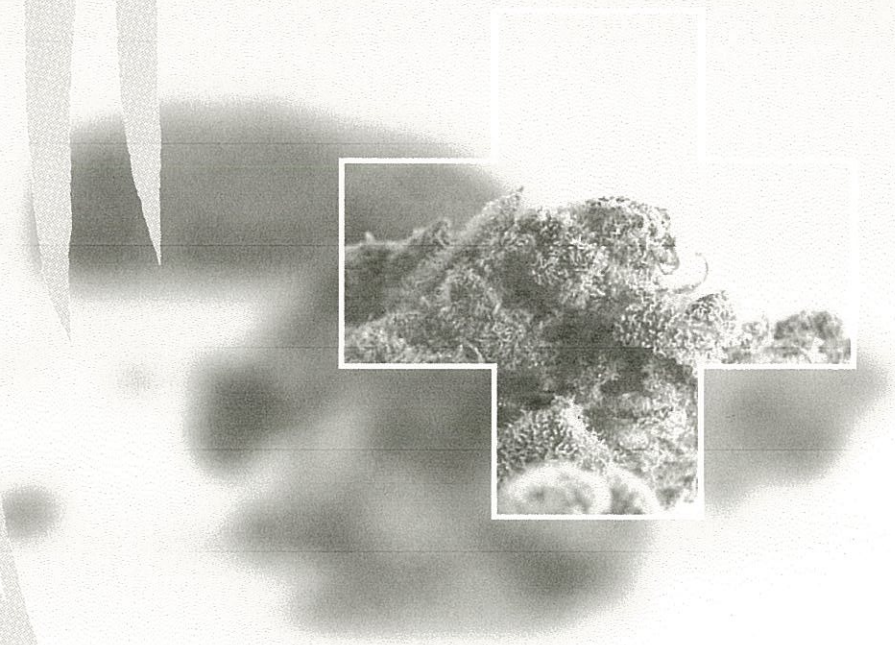
Medicinale Cannabis

Informatiebrochure voor patiënten



Ministerie van Volksgezondheid, Welzijn en Sport
DGV, Nederlands instituut voor verantwoord medicijngebruik

Ministry of Health, Welfare and Sports
Office of Medicinal Cannabis
P.O. Box 16144
NL-2500 BC The Hague
The Netherlands

Medicinal Cannabis

Information for Health Care Professionals version date: 15 November 2007

1. Name of drug

Cannabis, dried flowers (Cannabis flos)
Cannabis is supplied in three varieties:

Variety	Dronabinol /THC	Cannabidiol/CBD
Bedrocan	approx. 18%	<1%
Bedrobinol	approx. 11%	<1%
Bediol (granulate)	approx. 6%	approx. 7.5%

2. Qualitative and quantitative composition

Cannabis is made up of the dried inflorescences of the female *Cannabis* sativa L. plant, and is cultivated and processed under standardised conditions in order to obtain a consistent product. Cannabis contains several constituents including substances that belong to the cannabinoids, such as dronabinol (delta-9-tetrahydrocannabinol, THC) and cannabidiol (CBD). The content of cannabinoids depends on the type of cannabis.

3. Pharmaceutical form

Dried female flowers (gamma-irradiated)

4. Clinical information

4.1 Therapeutic indications

The efficacy of cannabis-components has been examined in various small and large scale clinical studies. Results from these studies indicate that medicinal cannabis may have a positive therapeutic effect on the symptomatic treatment of:

- disorders that involve slight spasticity with pain (multiple sclerosis, spinal chord injuries)
- nausea and vomiting (resulting from chemotherapy, radiotherapy and HIV combination therapy)
- chronic pain (in particular neurogenic pain)
- Gilles de la Tourette syndrome
- palliative treatment of cancer and AIDS

The use of cannabis is indicated only when the results with current treatment protocols are unsatisfactory or when too many side-effects occur.

Medical literature also mentions a significant number of other indications. However, the scientific basis for application in the case of these indications is still small, and more research is needed.

The variety of cannabis to be used must be established by experience. So far, no scientific evidence exists which point towards preference of one of the varieties for a certain indication. Recent data indicate that DRONABINOL and CBD in combination improve pain and spasm in MS-patients. Inhaling cannabis with a high content of dronabinol increases the risk of psychological side-effects. This can be avoided when using cannabis for the first few times, by choosing a variety with a low content of dronabinol or through oral administration in tea.

4.2 Dosage and method of administration
The required amount of cannabis per day should be determined on an individual basis. The initial dosage should be low and can be increased slowly as symptoms indicate. The dosage needed to achieve the desired effects is often different/ lower than the dosage at which psychological side-effects occur (become high).

Two methods of administration are recommended: orally or via inhalation. Inhaling cannabis exhibits a stronger and faster therapeutic effect compared to oral administration.

Oral (tea): (see also 6.6)
drink 1 cup (0.2 litre) of tea in the evening, hot or cold
When using this method, keep in mind that it takes an average of two weeks before the maximum effect is achieved; if after roughly two weeks the result is too limited or unsatisfactory, drink one extra cup (0.2 litre) in the morning.

Inhalation (vaporizer): (see also 6.6.)
1-2 times a day, inhale a few times until the desired effect is reached or until psychological side-effects occur. Wait 5-15 minutes after the first inhalation and between inhalations.
When using the inhalation method, the strength of the cannabis must be kept in mind. Be careful about the dosage when switching from one variety of cannabis to another, especially if cannabis with a lower content of dronabinol was used earlier.
With repeated administration of cannabis, it takes 2 weeks to arrive at steady-state concentrations of dronabinol. This must be kept in mind when evaluating the activity of the drug.

4.3 Contra-indications
The use of cannabis is not recommended for patients predisposed to psychotic disorders. Use cautiously in patients with underlying psychological problems.

4.4 Special warnings and precautions when using cannabis
Patients with heart diseases (heart arrhythmias, angina pectoris) should avoid high doses of cannabis because of the cardiovascular side-effects (in particular tachycardia). Tolerance to these effects develops within a few days to weeks. The dosage may only be increased slowly as indicated by the effects on the heart and only after consultation with the physician.
The psychological effects of cannabis can be disturbing for inexperienced users. It is advised to administer cannabis for the first time in a quiet and familiar setting, and in the presence of another person who can calm down the patient if necessary.
Smoking is not recommended. Cannabis smoke contains harmful combustion products, including carcinogens and carbon monoxide. As a result, frequent use of smoked cannabis over a long period of time presumably exposes users to health risks associated with smoking. Smoking cannabis can impair pulmonary function (histopathological changes in the mucous membranes) and reduce resistance to infection. Regular cannabis smokers can develop pharyngitis, rhinitis

and COPD (Chronic Obstructive Pulmonary Disease). To limit the damage caused by combustion products, cannabis can be inhaled by a vaporizer.

4.5 Interactions with other drugs and other forms of interaction
There are known cumulative effects when cannabis is used at the same time with other tranquillizing substances such as alcohol, benzodiazepines and opiates. *Basically there is only one research into interactions with other drugs. Finding was that there were no potential effects of medicinal cannabis on the pharmacokinetics of concomitantly administerd irinotecan and docetaxel, or other (anticancer) drugs.*
The effective dosage of opiates was found to be significantly decreased in the case of combination of opiates with cannabis in animal studies.

Because of the high first-pass effect in the liver, particularly in the case of oral administration of cannabis, it is possible that pharmacokinetic interactions could occur with drugs, which are broken down by the isoenzymes CYP2C9 and CYP3A4 in the cytochrome P450 system. Drugs that inhibit these isoenzymes are macrolides (in particular claritromycin and erythromycin), antimycotics (itraconazole, fluconazole, ketoconazole and miconazole), calcium antagonists (in particular diltiazem and verapamil), HIV protease inhibitors (in particular ritonavir), amiodarone and isoniazid. Simultaneous use of the enzyme inhibitors mentioned above can increase the bioavailability of dronabinol and with that, the possibility of additional side-effects.

Drugs that accelerate the breakdown of dronabinol via the isoenzymes mentioned are rifampicin, carbamazepine, phenobarbital, phenytoin, primidone, rifabutin, troglitazone and Saint John's Wort. When a patient stops taking these drugs, an increase in the bioavailability of dronabinol may be expected.
Interactions are also possible with drugs which (like dronabinol) are strongly bound to plasma proteins.

4.6 Pregnancy and breastfeeding
Use of cannabis during pregnancy should be avoided. Dronabinol is known to reach the fetus via the umbilical cord. There are no indications that the use of cannabis during pregnancy causes deformities. Research has not shown any unequivocal effect on growth parameters. School-aged children who were exposed to cannabis while in utero have a normal overall IQ but score lower on certain aspects (in particular, in their ability for abstract-visual reasoning, memory function, and the executive function, which is the ability to demonstrate flexible, purposeful behaviour). Hyperactivity, concentration problems and impulsivity are also reported in 10-year olds.
Dronabinol has been detected in breast milk. Therefore, the use of cannabis while breastfeeding is not recommended.

4.7 Effect on ability to drive and operate equipment
The use of cannabis can reduce reaction-time and lower concentration. This may create problems in carrying out everyday activities. Participating in traffic is forbidden in the Netherlands and operating equipment is not recommended.

4.8 Side-effects

The psychological side-effects of cannabis can vary widely, and depend on several factors: the amount of cannabis used, the method of administration, the user's experience with cannabis and personal constitution, such as the person's state of mind at the time of use and how open the user is to experiencing the effects. A person can become "high" after using cannabis. This is a feeling of euphoria that slowly changes into a pleasant sensation of calm and rest. Users can also experience other effects while "high", such as sedation, cheerfulness with fits of laughter, hunger, a heightened sensitivity to perceptions of colour and music, a disrupted sense of time and space, and lethargy. This altered perception can give rise to a sense of anxiety, panic and confusion. Restlessness and insomnia are also reported. Cannabis can sometimes provoke a psychotic reaction, characterized by delusions and hallucinations. A genetic relationship between cannabis use and schizophrenia has been established, although it is not clear whether the relationshop is causal.

Physical side-effects of cannabis are:

- tachycardia
- orthostatic hypotension
- headache
- dizzyness
- sense of hot or cold in hands and feet
- red burning eyes
- muscle weakness
- dry mouth
- in cannabis smokers (and after inhaling): irritation of the airways

These effects are temporary and disappear a few hours after use.
Long-standing, intensive use of cannabis is presumed to have an effect on cognition, but this is reversible. In some cases, cannabis use results in dependence and abuse. Chronic users who stop can experience physical withdrawal symptoms such as mild forms of restlessness, irritability, insomnia and nausea.

4.9 Overdose

An overdose of cannabis may cause depression or feelings of fear, to the point of panic and fainting. The symptoms should spontaneously disappear in a few hours. In case of overdose, benzodiazepines (diazepam IV) can be administered if needed. Tachycardia can be treated with a beta blocker (propranolol IV).

5. Pharmacological properties

5.1 Pharmacodynamic properties

Cannabinoids act on the cannabinoid receptors. At least two different receptors (G-protein coupled receptors) have been identified: CB_1 and CB_2 receptors. CB_1 receptors are found particularly in the central nervous system, while the CB_2 type are peripheral and located mainly in the immune system and gastrointestinal tract.

5.2 Pharmacokinetic properties

Absorption

The absorption of cannabinoids in the body is determined by the method of administration. When cannabis is *inhaled,* the cannabinoids are absorbed into the blood within minutes via the lungs and transported to the brain. The concentration of cannabinoids in the brain reaches a maximum within 15 minutes, which coincides with the peak of the psychological and physiological effects.
Absorption varies greatly per individual and depends on various factors, including the heating of the cannabis, the number of inhalations, the waiting time between inhalations, the inhalation time and lung capacity.

When cannabis is taken *orally,* absorption of cannabinoids in the blood is slow and more unpredictable. This results in the psychoactive effect being delayed 30 to 90 minutes with the maximum effect being experienced two or three hours later, and then lasting four to eight hours. Dronabinol concentrations in the blood with oral intake are 25-30% of those seen after inhalation. This is caused, in part, by the large first-pass effect in the liver.

Distribution

After being absorbed, the cannabis constituents are distributed through-out the body. The concentration of cannabinoids rises most quickly in the tissues with the largest blood supply: the brain, lungs, liver and kidneys. A substantial portion of the dronabinol is stored in fatty tissue. Dronabinol and its metabolites are strongly bound to plasma proteins. The distribution volume of dronabinol is 10 liter per kilogram of body weight.

Elimination

In the liver, isoenzymes CYP2C9 and CYP3A4 of the cytochrome P450 system initially convert dronabinol to 11-hydroxy-THC (11-OH-THC), a metabolite that is biologically active. This connection probably contributes to some of the effects of cannabis. The metabolite 11-OH-THC is further converted to 9-carboxy-THC (THC-COOH), which is biologically inactive. A range of other inactive metabolites are also formed. The elimination half-time of dronabinol and 11-OH-THC is 25-36 hours. Dronabinol metabolites can be detected in the urine up to several weeks after the last use of cannabis.

6 Pharmaceutical information

6.1 List of excipients
Not applicable.

6.2 Cases of incompatibility
None.

6.3 Shelf life
Cannabis can decompose under the influence of light and moisture. Cannabis can be stored in the original packaging until the expiry date indicated on the package.

6.4 Special precautions for storage
The cannabis should be stored in the original packaging at room temperature (15-25 °C).

6.5 Type and content of the packaging
Cannabis is available for pharmacies in 5-gram packages.

6.6 Instructions for use and processing
In cannabis, the cannabinoids are primarily present as pharmacologically inactive acids (for example, THC acid). Heating gives rise to free molecules through decarboxylation. For this reason, a heating step must always be carried out before administration.

Use of vaporizer
See instructions for use enclosed with the device. The cannabis is heated, causing the active ingredients to evaporate. Subsequently, they can be inhaled without combustion. The right temperature has been reached when a vapour is just visible (a light mist) but no smoke has formed (thick clouds). For vaporizers with a thermostat, the temperature should be set at 180-195 °C. It is possible to re-use the same cannabis 2-3 times in the inhaler.

Making the tea
Boil half a gram of cannabis for 15 minutes in half a liter of water in a covered pan. Before using, strain the solid ingredients from the tea. Sweeten the tea as desired with honey or sugar. The leftover tea can be kept in a thermosflask when consumed the same day.
When the tea is made for several days it is possible to store it in the refrigerator for up to 5 days. A fatty substance such as milkpowder should be added to the tea in order to keep the active ingredients in solution.

7. Particulars

Import
Import of medicinal cannabis from the Netherlands by an foreign company/ pharmacy is possible through the OMC.

For this, the following documents are needed:

- 2 original duplicates of an import licence from the requesting country
- A letter with the amount of medicinal cannabis needed, and the indication of the patient.

After we have received those documents we will apply for an export licence with the Netherlands Health Care Inspectorate. Subsequently, we will draw up a contract and send this together with an invoice. When we have received the signed contract in return and the invoice is paid we can send the medicinal cannabis.

Ministerie van Volksgezondheid, Welzijn en Sport
Bureau voor Medicinale Cannabis

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Specification sheet

Product: **Cannabis flos, variety Bedrocan (hemp flowers)**
Country: to be sold on the Dutch market
Strength: dronabinol: approx. 18% cannabidiol: < 1.0%
Dosage form: flowers
Package size: approx. 5 grams in container

	Method	Specification	
Appearance	monograph[1]	brown green clustered flowers of 1,5 to 3 cm with a characteristic smell	
Identity			
microscopy	monograph	mainly gland hairs visible	
thin layer chromatography	monograph	monograph	
Foreign material	monograph	stalks, insects and other vermin are absent	
Fineness	monograph	• no leaves shooting out more then 20% of the length of the flowers • stalks are cut away directly under the bottom flowers of the inflorescence	
Absence of pesticides	monograph	Ph. Eur (current ed.) 2.8.13	
Microbiological purity	Ph. Eur (current ed.) cat 2 and 4A:		
molds and aerobic bacteriae	2.6.12	$\leq 10^2$ cfu/gram	
Enterobact. and gram negatives	2.6.13	≤ 10 cfu/gram	
P. aeruginosa, S. aureus	2.6.13	absent	
Absence of heavy metals			
lead	Ph. Eur (current ed.) "Heavy metals in herbal drugs and fatty oils" (2.4.27)	max. 20.0	ppm
mercury		max. 0.5	ppm
cadmium		max. 0.5	ppm

[1] Analytical monograph by BMC/Farmalyse, version 6 of February 20, 2007

Postbus 16114
2500 BC DEN HAAG
Telefoon (070) 340 51 13
Fax (070) 340 74 26

Bezoekadres:
Wijnhaven 16
2511 GA DEN HAAG

Correspondentie uitsluitend richten aan het postadres met vermelding van de datum en het kenmerk van deze brief.

Internetadres:
www.minvws.nl
www.cannabisbureau.nl
e-mail:
info@cannabisbureau.nl

Ministerie van Volksgezondheid, Welzijn en Sport

Bureau voor Medicinale Cannabis

Specification sheet

Product: **Cannabis flos, variety Bedrobinol (hemp flowers)**
Country: to be sold on the Dutch market
Strength: dronabinol: approx. 11% cannabidiol: < 1.0%
Dosage form: flowers
Package size: approx. 5 grams in container

	Method	Specification	
Appearance	monograph[2]	brown green clustered flowers of 1,5 to 3 cm with a characteristic smell	
Identity			
microscopy	monograph	mainly gland hairs visible	
thin layer chromatography	monograph	monograph	
Foreign material	monograph	stalks, insects and other vermin are absent	
Fineness	monograph	• no leaves shooting out more then 20% of the length of the flowers • stalks are cut away directly under the bottom flowers of the inflorescence	
Absence of pesticides	monograph	Ph. Eur (current ed.) 2.8.13	
Microbiological purity	Ph. Eur (current ed.) cat 2 and 4A:		
molds and aerobic bacteriae	2.6.12	$\leq 10^2$ cfu/gram	
Enterobact. and gram negatives	2.6.13	≤ 10 cfu/gram	
P. aeruginosa, S. aureus	2.6.13	absent	
Absence of heavy metals			
lead	Ph. Eur (current ed.) "Heavy metals in herbal drugs and fatty oils" (2.4.27)	max. 20.0	ppm
mercury		max. 0.5	ppm
cadmium		max. 0.5	ppm
Loss on drying	Ph. Eur (current ed.) "Loss on drying" meth. C (2.2.32)	≤ 10.0	%
Assay (UPLC)			

[2] Analytical monograph by BMC/Farmalyse, version 6 of February 20, 2007

Ministerie van Volksgezondheid, Welzijn en Sport

Bureau voor Medicinale Cannabis

	Method	Specification	
Loss on drying	Ph. Eur (current ed.) "Loss on drying" meth. C (2.2.32)	≤10.0	%
Assay (UPLC)			
fingerprint	monograph	similar	
dronabinol (after heating)	monograph	approx. 18	%
cannabidiol (CBD)	monograph	<1.0	%
Related substances (UPLC)			
cannabinol (CBN)	monograph	<1.0	%

Ministerie van Volksgezondheid, Welzijn en Sport

Bureau voor Medicinale Cannabis

	Method	Specification	
fingerprint	monograph	similar	
dronabinol (after heating)	monograph	approx. 11	%
cannabidiol (CBD)	monograph	< 1.0	%
Related substances (UPLC)			
cannabinol (CBN)	monograph	< 1.0	%

Ministerie van Volksgezondheid, Welzijn en Sport

Bureau voor Medicinale Cannabis

Specification sheet

Product: **Cannabis flos, variety Bediol (hemp flowers), granulated**
Country: to be sold on the Dutch market
Strength: dronabinol: approx. 6% cannabidiol: approx. 7.5%
Dosage form: flowers
Package size: approx. 5 grams in container

	Method	Specification	
Appearance	monograph[3]	brown green granulated material of the flowers (about 5mm) with a characteristic smell	
Identity			
microscopy	monograph	mainly gland hairs visible	
thin layer chromatography	monograph	monograph	
Foreign material	monograph	stalks, insects and other vermin are absent	
Fineness	monograph	Fine granulated flowers without stalks longer than 1.0-1.5 cm	
Absence of pesticides	monograph	Ph. Eur (current ed.) 2.8.13	
Microbiological purity	Ph. Eur (current ed.) cat 2 and 4A:		
molds and aerobic bacteriae	2.6.12	$\leq 10^2$ cfu/gram	
Enterobact. and gram negatives	2.6.13	≤10 cfu/gram	
P. aeruginosa, S. aureus	2.6.13	absent	
Absence of heavy metals			
lead	Ph. Eur (current ed.) "Heavy metals in herbal drugs and fatty oils" (2.4.27)	max. 20.0	ppm
mercury		max. 0.5	ppm
cadmium		max. 0.5	ppm
Loss on drying	Ph. Eur (current ed.) "Loss on drying" meth. C (2.2.32)	≤10.0	%
Assay (UPLC)			
fingerprint	monograph	similar	
dronabinol (after heating)	monograph	approx. 6	%
cannabidiol (CBD)	monograph	approx. 7.5	%

[3] Analytical monograph by BMC/Farmalyse, version 6 of February 20, 2007

Ministerie van Volksgezondheid, Welzijn en Sport

Bureau voor Medicinale Cannabis

	Method	Specification
Related substances (UPLC)		
cannabinol (CBN)	monograph	< 1.0 %

BEDROCAN BV MEDICINAL CANNABIS

Bedrocan ®

cultivated to the highest standards – safe for the environment and the end user.

Introduction

Bedrocan BV Medicinal Cannabis is used for medicinal purposes and must therefore meet a number of important (pharmaceutical) requirements. The most important requirement is that the different types of cannabis produced by Bedrocan BV must always contain exactly the same ingredients.

To achieve this, cannabis must always be cultivated and processed under the same conditions. For this reason, Bedrocan BV Medicinal Cannabis is not grown outside or in a greenhouse. Our country is characterised by varying amounts of daylight, shortening and lengthening of days and different soil types; these factors greatly influence the way in which cannabis makes its active ingredients. To eliminate the effects of varying conditions, Bedrocan BV's cannabis is grown inside on hydroculture under artificial light, with a fixed regime of day and night temperatures, growth period and day length.

An important principle in this process is to prevent damage to the environment where possible.

Cultivation method and energy consumption

Bedrocan BV's nursery has been built and insulated in such a way that as little energy as possible is required, both in summer and in winter, to regulate the temperature in the different nursery rooms according to set values. The heat of the plant growth lights is sufficient to maintain a subtropical climate in the nursery day and night, even in winter. It is even sufficient to heat part of the business premises. As a result, total consumption of natural gas in our entire company over the past year (June 2003 – June 2004) was only 1875 m3.

Together with the Hortilux firm, we devised a growth lighting system that combines the lowest possible energy consumption with the highest possible light emission. Our total power consumption, depending on the type of cultivar, lies between 1 and 1.2 kilowatt per gram of cultivated cannabis.

Materials used for cultivation

We cultivate the plants on rockwool beds so that the cannabis plant always receives exactly the same nutrients. A rockwool bed does not change during the cultivation process. Soil consists for a large part of organic material that decomposes during cultivation. This releases fertilizers, an undesired process that cannot be controlled. It is practically impossible to give each crop of cannabis grown on soil the same "diet".

Bedrocan BV uses rockwool made from basalt rock, a natural product. Horticultural rockwool is a 100 % inert product that does not release any foreign substances. Because the plants are connected to a fully automated irrigation system, only a small amount of rockwool is needed per plant: rockwool consumption per cannabis gram amounts to no more than 5 grams. After use, the rockwool is recycled as a filler in road construction.

The pots in which the plants are grown are made of recyclable PE plastic. After cultivation, the rockwool and the pots are separated and brought to a waste processing plant for recycling. Consumption of this plastic is around 1.5 grams per gram of cannabis produced.

The required nutrients are administered to the plants via irrigation water. This system ensures that the plant receives exactly the right amount of food. Total fertilizer consumption in our company amounts to exactly one gram of fertilizer for each gram of cannabis produced. Excess irrigation water is carried off to the local purification plant via the sewer and is therefore not harmful to the environment.

Pesticides

Bedrocan BV does not use pesticides in the cultivation of medicinal cannabis. In the Netherlands, the use of chemical pesticides in arable farming and market gardening is prohibited, unless the manufacturer has the government's permission to use a certain substance in the cultivation of a specific product. In the Netherlands, no substance whatsoever is permitted for cultivating medicinal cannabis.

During cultivation, hygiene is our most important weapon against diseases and infestations. After each cultivation cycle, nursery rooms are decontaminated with an agent based on formic acid and hydrogen peroxide. When exposed to light, these substances decompose into harmless products within a few minutes: water and carbon dioxide. Again, the rinsing water is carried off to the local water purification plant via the sewer.

Combating diseases and infestations is done manually. All nursery rooms are inspected daily for the presence of harmful insects and pests. Plants are

checked daily for symptoms of diseases and fungal growth. Using filters and various types of glue traps, insects are kept outside the nursery. We do not have pest control based on predators, for example wasps, as cannabis plants have resin glands to which insects can become stuck. We prevent contamination of our plants by keeping insects out altogether.