March 31, 2022

Alan Fredricksen
Land Use Administrator
North Haven Town Hall Annex
5 Linsley St.
North Haven, CT 06473

Dear Alan and Planning and Zoning Commission:

Hope all is well! Attached, please find 10 copies of studies and reviews regarding the risks of Medical Marijuana for mood and anxiety disorders, effects of Cannabis on the adult brain, and the effects of Cannabis on the adolescent brain.

Also, please find quoted sections relative to the Planning and Zoning Commission's authority and considerations of the public's health, safety and welfare regarding Zoning regulations from www.cga.ct.gov Chapter 124 Zoning Sec. 8-2 Regulations (a).

And included from the CT Insider is a list of Towns who have banned Cannabis establishments as well as those who have moratoriums.

Please distribute a set to each Planning and Zoning Commission member and to town staff so the information can be reviewed prior to Monday's PZC meeting.

Mr. Chairman, Vern Carlson, I would like to kindly request less than 10 minutes to speak of the results of each study and review so as to enter them into the record during public comment. Thank you!

With Kind Regards,
Mary White
Summer Lane
North Haven, CT
203-239-4160

The names of studies and reviews include:
"Study raises questions about risks of using medical marijuana for mood and anxiety disorders"

"Using marijuana may affect your ability to think and plan, study says"
"The acute effects of cannabinoids on memory in humans: a review"
"The Psychotomimetic Effects of Intravenous Delta-9-Tetrahydrocannabinol in Healthy Individuals: Implications for Psychosis"
"Cancerous toxins linked to cannabis extract"
"Evidence on the acute and residual neurocognitive effects of cannabis use in adolescence and adults: a systematic meta-review of meta-analysis"

"Vaping marijuana by teens doubles in last seven years, with potentially harmful consequences, study says"
"Prevalence of Adolescent Cannabis Vaping A Systematic Review and Meta-analysis of US and Canadian Studies"
"Vaping marijuana linked to lung injury in teens, study says"
"Association of Cannabis Use With Self-harm and Mortality Risk Among Youths With Mood Disorders"
"Teen brain volume changes with small amount of cannabis use, study finds"
"Grey Matter Volume Differences Associated with Extremely Low Levels of Cannabis Use in Adolescence"

www.cga.ct.gov

Chapter 124
Zoning

Sec. 8-2 Regulations (a)

"...planning and zoning commission or zoning board of appeals, whichever commission or board the regulations may, notwithstanding any special act to the contrary, designate subject to the standards set forth in the regulations and to conditions necessary **to protect the public health, safety,** convenience and property values."
"Such regulations shall be designed to lessen congestion in the streets; **to secure safety from fire, panic, flood and other dangers; to promote health and the general welfare**; to provide adequate light and air; to prevent the overcrowding of land; to avoid undo concentration of population and to facilitate the adequate provision of transportation, water, sewerage, schools, parks, and other public requirements."

The Town's Planning and Zoning Commission has the authority to make zoning regulations regarding the way the land shall be developed and utilized and has the great responsibility to ensure those regulations **promote health and the general welfare and protect the public health and safety.**

Therefore, please ban Cannabis establishments in the Town of North Haven because Cannabis is addictive, Cannabis use impairs driving, impairs cognition, has a negative impact on the brain's higher levels of thinking - executive functions which include the ability to make decisions, remember important data, plan, organize and solve problems, as well as control emotions and behavior, Cannabis negatively effects adolescents even those who have just consumed one or two joints in that it changes the gray matter volumes in their brains negatively effecting fear and other emotion–related processes and memory development and spatial abilities, Cannabis use increases self-harm and mortality among youths with mood disorders, and adolescents are vaping marijuana at double the rate with harmful consequences such as lung injury.

CTInsider

Which Connecticut cities and towns have banned recreational cannabis businesses?

Some towns have banned recreational cannabis businesses while others have instituted moratoriums as they work to decide.

By Ginny Monk, Julia Bergman, Andrew DaRosa, Derek Turner | Feb. 11, 2022 | Updated: March 18, 2022 3:50 PM

Since the Connecticut legislature passed legal recreational cannabis in Connecticut last year, cities and towns across the state have been making decisions on how they will handle the new law.

Some municipalities outright banned legal cannabis businesses in their town, while others passed a moratorium to give local leaders more time to study and create regulations.

Cities and towns cannot restrict delivery of legal cannabis, even if they ban businesses and consumption on public property.

NO BAN
Andover
"Andover has not banned cannabis sales or farming in fact we've gone the other way and we're actively recruiting cannabis businesses in the town," Eric Anderson, the Town Administrator, said.

NO BAN
Ansonia
A moratorium was proposed, but nothing has been decided yet.

MORATORIUM
Beacon Falls
Beacon Falls' moratorium extended to Sept. 11, 2022.

MORATORIUM
Berlin
Planning and development unanimously approved a nine-month moratorium on adult-use cannabis sales on Nov. 24, 2021.

MORATORIUM
Bethany
Bethany passed a 365-day moratorium on Oct. 1, 2021.

BANNED
Bethel
No change.

MORATORIUM
Bloomfield
Moratorium in effect through April 1, 2022.
NO BAN
Bolton
"As far as I am aware this topic has not been brought up to the Board of Selectmen," Gary Silver, the Media Coordinator for the Town of Bolton, said.

NO BAN
Bridgeport
Bridgeport is taking measures to ban cannabis sales in certain zones, including near schools.

MORATORIUM
Bristol
Bristol's moratorium expires March 31, 2022.

MORATORIUM
Burlington
Burlington's moratorium expires Sept. 8, 2022.

NO BAN
Canaan
The Planning and Zoning Committee is looking into the issue to consider a ban.

MORATORIUM
Cheshire
The town's Planning and Zoning Committee is working on this. They held a public meeting on the topic in late January, and discussed setting the ban, with an exception for cultivators, per a request during the public comment session with a business owner who wants to apply for the micro-cultivator license.

MORATORIUM
Chester
The moratorium is in effect for at least six months [beginning Oct. 1, 2021] and up to one year, or until the Planning and Zoning Commission adopts regulations to govern recreational cannabis sales.

BANNED
Clinton
The town council passed it as a land-use ban for any type of cannabis sales or production facilities.

NO BAN
Colebrook
"Currently our Zoning Board is working on drafting and implementing a moratorium which will prohibit the use, sale or farming of cannabis. The plan is for the moratorium to be in place for one year or until the town approves an acceptable regulation for cannabis," Chris Johnstone, Colebrook's First Selectman, said.

MORATORIUM
Danbury
Danbury's moratorium expires July 29, 2022.

BANNED
Darien
The town's zoning laws have never allowed marijuana dispensaries. The planning and zoning commission will likely take the issue up formally in 2022, Jeremy Ginsberg, director of land use said.

MORATORIUM
Durham
Durham's moratorium expires Feb. 23, 2022.

MORATORIUM
East Granby
Six month moratorium began on Jan. 8, 2022.

NO BAN
East Haddam
"We do not currently have a ban. We just had a public hearing last week and on Feb. 16 we have a board of selectmen meeting to determine the next step. We are anticipating a referendum for the people of East Haddam to vote on it. No anticipated date on that referendum yet," East Haddam First Selectman Irene Haines said.

BANNED
Eastford
Eastford bans the sale of cannabis.

MORATORIUM
Ellington
6-month moratorium beginning Oct. 1, 2021.

NO BAN
Enfield
On Dec. 6, 2021, the Enfield Town Council flipped its decision on banning cannabis businesses.

MORATORIUM
Fairfield
Fairfield's moratorium, which becomes effective in February, is set to last for one year.

NO BAN
Farmington
Farmington instituted regulations for recreational cannabis into the existing medical regulations.

MORATORIUM
Glastonbury
Glastonbury's moratorium, which became effective in September, is set to last for 18 months.

MORATORIUM
Granby
Granby's moratorium is set to expire Aug. 31.

MORATORIUM
Griswold
Griswold set a one-year moratorium, which became effective in September.

BANNED
Groton
Groton's ban became effective in November.

MORATORIUM
Hamden
Hamden passed its moratorium in December, and it is set to last for a year.

MORATORIUM
Harwinton
Harwinton's moratorium, which became effective in December, is set to last for a year.

MORATORIUM
Hebron
Hebron's moratorium, which became effective in August, is set to last for nine months.

BANNED
Kent
Retail stores are banned from selling recreational cannabis.

NO DATA
Killingly
Moratorium adopted August 16, 2021 through Dec. 31, 2022.

BANNED
Lebanon
The ban on marijuana sales was enacted before the state's adult-use law was passed.

MORATORIUM
Ledyard
Ledyard's moratorium, which became effective in December, is set to last for a year.

MORATORIUM
Madison
Madison's moratorium is set to last for nine months.

MORATORIUM
Middlebury
The town set a moratorium, which expires March 31, and is considering an amendment to extend the moratorium to Dec. 30.

BANNED
Monroe
Cannabis dispensaries and production facilities are banned.

NO BAN
Montville
Montville already has a medical dispensary in town, and Town Clerk Katie Haring said there is consideration for rewriting the zoning rules to allow for manufacturing and production facilities.

NO BAN
Morris
The town's Planning and Zoning Committee is working on this.

MORATORIUM
New Fairfield
New Fairfield's moratorium, which became effective in November, is set to last for one year.

NO BAN
New London
"We are courting the industry and will be adopting regulations soon," Mayor Michael Passero said.

NO BAN
New Milford
New Milford Mayor Pete Bass said at the end of January that the town was still researching recreational cannabis sales but had not proposed a moratorium. The town was working on an ordinance to ban the use of cannabis on town property, Bass said at the time.

MORATORIUM
North Branford
North Branford's moratorium is set to last for one year and became effective in October.

MORATORIUM
North Stonington
North Stonington's moratorium became effective Feb. 3 and is scheduled to last for six months.

NO BAN
Norwalk
Norwalk is in the process of instituting a moratorium, which does not yet have full approval.

NO BAN
Norwich
Norwich is openly welcoming the industry.

MORATORIUM
Old Saybrook
Old Saybrook's moratorium is scheduled to expire at the end of May.

MORATORIUM
Orange
Orange's moratorium, which went into effect in November, is set to last for one year.

MORATORIUM
Oxford
Oxford has set an 18-month moratorium on "retail sales, manufacture, and cultivation" of cannabis.

MORATORIUM
Preston
Preston's moratorium is set to expire March 20.

MORATORIUM
Putnam
Putnam's moratorium is set to expire Sept. 18.

NO BAN
Redding
"Redding has not banned or instituted a moratorium on cannabis businesses," First Selectman Julia Pemberton said on Jan. 27.

MORATORIUM
Sharon
Sharon's moratorium, which became effective in January, is set to last for six months.

NO BAN
Stonington
A public hearing is scheduled in March to solicit feedback on a proposal to impose a six-month moratorium. The moratorium would give the Planning and Zoning Commission time to establish regulations.

MORATORIUM
Thomaston
Thomaston's moratorium is set to expire June 2.

MORATORIUM
Torrington
Torrington's moratorium is set to expire Sept. 25.

MORATORIUM
Trumbull
Trumbull's moratorium is set to expire Sept. 1.

MORATORIUM
Waterbury
Waterbury's moratorium expires Aug. 9.

BANNED
Westport
Westport's ban excludes medical dispensaries.

MORATORIUM
Wilton
Wilton's moratorium is set to expire Sept. 29.

MORATORIUM
Woodstock
Woodstock's moratorium is set to expire Oct. 31.

NOTE:
BANNED
Guilford has a ban on Medical Marijuana Dispensary Facilities and Medical Production Facilities.

Study raises questions about risks of using medical marijuana for mood and anxiety disorders

By Sandee LaMotte, CNN
Updated 9:47 AM ET, Sat March 19, 2022

(CNN) Some people with pain, anxiety or depression who obtain medical marijuana cards may overuse marijuana within a short time frame, leading to cannabis use disorder while failing to improve their symptoms, a new study found.

Cannabis use disorder, also known as marijuana use disorder, is associated with dependence on the use of weed. People are considered dependent on weed when they feel food cravings or have a lack of appetite, irritability, restlessness, and mood and sleep difficulties after quitting, according to the National Institute on Drug Abuse.

People who obtained medical marijuana cards immediately were twice as likely to develop cannabis use disorder than those who waited 12 weeks before getting cards, new research found.

Heavy use of marijuana by teens and young adults with mood disorders -- such as depression and bipolar disorder -- was linked to an increased risk of self-harm, suicide attempts and death, according to an earlier study published in 2021.

Under the current system of providing medical marijuana cards, people only require written approval by a licensed physician, the latest study said. But often that doctor is "not the patient's primary care provider but a 'cannabis doctor' who may provide authorization to patients with only a cursory examination, no recommendations for alternative treatments, and no follow-up," according to a statement released with the study.

"Indeed, the medical marijuana industry functions outside regulatory standards that apply to most fields of medicine," the statement said.

No changes in depression, anxiety or pain symptoms

The study, published Friday in the journal JAMA Network Open, followed 269 adults from the Boston area with an average age of 37 who wanted to obtain medical marijuana cards. Participants were divided into two groups: One was allowed to

get cards immediately and begin use; the other group waited for 12 weeks before obtaining cards.

Using marijuana may affect your ability to think and plan, study says

"The waitlist group was our comparison group, like a placebo group, but we couldn't do 'placebo' cannabis," said lead author Jodi Gilman, an associate professor at Harvard Medical School/Massachusetts General Hospital with the Center for Addiction Medicine.

"The waitlist group continued their usual treatment, whether it was counseling, medication, etc.," she said in an email.

All participants were able to choose their choice and dose of cannabis products from a dispensary as well as frequency of use. They could also continue their usual medical or psychiatric care.

People who obtained cards immediately were twice as likely to develop cannabis use disorder, the study found. Ten percent had developed the disorder by week 12, and that figure rose to 20% if they were using marijuana for anxiety or depression.

Those who got cards immediately saw "no significant changes in pain severity or anxiety or depressive symptoms" but did report improvement in insomnia and greater well-being, according to the study. The benefits for sleep and well-being need further follow-up, the study said.

It's possible that medical marijuana use may "pose a high risk or may even be contraindicated for people with affective disorders. This finding is important to replicate because depression has been reported as the third most common reason that people seek a medical marijuana card," the study said.

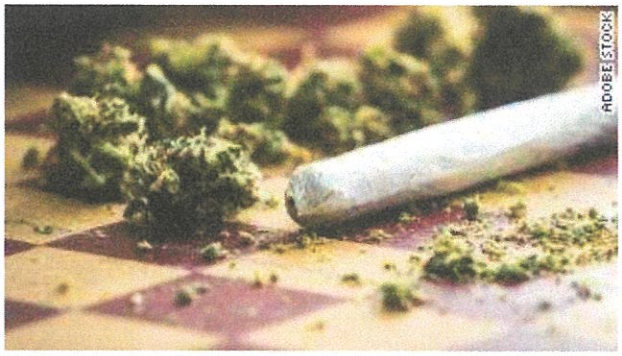
"Our study underscores the need for better decision-making about whether to begin to use cannabis for specific medical complaints, particularly mood and anxiety disorders, which are associated with an increased risk of cannabis use disorder," Gilman said in a statement.

"There needs to be better guidance to patients around a system that currently allows them to choose their own products, decide their own dosing, and often receive no professional follow-up care," Gilman added.

Using marijuana may affect your ability to think and plan, study says

By Sandee LaMotte, CNN
Updated 12:02 AM ET, Thu January 20, 2022

(CNN) Remember those classic stoner dudes -- Cheech and Chong, anyone? -- spending their days in a weed-drenched room (or car), capable of little besides finding that next great high?



Weed can affect your ability to make decisions, solve problems and perform other cognitive functions, a study found.

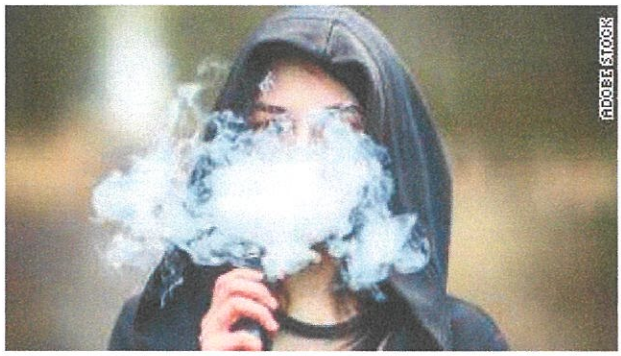
If you don't, that's not surprising. As more and more states move to legalize marijuana, the stereotypical mind-numbing effects of weed have become passé, often replaced by an acceptance of the drug as an acceptable way to socialize, relax and get better sleep.

But while society may have forgotten the impact that weed can have on the brain, science has not.

Studies have long shown that getting high can harm cognitive function. Now, a new review of research, published Thursday in the journal Addiction, finds that impact may last well beyond the initial high, especially for adolescents.

"Our study enabled us to highlight several areas of cognition impaired by cannabis use, including problems concentrating and difficulties remembering and learning, which may have considerable impact on users' daily lives," said coauthor Dr. Alexandre Dumais, associate clinical professor of psychiatry at the University of Montreal.

"Cannabis use in youth may consequently lead to reduced educational attainment, and, in adults, to poor work performance and dangerous driving. These consequences may be worse in regular and heavy users," Dumais said.



Vaping marijuana by teens doubles in last seven years, with potentially harmful consequences, study says

Weed's impact on the brain can be particularly detrimental to cognitive development for youth, whose brains are still developing, said Dr. Megan Moreno, a professor of pediatrics at the University of Wisconsin School of Medicine and Public Health, who was not involved in the study.

"This study provides strong evidence for negative cognitive effects of cannabis use, and should be taken as critical evidence to prioritize prevention of cannabis use in youth," Moreno said. "And contrary to the time of Cheech and Chong, we now know that the brain continues to develop through age 25.

"Parents should be aware that adolescents using cannabis are at risk for damage to their most important organ, their brain."

Higher-level thinking

The newly published review looked at studies on over 43,000 people and found a negative impact of tetrahydrocannabinol or THC, the main psychoactive compound in cannabis, on the brain's higher levels of thinking. Those executive functions include the ability to make decisions, remember important data, plan, organize and solve problems, as well as control emotions and behavior.

Uncontrollable vomiting due to marijuana use on rise, study finds

Can you recover or reverse those deficits? Scientists aren't sure.

"Research has revealed that THC is a fat-soluble compound that may be stored in body fat and, thus, gradually released into the bloodstream for months," Dumais said, adding that high-quality research is needed to establish the long-term impact of that exposure.

Some studies say the negative effects on the brain may ease after weed is discontinued, but that may also depend on the amount, frequency and years of marijuana use. The age in which weed use began may also play a role, if it falls within the crucial developmental period of the youthful brain.

"Thus far, the most consistent alterations produced by cannabis use, mostly its chronic use, during youth have been observed in the prefrontal cortex," Dumais said. "Such alterations may potentially lead to a long-term disruption of cognitive and executive functions."

In addition, some studies have shown that "early and frequent cannabis use in adolescence predicts poor cognition in adulthood," he added.

While science sorts this out, "preventive and interventional measures to educate youths on cannabis use and discourage them from using the substance in a chronic manner should be considered ... since youths remain particularly susceptible to the effects of cannabis," Dumais said.

Psychopharmacology
DOI 10.1007 s00213-006-0508-y

REVIEW

The acute effects of cannabinoids on memory in humans: a review

Mohini Ranganathan · Deepak Cyril D'Souza

Received: 23 February 2006 / Accepted: 28 June 2006

Abstract
Rationale Cannabis is one of the most frequently used substances. Cannabis and its constituent cannabinoids are known to impair several aspects of cognitive function, with the most robust effects on short-term episodic and working memory in humans. A large body of the work in this area occurred in the 1970s before the discovery of cannabinoid receptors. Recent advances in the knowledge of cannabinoid receptors' function have rekindled interest in examining effects of exogenous cannabinoids on memory and in understanding the mechanism of these effects.
Objective The literature about the acute effects of cannabinoids on memory tasks in humans is reviewed. The limitations of the human literature including issues of dose, route of administration, small sample sizes, sample selection, effects of other drug use, tolerance and dependence to cannabinoids, and the timing and sensitivity of psychological tests are discussed. Finally, the human literature is discussed against the backdrop of preclinical findings.
Results Acute administration of Δ-9-THC transiently impairs immediate and delayed free recall of information presented after, but not before, drug administration in a dose- and delay-dependent manner. In particular, cannabinoids increase intrusion errors. These effects are more robust with the inhaled and intravenous route and correspond to peak drug levels.
Conclusions This profile of effects suggests that cannabinoids impair all stages of memory including encoding, consolidation, and retrieval. Several mechanisms, including effects on long-term potentiation and long-term depression and the inhibition of neurotransmitter (GABA, glutamate, acetyl choline, dopamine) release, have been implicated in the amnestic effects of cannabinoids. Future research in humans is necessary to characterize the neuroanatomical and neurochemical basis of the memory impairing effects of cannabinoids, to dissect out their effects on the various stages of memory and to bridge the expanding gap between the humans and preclinical literature.

Keywords Cannabinoids · Cannabis · Marijuana · Δ-9-THC · Memory · Learning · Cognition

Abbreviations

CB	cannabinoid
CB1	cannabinoid 1 receptor
Δ-9-THC	delta-9-tetrahydrocannabinol
CBD	cannabidiol

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Introduction

Cannabis or marijuana is the most widely used illicit drug in the Western hemisphere, and among its many effects it is known to produce cognitive effects. The most robust cognitive effects of cannabis are on memory. However, the mechanism of action of these compounds has long remained an enigma. Recent advances in the understanding of cannabinoid receptor function have renewed interest in the effects of cannabis and other cannabinoids on cognition.

Reviewing the effects of cannabinoids on memory is relevant to both normal physiology and pathological states. Cannabis use disorders are not uncommon; therefore, understanding the effects of cannabinoids on memory is important. More recently, there is growing interest in the association between cannabis use and schizophrenia, a disorder characterized by memory impairments that are considered to be core manifestations of the illness. In fact, laboratory studies with cannabinoids are receiving increasing scrutiny as possible "models" of schizophrenia. Preclinical findings suggest a role for the endocannabinoid system in memory processes. Finally, with an explosion in preclinical research on the endocannabinoid system, it seems timely to revisit and review the literature on the effects of cannabinoids on memory in humans.

The objective of this paper is to review the acute effects of cannabinoids on short-term memory in humans and to examine their effects on the various stages of memory. One other objective of this paper is to draw attention to the possible role of the endocannabinoid system in the physiology of memory by briefly discussing the preclinical literature. While there is considerable debate about the long-term effects of cannabinoids, this paper only reviews the acute effects of cannabinoids. Similarly, while there is evidence that cannabinoids impair other cognitive function, e.g., attention and time perception, this paper only reviews the effects of cannabinoids on short-term memory. The literature on the cognitive effects of cannabinoids is divided into roughly two eras; one predominantly in the 1970s and one after the discovery and characterization of a brain endocannabinoid system in the 1990s. These two phases, while valuable and informative, are challenging to compare because of widely differing methodologies including differences in tasks, controls, etc., which will be discussed later. Furthermore, relative to other drugs known to impair memory, e.g., benzodiazepines and ketamine, the cannabinoid literature presents some unique challenges. The cannabinoid literature includes studies using herbal cannabis, unassayed amounts of delta-9-tetrahydrocannabinol (Δ-9-THC) and varying routes of administration, which as discussed later, make the interpretation of the literature difficult. In this paper, the clinical literature will first be reviewed, followed by a review of the more recent preclinical literature with the goal of providing potential mechanistic explanations and stimulating further research in the field.

As a prelude to reviewing the studies about the effects of cannabinoids on memory, we first review the constituents of cannabis, issues related to the dose and route of administration of cannabinoids, and cannabinoid receptor function. The large majority of pharmacological studies were conducted with herbal cannabis and its principal active ingredient Δ-9-THC. Herbal cannabis contains more than 600 compounds, more than 70 of which are cannabinoids. Of these, Δ-9-THC is thought to be the ingredient responsible for most of the cognitive and behavioral effects of cannabis.

In addition to the classic natural cannabinoids found in herbal cannabis, there are a number of synthetic cannabinoids that have been studied in man. These include dronabinol, nabilone, and levonantradol. Dronabinol is synthetic Δ-9-THC. The 9-trans keto-cannabinoid nabilone is a synthetic analog of Δ-9-THC that was developed as an antiemetic and is available in Europe as Cesamet. Levonantradol was developed as an analgesic agent, but was abandoned because of a high incidence of intolerable behavioral side effects.

Δ-9-THC has a long half-life of approximately 4 days (Johansson et al. 1988). Its principal active metabolite, 11-hydroxy-THC, is more potent than Δ-9-THC. The time course of 11-hydroxy-THC blood levels correlates well with the psychological effects of inhaled and oral Δ-9-THC (reviewed in Agurell et al. 1986). Therefore, in relating cognitive or behavioral data with blood levels, both Δ-9-THC and 11-hydroxy-THC blood levels need to be considered.

Route of administration

The pharmacokinetics and effects of Δ-9-THC vary as a function of its route of administration. In attempting to quantify the dose of Δ-9-THC extracted from a typical cannabis cigarette, several factors need to be considered including, but not limited to, the weight of a cannabis cigarette, the potency of Δ-9-THC in the herbal cannabis preparation, and the presence of other cannabinoids (Kamiol and Carlini 1973; Kamiol et al. 1974, 1975; Turner et al. 1980). Furthermore, the amount of Δ-9-THC delivered is influenced by several factors including the rate of inhalation, depth of puffs, duration of puffs, volume inhaled, extent of breath-holding after inhalation, the amount lost by smoke escaping into the air or respiratory dead space, vital capacity, the length of cigarette smoked, the adeptness of smoking, and the subject's overall experience in titrating the dose. A typical cannabis cigarette contains varying doses of Δ-9-THC (0.3% to as much as 10% in hashish). Standard NIDA cigarettes, which have been used in many of the studies to be

discussed, weigh about 0.35 g and contain various concentrations of Δ-9-THC. Only 10–25% of the Δ-9-THC content of a cannabis cigarette enters the circulation when smoked (Adams and Martin 1996). With smoking, peak plasma concentrations of Δ-9-THC are reached within 3–10 min. Psychotropic effects start within seconds to a few minutes, reaching a peak after 15–30 min and then tapering off within 2–3 h. With oral consumption, the absorption of Δ-9-THC is slower and its bioavailability is lower (about 4–12%). An extensive first pass metabolism further reduces bioavailability after oral administration (McGilveray 2005). Peak plasma concentrations occur after 1–2 h and multiple peaks may be seen (Agurell et al. 1986; Grotenhermen 2003). With oral ingestion, psychotropic effects set in with a delay of 30–90 min, reach their maximum after 2–3 h, and last for about 4–12 h (Agurell et al. 1986; Hollister et al. 1981; Ohlsson et al. 1980, 1981). Intravenous dosing follows the pharmacokinetics and pharmacodynamics (Fig. 1) of the inhaled route, though blood levels tend to be higher. While Δ-9-THC is consumed by the oral or inhaled route, nabilone is administered by oral route, and levonantradol is administered by intramuscular route.

Given that cannabinoids have been studied using the oral, sublingual, inhaled, intramuscular, and intravenous routes, the literature on the effects of cannabinoids on memory is a little more challenging to interpret than studies with other drugs known to impair memory. For example in most studies with ketamine, the drug is administered by the intravenous route; therefore, these studies are easier to compare. Thus, the intensity, onset, and duration of cannabinoid effects on memory should be interpreted in the context of the route of drug administration.

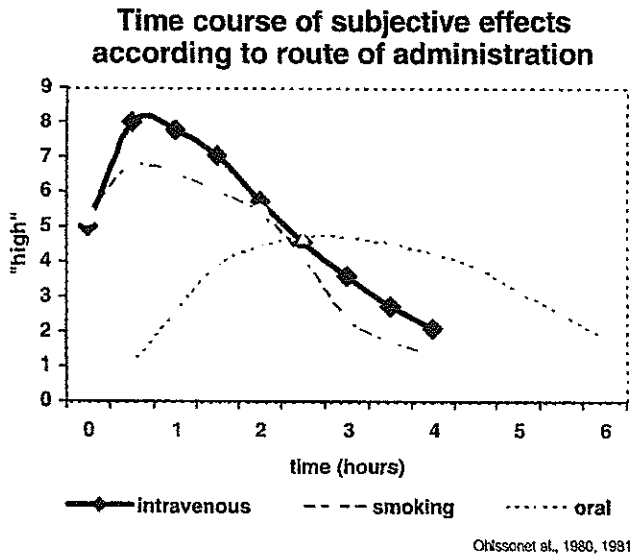


Fig. 1 Figure shows the time course of the acute behavioral effects of Δ-9-THC (feeling high) as a function of route of administration (intravenous, inhaled and oral)

Withdrawal, tolerance, and dependence

There is evidence of a withdrawal syndrome, albeit mild, with the cessation of cannabis use (Budney et al. 2003, 2004), as well as tolerance to the effects of cannabinoids (reviewed in Howlett 2004; Iversen 2003, 2005; Tanda and Goldberg 2003). In fact, tolerance to the memory disruptive effects of cannabinoids has been shown in animals to involve adaptation by specific hippocampal neurons (Hampson et al. 2003). The large majority of studies reviewed here included subjects who were using cannabis regularly and were therefore likely to be tolerant to some of the effects of cannabis. Furthermore, variability in defining subject samples with regard to extent of cannabis exposure and interval from last use may complicate comparison across studies. None of the studies that we are aware of included cannabis-naïve individuals. Therefore, in reviewing pharmacological studies involving cannabis users, it is important to consider whether withdrawal, tolerance, and residual carryover effects confound the results.

Cannabinoid receptors

Thus far, two cannabinoid receptors have been identified and cloned, and a third has been recently described. The CB1 receptor (Matsuda 1997; Matsuda et al. 1990) is a G-protein coupled receptor and is distributed extensively in the forebrain and the cerebellum (molecular layer), with the highest density in the basal ganglia, substantia nigra (pars reticulata), and hippocampus and peripherally in the spleen, tonsils and other viscera (Herkenham et al. 1990; Mailleux et al. 1992; Mailleux and Vanderhaeghen 1992; Tsou et al. 1998; Fig. 2). The behavioral, cognitive, and physiological effects of cannabis are believed to be primarily mediated via this receptor.

The hippocampus includes the CA1–CA3 regions and the dentate gyrus. Information entering the hippocampus flows through the dentate gyrus proceeding through the CA3 and CA1 regions to the subiculum. The CA1–CA3 regions have pyramidal cells as their main neurons. Both the dentate gyrus and the CA1–CA3 regions (with CA3 being more dense than CA1) have higher densities of CB1 (Heyser et al. 1993), correlating well with the known effects of cannabinoids on learning and memory.

Anandamide and 2-arachidonoyl glycerol (2-AG) are the main endogenous agonists of CB1 receptors. Note that Δ-9-THC is a partial agonist with modest affinity (K_i=35–80 nmol) and low intrinsic activity (Compton et al. 1992; Gerard et al. 1991; Howlett et al. 2002; Matsuda et al. 1990; Mechoulam et al. 1995), while levonantradol is a full agonist (Fig. 3) and SR141716A (Rimonabant) is a potent antagonist.

The second cannabinoid receptor CB2 (Munro et al. 1993), distributed mainly peripherally (reviewed in Demuth

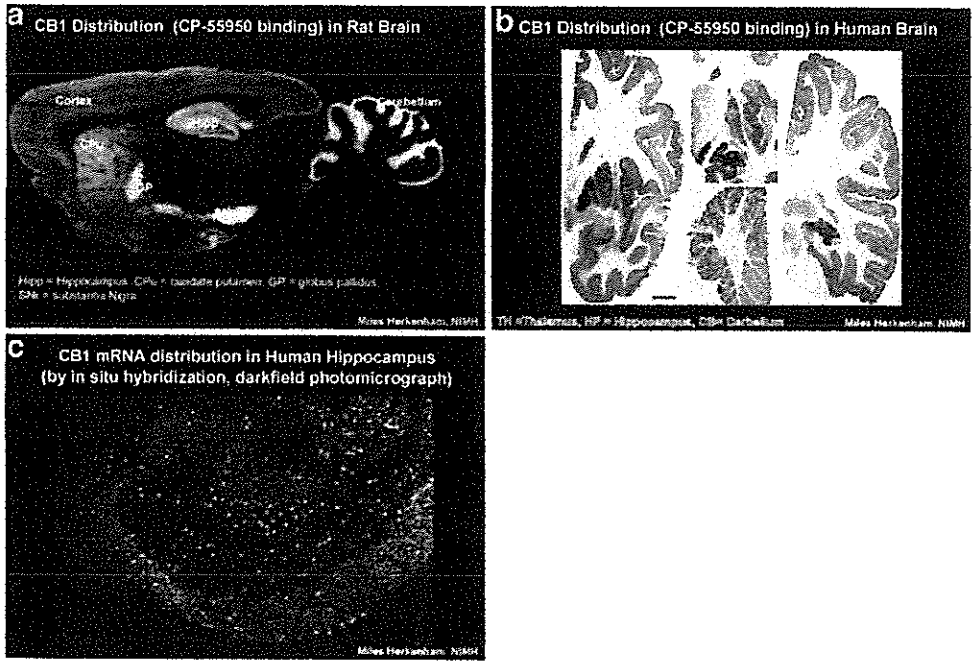


Fig. 2 The figures show the distribution of cannabinoids receptors in specific brain areas. a Distribution of CB1 receptor in the rat brain. b Distribution of CB1 receptor in the human brain. c Distribution of CB1 receptor mRNA in the human brain (Miles Herkenham, personal communication)

and Molleman 2005), is not relevant to the cognitive effects of cannabinoids. There are a number of putative novel non-CB1/CB2 receptors that have been identified, some of which may be relevant to the cognitive effects of cannabinoids (Baker et al. 2006).

Memory subtypes and processes

Since there is considerable variability in the terminology relating to memory in the published literature, we now briefly define some basic concepts related to memory subtypes and processes (reviewed in Atkinson and Shiffrin 1968; Baddeley 1999; Stout and Murray 2001). While there are several classifications of memory, for the purpose of this review we have classified memory into short term, long term, and working memory.

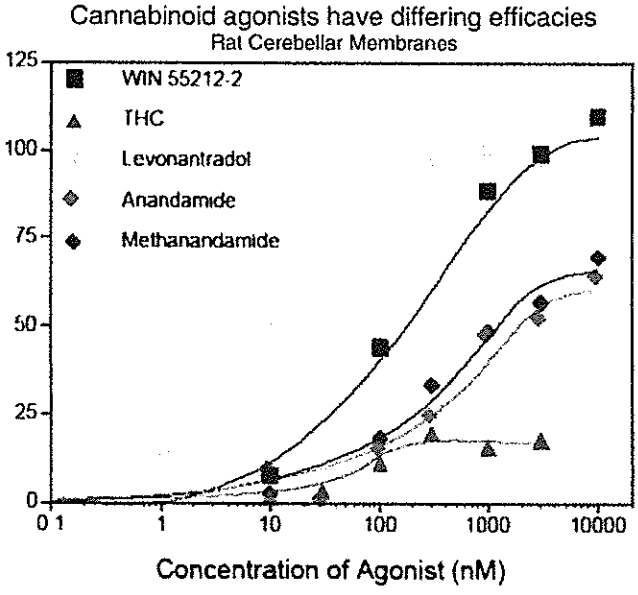


Fig. 3 Figures show varying efficacies of cannabinoid agonists at the CB1 receptor. Note that Δ-9-THC is a partial agonist

Short-term memory (STM) refers to that process or processes involved in the storage of a limited amount of information for a limited amount of time, usually considered less than a minute. To facilitate longer retention, information must be periodically rehearsed so that it will reenter the short-term store and be retained for longer periods of time. Furthermore, STM appears to have a limited capacity, which is estimated to be about seven "chunks" of information; the latter is roughly equivalent to about seven digits or about five to six words. In contrast, *long-term memory* (LTM) refers to the process or processes by which unlimited amount of information is stored indefinitely. However, the existence of a genuine distinction between STM and LTM remains controversial. One line of evidence supporting the existence of a short-term store is that anterograde amnesia affects LTM while leaving STM intact. Long-term memory can be divided into explicit and implicit memory. Explicit or declarative memory involves the conscious recollection of past events or experiences and is typically measured through recall or recognition. It includes semantic and episodic memory (Tulving 1972). Semantic memory refers to the memory of the meaning of words, facts, rules, or abstract concepts. Episodic memory or autobiographical memory is the memory of temporally dated events or episodes (Tulving and Markowitsch 1998). It includes time, place and

associated emotions. In contrast, implicit memory or procedural memory involves demonstrations of learning or facilitation of performance in the absence of conscious recollection.

Working memory (WM) in this review refers to processes that subserve a very limited capacity system to store and manipulate information for short durations. WM is distinct from STM in that it places emphasis on the manipulation of the stored information (Baddeley 1999; Baddeley et al. 2001). It is central to cognitive function and its disruption can result in impaired processing across many other cognitive domains. It is believed that there are distinct circuits underlying the manipulation and maintenance components of working memory with manipulation corresponding with dorsolateral prefrontal cortex activity and maintenance corresponding with ventral prefrontal activity (reviewed in Fletcher and Henson 2001).

The cannabinoid literature is dominated by studies examining cannabinoid effects on short-term, episodic and verbal memory. A small number of studies examined cannabinoid effects on short-term spatial episodic memory, working memory, and long-term semantic memory. There is a paucity of data on whether cannabinoids impair procedural or implicit memory. While a multitude of tests were used to study the effects of cannabinoids on memory, making comparisons across studies somewhat difficult, verbal memory is most commonly tested using a number of word list tasks. Typically, subjects learn a supraspan list of words presented over multiple trials. Capacity for learning is assessed with immediate free recall, delayed free recall, cued and recognition recall (reviewed in Stout and Murray 2001). In verbal recall tasks, word lists are sometimes semantically organized into categories. On immediate recall tasks, subjects are presented with information, which they are asked to recall immediately. Sometimes the information is presented across trials to aid learning, and in such cases, the sum of information recalled across trials (total immediate recall) is used as an index of learning. During this task, recall of information not previously presented in the list to be learned is referred to as intrusions. Typically, after a variable delay (1–30 min), subjects are asked to recall the information previously presented without cues (delayed free recall) and then with the help of cues (delayed cued recall). Finally, in the recognition recall task, subjects are presented with a list of items that includes some of the items initially presented for learning; erroneously recognized information on this task is referred to as false positive. Immediate recall yields items from short-term memory, while delayed recall yields items from long-term memory. A minority of studies of cannabinoid effects have employed nonverbal memory tasks such as reproduction of previously learned geometric designs, e.g., the Rey Osterrieth complex figure tests.

The processes involved in learning and memory include encoding, storage or consolidation and retrieval, and relevant to long-term memory, reconsolidation. These processes may not be entirely dissociable, but are important constructs in understanding memory. Encoding refers to the stage of processing during which information is initially learned, followed by a series of changes that consolidate the new information against disruption and decay. Retrieval refers to the access of previously encoded memories. Dissociating these effects can be accomplished to some extent with a variety of manipulations. Various cognitive manipulations have been used in an attempt to locate episodic memory deficits with respect to encoding and retrieval stages. However, identification of stage-specific deficits is problematic because a performance deficit could reflect impaired encoding, consolidation, or retrieval (or all). Usually, deficits in immediate recall after learning trials on memory tasks are attributed to encoding deficits. Thus, administering a drug during encoding but terminating its effects before consolidation and retrieval would isolate encoding deficits. However, given the long half-life of Δ-9-THC, this would be difficult to do. The preservation of immediate free recall combined with impairments in delayed free recall implicates consolidation/storage deficits. Impairment on free recall combined with intact recognition memory implicates retrieval deficits. Impaired recall of information encoded before drug administration would suggest storage or retrieval deficits.

With this background, we now review the effects of cannabinoids on memory. Results from studies on the effects of cannabinoids on short-term, episodic memory and working memory are discussed below.

Short-term, episodic memory

These results of studies have been organized according to the task: word, prose, digit recall. In addition, Table 1 provides a list of studies reviewed with route and dose of Δ-9-THC, test administered and results.

Word recall

In the early 1970s, Abel (1971) tested the effect of unassayed doses of cannabis on recall of word lists learned before (n=49) and after ad lib smoking (n=10) in cannabis users. Relative to placebo, cannabis had no effect on the accuracy of delayed recall (both free and recognition) of word lists that had been presented before smoking. In the subsequent placebo-controlled study, the author tested the effect of cannabis on encoding. Both free and recognition recall of word lists presented after Δ-9-THC administration were significantly impaired by cannabis. The lack of effects

Table 1 List of studies reviewed with route and dose of Δ-9-THC, test administered, and results

Year	Author	Subjects	Estimated Δ-9-THC dose	Route	Tests	Results (only THC effects)			
						IFR	DFR	Recognition recall	Miscellaneous
1970	Tinklenberg et al.	Eight infrequent users	0, 20, 40, 60 mg	O	Digit span	↓ Forward and backward span at all doses			
1971	Abel et al.	49 users and nonusers 10 users	unassayed	I	Word lists (10 per list) Word lists (12 per list)	↓	Trend ↓ ↓	↔ DRR	↑ False positive in DRR
1972	Tinklenberg et al.	15 users	0, ~26 mg: ETOH	O	GDSA, RMS	↔ GDSA errors, ↔ digit (RMS) span			
1973	Darley et al.	12 regular users	0, 20 mg	O	Word lists	↔ Accuracy, ↑ RT			
1974	Darley et al.	48 occasional users	0, 20 mg	O	Word lists (20 per list), fixed vs. free rehearsal	↓	↔	↔	Recency effect maintained; fixed rehearsal did not reduce THC effects
1974	Vachon et al.	8 occasional users	0, 25 mg	I	CPT DSST	↔CPT ↓ Learning curve, ↓ accuracy and speed on DSST			
1976	Miller et al.	40 moderate users	0, 9.4 mg	I	Word lists: cued (first letter) vs uncued	↓ IFR in cued and uncued condition. Cues did not significantly improve the impaired recall in the THC condition; ↑ intrusions			
1977	Pfefferbaum et al.	16 users	0, 0.3 mg·kg	O	Word lists—with overt and associative rehearsal	↓			↑ Intrusions
1977	Miller et al.	28 moderate users	0, 14 mg	I	40 words, 40 pictures, 5 trials	↓ (both pictures & words)			Learning was better for words than pictures over trials.
1977	Miller et al.	34 heavy and regular users	0, 14 mg	I	Word lists (15 words per list), first list repeated 4 times at intervals	↓	↓	↔	No practice effect on repeated list, ↔ shape of serial position curve, ↑ external intrusions in IFR, ↑ intrusions in DR, ↑ false positive in recognition recall
1978	Miller and Cornett	16 regular users	0, 5, 10, 15 mg	I	Word lists (40 words)	↓	↓	↔	↑ Intrusions
1977	Darley et al.	16 occasional users	0, 0.3 mg·kg	O	Common facts recall test	↔ Recall of facts, ↔ ability to assess memory			
1977	Sulkowski et al.	6 occasional users	0, 10 mg and propranolol. 120 mg (PO)	I	DSST (with and without memory), CPT, digit matching	↓ Learning in DSST, ↔ CPT, ↔ matching task (no effect of propranolol on THC-induced impairment)			
1977	Miller et al.	40 moderate and regular users	0, 10.2 mg, days 1 and 2	I	Prose recall	↓	↓		↑ Intrusions; cues did not improve recall in the THC condition
1978	Miller et al.	22 moderate and regular users	0, 14 mg	I	2-D design recall with 10 trials	↓			↑ Intrusion errors in first 5 trials, ↑ errors in field dependent group
1979	Miller et al.	12 Moderate and regular users	0, 10 mg	I	Word lists (15 words per list)	↓	↓	↔	↑ Intrusion errors
1980	Belmore and Miller	16 Moderate and regular users	0, 14 mg	I	Word lists (16 words per list)—processing questions for each word	↓	↓		Better recall of later lists semantic processing more impaired by THC
1982	Wetzel et al.	41 Moderate–heavy regular users	0, 6 mg	I	Word list (14 words per list) Long-term recall	↓			↔ Shape of serial position curve, ↓ long-term recall
1987	Hooker and Jones	12 Infrequent Users	0, 10.7 mg	I	PASAT SCWT	↔ ↓ Rate of reading, ↑ interference effect			

					Randt memory battery	↔	↔	↑ Intrusions in word recall, ˆ intrusions and omissions in prose DFR
					COWAT	↔		
1990	Kelly et al.	15 Regular users	0, 1.3, 2.3, 2.7%	I	DSST, vigilance, sorting task	DSST: ↑ errors, ↔ vigilance and sorting		
1990	Heishman et al.	3 Moderate and regular users	0, 25.7 mg—1, 2, 4 cigs	I	PAB—two-letter search, digit recall, SAS, logical reasoning	↓ Accuracy on serial addition subtraction and digit recall, ↔ all other tests		
1992	Block et al.	48 Moderate and regular users	0, 19 mg	I	Buschke selective reminding task	↓	↓	Consistent long-term retrieval, no effect of breath-holding on THC effects
					Text learning	↓	↓	
					PAL	↓ Learning of associations		
1994	Wilson et al.	10 users	0, 1.75%, 3.55% ad lib	I	DSST	↓ Performance on DSST with memory;↑ RT		
					CPT	↔		
					Tracking task	Dose-dependent impairment		
					Keyboard RT	Dose-related ↑ RT		
1994	Chait et al.	14 Occasional and heavy users	0, 36 mg (4 puffs) + ETOH	I	Free recall	↔ On recall		
					DSST	↓ Percentage of correct responses		
					Backward digit span	↔		
					Divided attention	↔ RT, ↑ false positives		
					Logical reasoning	↔		
1997	Heishman et al.	5 Regular users (variable)	0, 35.5 mg THC—4, 8, 16 puffs + ETOH	I	Word recall	↓		
					DSST	↓ Speed and accuracy on DSST		
					RT task	↔		
					Number recognition	↔ Number recognition		
1998	Fant et al.	10 Social users	0, 15.6 mg and 25.1 mg	I	Walter Reed PAB- rapid arithmetic skill, digit recall, logical reasoning	↔ All tests		
2000	Greenwald and Stitzer	5 Moderate users	0, ~35 mg	I	Digit span	↔		
					DSST	ˆ RT, ↔ Accuracy		
					Divided attention	↑ RT		
2001	Hart et al.	18 Infrequent and heavy users	0, 18 and 39 mg	I	Digit recall	↓ Digit recall only with 39 mg		
					Micro Cog battery	↑ Premature responses and ↑ time to complete microcog battery		
					DSST	↔		
					Reasoning and flexibility task	↔		
2002	Curran et al.	15 Infrequent users	0, 7.5 and 15 mg	O	Buschke selective reminding task	↔	↓	↓ Learning (15 mg)
					Prose recall	↓	↓	Persisting up to 6 h
					Baddeley logical reasoning task	↑ RT logical reasoning		
					Serial sevens	↔		
					RVIP	↔		
					Choice RT	↔		
					Single/double digit cancellation task	↔		

Table 1 (continued)

Year	Author	Subjects	Estimated Δ-9-THC dose	Route	Tests	Results (only THC effects)			
						IFR	DFR	Recognition recall	Miscellaneous
2004	D'Souza et al.	22 Infrequent users	0, 2.5, 5 mg	IV	HVLT	↓	↓		↓ DCR, ↑ false positives; ↑ intrusions
					CPT	↔			
					DMTS	↓ Accuracy in easy WM task, ↔ RT; ↔ hard subtask			
2004	Ilan et al.	10 Casual users <1/week	0, 3.45%	I	Word recognition (20 words per list)	↔ Learning;↑ false alarms			
					Spatial N-back	↑ RT,↓ accuracy in high load task			
					EEG	Altered θ and α power, ↓ N100			
2005	Ilan et al.	24 Chronic regular users	THC 0, 1.8/3.6%; CBC: <0.2/0.5%; CBD:<0.4/>1%	I	EM: WP/WR—24 per list	WP, ↓ ERP amplitudes: WR, ↓ accuracy to new words, ↔ RT; WR, Slow wave ↓, N400 to new words ↓			
					WM: spatial, N-back	↓ Accuracy, ↑ RT: WM:↓ P300			
2005	Lane et al.	5 occasional users	0, ~11 mg, ~38.9%	I	DMTS	↓ Percentage of correct responses as a function of delay interval			
2005	D'Souza et al.	13 Schizophrenia patients with previous exposure but no abuse	0, 2.5, 5 mg	IV	CPT	↑ Omission errors, ↔ commission			
					HVLT	↓	↓	↓ DRR	↓ DCR, ↓ learning, ↑ intrusions and false positives
2006	Makela et al.	19 Occasional regular users	0, 5 mg; as 8.5 mg/ml sublingual spray	S/L	CANTAB-spatial WM task	↑ accuracy- in women			
					Spatial span task	↔ Span, ↓ errors in men, ↑ errors in women			

↑ = Increase, ↓ = decrease, ↔ = no change

CANTAB Cambridge Neuroscience test battery, *COWAT* controlled oral word assessment test, *CPT* continuous performance test, *DSST* digit symbol substitution test, *DFR* delayed free recall, *EM* episodic memory, *DRR* delayed recognition recall, *FR* free recall, *HVLT* Hopkins verbal learning test, *GDSA* goal-directed serial alternation, *I* inhaled, *IFR* immediate free recall, *IM* intramuscular, *IRR* immediate recognition recall, *IV* intravenous, *MicroCogBattery* a computer administered cognitive battery testing attention, *RT* reasoning, spatial ability and memory (the tests for memory include digit span, California verbal learning task and story recall), *O* oral, *PAB* performance assessment battery, *PAL* paired associate learning, *PASAT* paced auditory serial addition test, *RMS* running memory span, *RT* reaction time, *DMTS* delayed match to sample task, *RVIP* rapid visual information processing task from CANTAB, *SCWT* Stroop color word test, *SL* sublingual, *WM* working memory, *WP* word presentation, *WR* word recognition

on retrieval of information learned under normal conditions and the impairment in recall of information learned under the influence of Δ-9-THC was interpreted as an effect on encoding rather than retrieval. The use of unassayed cannabis, the differences in the composition of the placebo and Δ-9-THC groups, and the selective inclusion of only those subjects who reported feeling "high" in the analysis confound the interpretation of the results. In addition, the author used the serial position curves to investigate the effects of cannabis. While the recency effect of lists remained unaffected, recall of earlier lists (primacy effect) was significantly decreased by cannabis. The author interpreted this effect on the serial position of word lists as evidence that cannabis did not impair recall from short-term memory, but did impair recall of information that should have been transferred into long-term memory.

Darley et al. (1973) used the Sternberg (1966) task in an attempt to evaluate which stage of short-term memory was impaired by cannabis. The Sternberg task, a short-term recognition memory paradigm, has periods of encoding, retention, and recognition that are all separated in time. In this task, subjects are asked to memorize a set of items that are presented on a computer screen. After this, a series of items appear one at a time, and the subject has to tap a "Yes" or "No" button to indicate whether the item was from the memorized set. Response times and numbers of errors are recorded. Two measures are derived from a plot of reaction time (RT) against size of memory set: (1) the slope representing the time taken to compare the test item with memorized set, and (2) the intercept on the *Y*-axis, i.e., time taken to encode test stimulus and respond. Darley et al. (1973) utilized memory sets comprised of word lists. The effects of both single and daily (for 5 days) doses of Δ-9-THC were studied. Subjects were tested first on day 1 both before and after they all received 20 mg of Δ-9-THC. After this, half the subjects received 20 mg Δ-9-THC daily on days 2–5 and the other half received placebo. On day 5, subjects underwent the same test as on day 1 before and after they received the same study drug (Δ-9-THC or placebo) that they had been randomized to. Accuracy of response on the Sternberg task was unaffected by Δ-9-THC by both single and repeated daily dosing with Δ-9-THC. There were no other significant effects of Δ-9-THC except that on day 5, i.e., cumulative dosing (5 days × 20 mg/day) Δ-9-THC appeared to increase the time to encode and respond.

The work of Miller and colleagues (Miller et al. 1977a,c,d, 1979; Miller and Cornett 1978) has been a major contribution to the literature on the effects of cannabinoids on memory. Using word lists, they found that relative to placebo, Δ-9-THC at varying doses decreased immediate free recall of word lists without affecting recognition recall and increased the number of intrusions (Miller and Cornett 1978; Miller et al. 1977c, 1979). Although lower than placebo at all time points, the shape of the serial position curve was unaltered by Δ-9-THC (Miller et al. 1977c). The authors speculated that the observed effects on recall might be a consequence of Δ-9-THC's effects on processing the information to be learned. To test this hypothesis, Δ-9-THC (14 mg) or placebo was administered to 16 moderate to heavy users in 2 sessions separated by 1 week. Word lists, where presentation of each word was followed by four kinds of strategies to facilitate meaningful processing, were used (Belmore and Miller 1980). The strategies were yes/no answers to questions about the number of letters making up the word, rhyming with other words, syntax and semantics. Δ-9-THC significantly decreased both immediate and delayed free recall as in previous studies. Furthermore, more meaningful processing (i.e., semantic and syntactic processing) improved immediate free recall regardless of drug condition. However, subjects under the influence of Δ-9-THC were especially impaired on delayed free recall of more meaningfully processed words from the lists presented later. Block et al. (1992) also examined the acute effects of Δ-9-THC on verbal memory, associative learning, text learning, and RT. In addition, they examined the effect of different durations of breath-holding on the effects of smoked cannabis. While Δ-9-THC (19 mg) significantly affected performance in most domains tested relative to placebo, breath-holding did not seem to affect this impairment.

Rehearsal is necessary for information to reenter the short-term store and to be retained for longer periods. The effect of Δ-9-THC on recall was proposed to be mediated by deficient rehearsal during the encoding process. Thus, fixed rehearsal was expected to reduce or eliminate Δ-9-THC-induced recall impairments. Darley et al. (1974) studied the effects of rehearsal and state on learning. Fixed rehearsal did not improve Δ-9-THC-induced recall deficits on a verbal learning task. In two separate sessions spaced 3 days apart, occasional cannabis users were presented with a total of 20 word lists. On the first day (day 1), subjects were instructed to learn the lists alternately by free or fixed rehearsal. After being presented with each list, subjects were asked to recall the list. At the end of the tenth list, subjects were asked to recall all ten previously presented lists. Subjects were then administered a single dose of 20-mg Δ-9-THC, followed 90 min later by another ten lists. Δ-9-THC significantly decreased immediate free recall, an effect that was not improved by the fixed rehearsal procedure. However, fixed rehearsal altered the serial position effect reducing the primacy effect, a phenomenon that is described in further detail later. Subjects returned 3 days later (day 4) and half of them received Δ-9-THC (20 mg) and the other half placebo. This was followed by delayed free and recognition recall of all 20 word lists: the

first ten lists that had been presented before Δ-9-THC on day 1, and the second ten lists that had been presented after Δ-9-THC administration on day 1. To determine if recall was state-dependent, day 4 delayed free recall and delayed recognition recall were analyzed separately for lists 1–10 (day 1, pre-Δ-9-THC lists) and 11–20 (day 1, post Δ-9-THC list). Δ-9-THC administered on day 4 did not impair delayed free or recognition recall of lists learned on day 1 in the pre-Δ-9-THC condition. Similarly, the type of rehearsal procedure (free or fixed) did not impair delayed free or recognition recall of lists learned on day 1 in the pre-Δ-9-THC condition. However, relative to placebo, Δ-9-THC on day 4 was associated with better free recall of lists learned under the influence of Δ-9-THC on day 1. These data support a state-dependent learning hypothesis according to which information learned under the influence of Δ-9-THC is also recalled better under the influence of Δ-9-THC. One limitation of this study was a floor effect on delayed recall, which may have masked the detection of other effects. Of note, the delay period in this study far exceeds the delay period in other studies of cannabinoid effects and the lack of an effect on recognition recall is consistent with the vast majority of studies.

More recently, Curran et al. (2002) studied the effects of oral 7.5 and 15 mg of Δ-9-THC on measures of working memory, attention, executive functioning, reaction time, learning, and recall in infrequent cannabis users in a double-blind, placebo-controlled study. Only the higher dose of Δ-9-THC significantly impaired learning across trials on Buschke's selective reminding task (Buschke and Fuld 1974). These effects peaked at 2 h coinciding with peak plasma Δ-9-THC levels, before returning to baseline at 6 h. In this task, subjects are read a list of 16 words and then asked to recall as many words as possible. The experimenter then reads out only those words not recalled, and the subject has to again recall the entire list. This procedure is repeated three times. Measures of recall from short- and long-term memory, as well as forgetting from long-term memory, are obtained. Δ-9-THC also altered the standard learning curve, i.e., the recall on the third trial was not greater than that on the first, demonstrating that ability to learn new material was impaired by Δ-9-THC.

D'Souza et al. (2004, 2005) studied the effect of IV Δ-9-THC (2.5 and 5 mg) in healthy subjects and schizophrenia patients in two separate studies. Unlike most of the published studies, in this study subjects with a lifetime history of any cannabis use disorder were excluded. Therefore, tolerance, withdrawal, or residual effects did not confound the acute Δ-9-THC effects. Learning and recall measured by the Hopkins verbal learning task, vigilance and distractibility (continuous performance task), verbal fluency and working memory (DMST) were assessed in the subjects who rarely used cannabis. Δ-9-THC significantly impaired immediate recall in a dose-dependent manner across all three trials of immediate recall in healthy individuals (Fig. 4). However, its effects on learning were not statistically significant. Δ-9-THC also impaired delayed (+30 min) free and cued delayed recall and cued recall in a significant, dose-dependent manner. However, its effect on delayed recognition recall showed a trend toward significance. Finally, Δ-9-THC increased the number of false positives and intrusions with a trend toward significance. Similar effects on immediate, delayed free, and delayed cued recall were seen in schizophrenic patients. However, learning over trials and delayed recognition recall were significantly impaired by Δ-9-THC only in the schizophrenia group. In fact on the 5-mg Δ-9-THC dose condition, there was no learning across trials.

Prose recall

While most studies have examined the effects of Δ-9-THC on word lists, a few have also studied its effects on prose recall. Miller et al. (1977b) demonstrated that Δ-9-THC (10.2 mg) significantly decreased both immediate and delayed prose recall in a group of 40 moderate users as compared to placebo. The second day, one half of the group received the same drug as they received on the first day, while the other half received the other drug condition. Thus, subjects who received Δ-9-THC on the second day consisted of subjects who received Δ-9-THC on both days and those who received placebo the first day and Δ-9-THC on the second. Δ-9-THC significantly impaired delayed prose recall of the story presented on day 1 in this group. Subjects who received Δ-9-THC on day 1 and placebo on day 2 also showed delayed recall impairments that persisted to day 2. These data suggest lasting effects of Δ-9-THC on prose recall. Similar to this, Curran et al. (2002) demonstrated that prose recall (story recall), which is more indicative of day-to-day memory continued to show Δ-9-THC-induced impairments even at 6 h, lasting longer than the other impairments. However, other studies of prose recall have had mixed results (Block et al. 1992; Hart et al. 2001).

Δ-9-THC is associated with an increase in both external and internal intrusion errors in the recall of word and prose recall and with false positives in recognition recall (Abel 1971; Hooker and Jones 1987; Miller and Cornett 1978; Pfefferbaum et al. 1977). This increase in intrusion errors appears to be a robust and relatively unique effect of cannabinoids.

Digit recall

In a randomized study of infrequent cannabis users, Tinklenberg reported that relative to placebo, oral Δ-9-

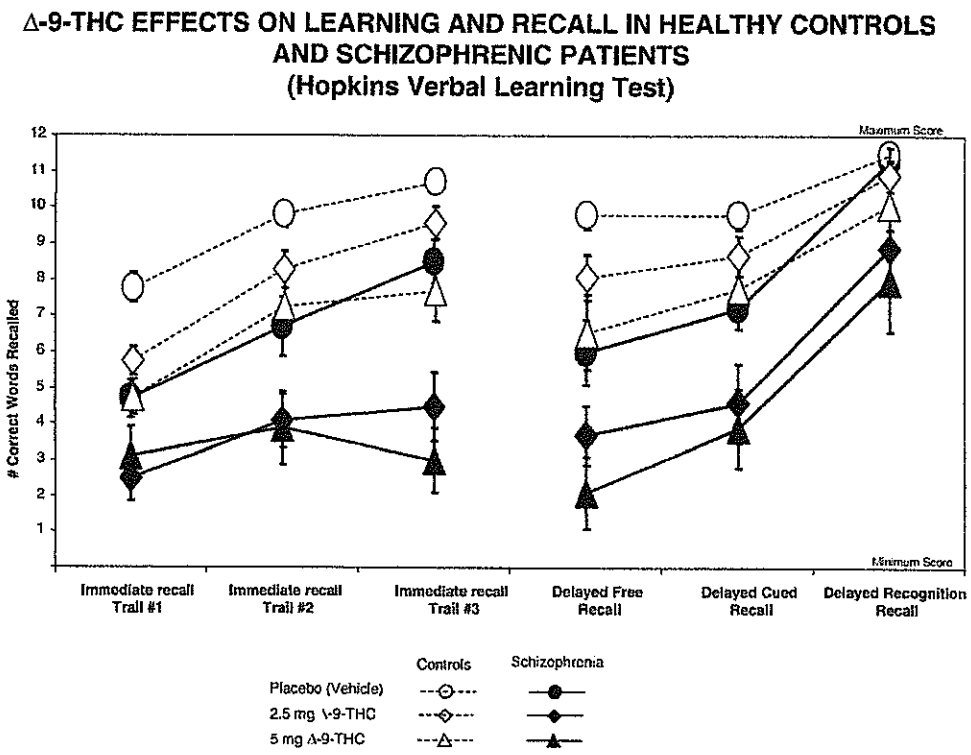


Fig. 4 Learning and recall with placebo and two doses of intravenous Δ-9-THC in health controls and schizophrenic patients (D'Souza et al. 2005)

THC significantly decreased both digit forward and backward recall at all doses (20, 40, and 60 mg) in a manner that is not dose-dependent (Tinklenberg et al. 1970). While the impairment of forward delayed free digit recall peaked at 1.5 h and returned to near baseline at 3.5 h, the impairment in backward recall persisted beyond 3.5 h.

Tinklenberg et al. (1972) did not find any significant 406 effects of oral Δ-9-THC (0.35 mg/kg body weight equivalent to 24.05 mg in a 70-kg individual) on a digit span task in cannabis users. Their observation that lowest recall corresponded with peak drug effects suggested impairments induced by Δ-9-THC. However, the effects did not reach significance and were attributed to a possible floor effect.

Heishman et al. (1990), in their small sample of infrequent users, reported that inhaled Δ-9-THC significantly impaired performance on a serial addition/subtraction task. The task difficulty i.e., the chunks of information that need to be learned and recalled, and the route of administration might account for the differences in results. On the contrary, Chait and Perry (1994) failed to find any effect of Δ-9-THC on backward digit recall.

Visual recall

In light of the notion that cannabis may facilitate mental and visual imagery, Miller et al. (1977d) tested the hypothesis that Δ-9-THC impaired picture recall to a lesser extent than verbal recall. Moderate users completed two test days (Δ-9-THC 14 mg or placebo) 1 week apart. Relative to placebo, Δ-9-THC impaired both verbal and picture recalls; however, while practice improved verbal recall in the Δ-9-THC condition, picture recall remained impaired. Subjective organization of information correlated with recall, but was not influenced by Δ-9-THC. The same group examined whether learning strategy, i.e., field dependent vs independent, influenced Δ-9-THC effects on figure recall (Miller et al. 1978). They hypothesized that the significant variability in recall deficits produced by Δ-9-THC might be explained by differences in cognitive style. Field independence is defined as the ability to overcome embedding contexts in perceptual function and is measured by the Witkin's embedded figures test (Witkin and Oltman 1967). In general, individuals who adopt a field-independent cognitive style perform better on free recall tasks. Consistent with their previous study, Δ-9-THC impaired immediate recall on figure recall, which improved with practice. However, field-dependent individuals made more recall errors on the Δ-9-THC condition, suggesting that learning strategy may influence response to Δ-9-THC.

Working memory

Of several cognitive measures, Wilson found the Digit Symbol Substitution Test (DSST) to be the most sensitive

to Δ-9-THC effects in occasional cannabis users (Wilson et al. 1994). In the DSST, subjects are presented a code of letters substituting for digits. Subjects are then presented the letters prompting them to respond by indicating the appropriate digit. In the easy version, the code is available for reference through task performance. Others have reported that Δ-9-THC increased error rates (Kelly et al. 1990) and decreased both speed and accuracy on the DSST (Heishman et al. 1997). In chronic cannabis users, Δ-9-THC was shown to decrease the number of attempts and correct responses on the DSST without changing overall accuracy (Greenwald and Stitzer 2000).

Lane et al. (2005) showed dose-dependent effects of Δ-9-THC in performance on a pattern recognition delayed match to sample task (DMTS). Occasional users of cannabis received placebo and two doses of Δ-9-THC via a paced smoking procedure. In the delayed match to sample task, two or more comparison stimuli are presented after the presentation of a sample stimulus. The subjects are required to correctly choose the stimulus that matches the previously shown sample. The sample and choice stimuli are separated by a delay period, which can be manipulated. Δ-9-THC disrupted DMTS performance in a dose- and delay-dependent manner. However, Heishman et al. (1997) found that in moderate to heavy cannabis users (one to six joints per week), inhaled Δ-9-THC administered by three paced smoking procedures did not impair performance on a DMTS task that used numbers instead of figures. Perhaps the differences in doses, degree of tolerance in the sample and task parameters account for the disparate results. Similarly, Curran et al. (2002) found that Δ-9-THC did not impair performance on the serial sevens task and a continuous performance task even though it impaired verbal recall. Finally, D'Souza et al. (2004) found that intravenous Δ-9-THC reduced the number of correct responses, but not response time, on a working memory task for figures in healthy subjects.

The continuous performance task (CPT), which is often used to test attention or vigilance, requires subjects to pay attention to sequentially presented items. Subjects are required to constantly utilize a "rule" (e.g., respond when a "9" is preceded by a "1") and also to keep the preceding item in mind while responding. Thus, it may be considered to have a small working memory component. Δ-9-THC does not appear to impair performance on CPT (D'Souza et al. 2004; Vachon et al. 1974; Wilson et al. 1994).

Finally, Ilan et al. (2004) studied the effects of Δ-9-THC on electrophysiological correlates of working and verbal memory. Occasional users of cannabis (n=10) performed the easy and hard versions of a spatial N-back task and word recognition task before and after smoking Δ-9-THC (3.45%) or placebo. The N-Back task, often used to test working memory, is one task where subjects are presented with a series of items (verbal or nonverbal). They are then required to attend to a particular aspect of these items such as description, color, or position, and to respond when the current item is similar to an item presented "n," i.e., 0, 1 or 2 trials before (Owen et al. 2005). Relative to placebo, Δ-9-THC decreased accuracy in performance on both the word recognition task and the high load version of the N-back task. Furthermore, Δ-9-THC also increased reaction times on both versions of the N-back task. Δ-9-THC attenuated several time-locked, event-related potentials (ERPs) under both task conditions. Δ-9-THC specifically decreased the N100, P300 amplitude associated with spatial N-back task performance. Δ-9-THC also attenuated the slow waves associated with the working memory and word recognition task. The attenuation of slow wave patterns associated with the working memory and word recognition task, as well as the P300 associated with the WM paradigm, is thought to reflect encoding processes and suggests that Δ-9-THC disrupts encoding. Recognition of old words relative to new words is associated with a broad positive shift of the ERP, referred to as the "memory-evoked shift." Δ-9-THC attenuated this memory evoked shift. Finally, Δ-9-THC attenuated the N400 to new words, which may reflect a diminished sense of novelty.

While most studies demonstrate that smoking Δ-9-THC cigarettes produces significant impairments in learning and recall (Heishman et al. 1990, 1997; Miller et al. 1977b,c), a few studies discussed below failed to find such effects (Chait and Perry 1994; Fant et al. 1998; Hart et al. 2001). Hart studied the effects of Δ-9-THC in heavy cannabis users (n=18) averaging 24 cannabis cigarettes per week, in a double-blind, randomized, balanced-order study. During the three sessions, each of which were separated by at least 72 h, participants smoked cannabis cigarettes containing 0, 1.8, or 3.9% Δ-9-THC in a paced smoking procedure. Subjects completed baseline computerized cognitive tasks, smoked a single cannabis cigarette, and completed additional cognitive tasks. The cognitive battery (microcog) consisted of tests for reaction time, attention, immediate digit recall, immediate prose recognition recall, delayed prose recognition recall, delayed recognition recall of names and addresses, visuospatial processing, reasoning, flexibility, and mental calculation. In addition, a standard computerized battery was used, which consisted of a digit recall task, a digit–symbol substitution task, a divided attention task, and a repeated acquisition task. Although Δ-9-THC significantly increased the number of premature responses and the time participants required to complete several tasks, it had no effect on accuracy on measures of cognitive flexibility, mental calculation, and reasoning. The absence of acute Δ-9-THC effects was most likely related to significant tolerance to cannabinoids in this sample of heavy users and or the limited sensitivity of the battery. Chait and

Perry (1994) also found no effect of Δ-9-THC on their measures. They studied subjects who varied in their usual Δ-9-THC use (1–16/month) and tested them more than an hour after smoking. Previous studies have demonstrated that the peak effects of inhaled Δ-9-THC occur within 30 min of smoking (Chait and Zacny 1992), and this may account for the lack of any effect seen.

Fant et al. (1998) compared Δ-9-THC and placebo administered by a paced smoking procedure in occasional cannabis users. Subjects received active Δ-9-THC in a fixed order design (15.6 mg followed by 25.1 mg) and were tested using the Walter Reed performance assessment battery, which includes a rapid arithmetic task, a digit recall task, logical reasoning, and a spatial perception task. Although Δ-9-THC produced behavioral and physiological effects, no effects were detected on the cognitive battery. The authors acknowledged that practice effects related to the fixed order of drug administration may have prevented the detection of Δ-9-THC effects.

Discussion

In summary, Δ-9-THC transiently impairs the learning and recall of both verbal and nonverbal information in a manner that is dependent on dose and task difficulty. These memory impairments cannot be accounted for by cannabinoid disruption of attentional processes (Chait and Perry 1994; Curran et al. 2002; D'Souza et al. 2004; Hart et al. 2001), though the latter could certainly contribute to the former.

Some, but not all, studies suggest that cannabinoids impair verbal learning across trials. Δ-9-THC clearly impairs free recall of information learned under the influence of the drug, and most studies demonstrate that Δ-9-THC does not appear to impair recognition recall. One interpretation of this profile of effects is that cannabinoids interfere with the retrieval of information without disrupting encoding. Furthermore, retrieval cues appear to facilitate recall of information learned under the influence of the drug but do not completely restore recall (Miller et al. 1976). The facilitatory effects of retrieval cues on recall suggest that cannabinoids may be disrupting access to memory traces or the organization of information. In contrast to information learned under the influence of Δ-9-THC, the recall of information learned under normal conditions is not impaired by Δ-9-THC. One interpretation of this profile of effects is that cannabinoids do not impair the retrieval of information once it is encoded. Cannabinoids preferentially impair primacy effects but not recency effects, suggesting that these compounds interfere with the process by which information is transferred into longer-term memory. Furthermore, one of the most consistent and unique effects of cannabinoids is an increase in intrusion errors during recall of both word list and prose recall. The increase in intrusion errors may reflect increased mental activity and subsequent irrelevant associations induced by cannabinoids, spilling over into the retrieval processes; a possible mechanism for these effects is discussed later. Taken collectively, the literature suggests that cannabinoids impair both encoding and retrieval. Finally, cannabinoids may also impair the process of consolidation, whereby immediate memory is stored for later retrieval. This process of consolidation is possibly strengthened by the rehearsal of information.

One issue that has received little attention is the role of motivation in test performance in these studies. Some have suggested that recall impairments under the influence of cannabinoids may reflect a reduced motivation state (Miller et al. 1977a). Alternatively, others have speculated that subjects under the influence of cannabinoids may compensate for perceived impairments by working harder, resulting in an underestimation of the extent of drug-induced impairments (Curran et al. 2002). Since their subjects reported an awareness of the drug-induced impairments, the authors went on to speculate that they actively compensated for these impairments resulting in the lack of observable Δ-9-THC effects on some measures. None of the studies reviewed used any procedures to control for effort on cognitive test performance. Recent brain imaging studies raise the intriguing possibility that despite similar cognitive test performance, groups may differ on the extent and degree of brain activation. Kanayama et al. (2004) studied brain activation during performance of a spatial working memory task in heavy cannabis users after recent (6 h) cannabis exposure using functional MRI (fMRI). While there were no significant differences in performance on the working memory task between cannabis users and controls, cannabis users had more prominent and widespread activation in response to the working memory task, including regions not usually used in working memory. The authors suggested that cannabis users recruit more regions in a more pronounced manner so as to meet the demands of the tasks as compared to controls.

The mechanisms underlying the memory impairing effects of cannabinoids

Studies with cannabinoids in animals provide a backdrop to understand the memory impairing effects of cannabinoids in humans. Natural and synthetic exogenous cannabinoids impair learning and memory processes in rodents and nonhuman primates (Aigner 1988; Brodkin and Moerschbaecher 1997; Castellano et al. 2003; Collins et al. 1994; Lichtman et al. 2002). These impairments occur

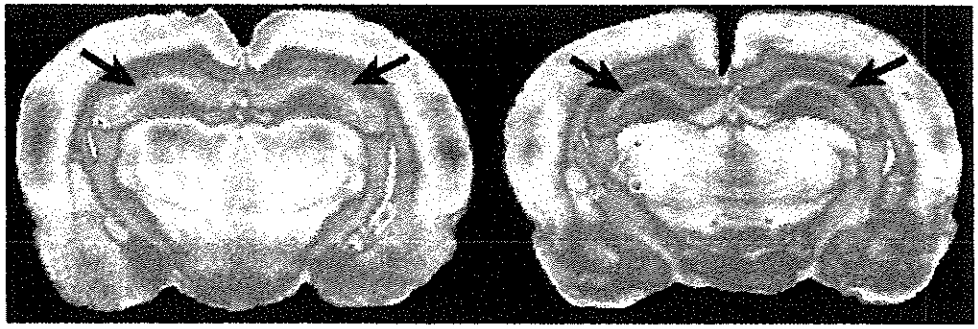
at doses lower than those required to elicit other well-characterized effects of cannabinoids including motor effects, analgesia, hypothermia and, therefore, suggest that cannabinoids have selective effects on memory. The most robust effects are on working memory and short-term memory, both of which require intact hippocampus and prefrontal cortex and both of these regions have high densities of CB1 receptors (Fig. 2).

Maze tasks specifically measure spatial learning and memory, both of which appear to be hippocampal-dependent tasks. Acute administration of Δ-9-THC and a number of synthetic cannabinoids impair performance on a number of maze tasks (Carlini et al. 1970a,b). Chronic administration of Δ-9-THC can result in the development of tolerance in rats. Finally, the fact that intrahippocampal administration of cannabinoids impairs maze performance in rats implicates the centrality of the hippocampus in some of the effects of cannabinoids (Fig. 5; Aigner 1988; Ferraro 1980; Ferraro and Grilly 1974; Winsauer et al. 1999; Zimmerberg et al. 1971).

Acute administration of Δ-9-THC and synthetic cannabinoids also impairs performance on the delayed match-to-sample (DMTS) and delayed nonmatch-to-sample tasks in rodents (Heyser et al. 1993) and nonhuman primates (Aigner 1988; Ferraro 1980; Ferraro and Grilly 1974; Winsauer et al. 1999; Zimmerberg et al. 1971). These impairments induced by Δ-9-THC on DMTS task performance are evident when the delay is long (Heyser et al. 1993). The absence of an effect at short delay times indicates that cannabinoids do not impair the ability to perform the basic task, but instead produce a selective learning and memory deficit. The lack of an effect of Δ-9-THC on DMTS task performance, after very brief delays between sample and match phases and increasing impairment of performance with increasing delay, is akin to the pattern of deficits produced by lesions of the hippocampus and related structures (Freedland et al. 2002; Margulies and Hammer 1991). Recordings from hippocampal complex spike cells indicated that DMTS deficit induced by Δ-9-THC is associated with a specific decrease in hippocampal cell discharge during the sample (but not match) phase of the task (Lichtman 2000; Terranova et al. 1996; Wolff and Leander 2003). These and other data support a central role for CB1 receptors located in the hippocampus and neighboring structures in the memory-impairing effects of cannabinoids. It is notable that people diagnosed with schizophrenia, an illness in which hippocampal dysfunction has been reported, are more sensitive to the learning and memory impairments induced by Δ-9-THC (D'Souza et al. 2005), suggesting that the hippocampus is the locus of the learning and memory impairments induced by cannabinoids. Exogenous administration of Δ-9-THC and other cannabinoid ligands produced widespread, dose-dependent alterations in brain function in the hippocampus, basal ganglia, cerebellum, amygdala, and striatum (Da Silva and Takahashi 2002; Davies et al. 2002). These changes parallel closely both the dose-dependent nature of the effects on cannabinoid-induced behaviors and the time course of the onset of these behaviors, indicating that these alterations in functional activity are the substrates of these behaviors. As discussed later, other areas, particularly the prefrontal cortex, are also likely to be involved.

Many of the effects of Δ-9-THC and other synthetic cannabinoids can be reversed or blocked by CB1 antagonists, supporting the view that the effects of cannabinoids on memory are indeed mediated via actions at CB1 receptors (Marsicano et al. 2002). Furthermore, some studies (Lichtman et al. 2002; Terranova et al. 1996; Wolff and Leander 2003), but not others (Da Silva and Takahashi 2002; Davies et al. 2002), suggest that the CB1R antagonist/inverse agonist, when administered on their own, may enhance memory on tasks that recruit memory processes that span minutes to hours. Systemic SR141716A has been shown to disrupt the extinction of aversive memories in mice (Bohme et al. 2000). More recently, SR141716A has been shown to improve spatial memory

Autoradiogram of rat brain showing reduced local cerebral glucose utilization after acute administration of Δ-9-THC.



Arrows point to Hippocampus

Adapted from Whitlow et al. 2002

Fig. 5 Effect of acute administration of Δ-9-THC 0.0 mg/kg (*left*) and 2.5 mg/kg (*right*) on rates of glucose utilization in the hippocampi of rats 15 min after administration. Note that the rate of glucose utilization decreases with active Δ-9-THC administration. Panel on the *right* shows the range of rates of cerebral glucose utilization

when administered before or immediately after the training, but not when administered before the test (Maccarrone et al. 2002; Reibaud et al. 1999). The profile of effects of SR141716A suggests that it facilitates the acquisition and consolidation of memory without affecting retrieval (Varvel and Lichtman 2002).

CB1 receptor knockout mice were reported to show enhanced long-term potentiation (LTP), a basic process that is discussed in further detail later. CB1 knockout mice showed enhancement of hippocampal LTP (de Oliveira Alvares et al. 2005) and improved performance on memory tasks that rely on hippocampal function test (Marsicano et al. 2002). In contrast Varvel and Lichtman (2002) showed that in the reversal test of the water maze task, another test that relies on hippocampal function, knockout mice spent significantly more time returning to the position where the platform was formerly located and showed impairments in locating the new platform position (Lichtman et al. 2002; Marsicano et al. 2002). Similarly, intrahippocampal administration of the highly selective cannabinoid antagonist AM251 was shown to disrupt induction of LTP in rodents (Dudai 2004; Dudai and Eisenberg 2004; Moscovitch 1995; Squire and Alvarez 1995). CB1 knockout mice showed impairments in both short- and long-term extinction of aversive memories (Hajos et al. 2000; Hoffman and Lupica 2000). These data from CB1 knockouts suggest that the endocannabinoid system may facilitate the extinction of learned behaviors and play a key role in the forgetting of information stored in the long-term memory, in addition to their role in encoding of memory (Wilson and Nicoll 2002). As discussed later forgetting irrelevant information is an important aspect of memory.

Memory consolidation begins when information, registered initially in the neocortex, is integrated by the hippocampal complex/medial temporal lobes and related structures to form a memory trace that consists of an ensemble of bound hippocampal complex-neocortical neurons (Spencer et al. 2003). This initial binding into a memory trace involves short-term processes, the first of which may be completed within seconds and the last of which may be completed within minutes or, at most, days. If every encoded internal representation is instantly stabilized and consolidated, then it is possible that the brain's computational space will be quickly consumed by useless/irrelevant information leading to rapid saturation of processing and storage capacity. Perhaps the endocannabinoid system, as studies with knockout mice have shown, contributes to the mechanisms that prevent the automatic and instantaneous consolidation of memory. Perhaps, similar to endocannabinoids, exogenous cannabinoids prevent or attenuate the consolidation of newly learned memory.

Behavioral studies in animals support the clinical literature and suggest, with respect to the hippocampus, that exogenous cannabinoid treatment selectively affects encoding processes. However, this may be different in other brain areas, for instance the amygdala, where a predominant involvement in memory consolidation and forgetting of information or the extinction of learned behaviors has been established. Extinction is believed to involve active suppression of previously learned associations and seems to involve molecular mechanisms distinct from those associated with normal learning (Abel and Lattal 2001). This possible mechanism may underlie the intrusion errors observed on recall tasks in humans under the influence of cannabinoids. All memories are susceptible to decay over time. If endocannabinoids modulate tonic forgetting, then a partial cannabinoid agonist such as Δ-9-THC may lower this tone. In doing so, this agonist permits "forgotten" information to "intrude" on the learning and recall of new information. This mechanism may underlie the robust increase in intrusion errors seen in studies with Δ-9-THC. If the endocannabinoid system were involved in forgetting and/or extinction processes, then disrupting it via pharmacological or genetic deletion of CB1 receptors may seem in some models to improve memory (Lichtman 2000; Reibaud et al. 1999; Terranova et al. 1996). This is because disruption of endocannabinoid signaling prolonged retention compared with control animals. Conversely, in tasks that require the suppression of previously learned responses, endocannabinoid inhibition may actually interfere with learning, as in the reversal test of this study. CB1 (−/−) mice demonstrated increased perseverance of an acquired spatial memory at the expense of learning a new one (Varvel and Lichtman 2002). Several other reports have demonstrated that disruption of CB1 receptor signaling impairs memory in fear-conditioning procedures. Previously, SR 141716-treated mice and CB1 (−/−) mice exhibited impaired extinction of conditioned freezing to a tone that had been paired with foot shock (Marsicano et al. 2002). Interestingly, presentation of the conditioned stimulus (CS) tone during extinction was sufficient to increase endogenous levels of anandamide and 2-AG in the amygdala. A subsequent study found that SR 141716 also impaired conditioned freezing to the test chamber in which the mice had received the shock (Suzuki et al. 2004). Given the extent to which the endocannabinoid system appears to modulate short-term and long-term forms of synaptic plasticity, it should not be surprising that this system plays a tonic role in mnemonic processes.

Neurochemical mechanisms contributing to the memory-impairing effects of cannabinoids

In the hippocampus, CB1R are located primarily on cholecystokinin containing GABAergic interneurons (Hajos et al. 2001; Katona et al. 2000, 2001). These GABAergic interneurons are believed to orchestrate fast synchronous

oscillations in the gamma range, a critical process in synchronizing pyramidal cell activity (Wilson and Nicoll 2002). Gamma oscillations are synchronized over long distances in the brain and are hypothesized to "bind" together sensory perceptions and to play a role in cognition reviewed in (Shen et al. 1996; Shen and Thayer 1999; Sullivan 1999, 2000). Abnormalities in gamma band synchronization have been reported in schizophrenia (Hajos et al. 2001). Activation of these presynaptic CB1Rs reduces GABA release by interneurons (Martin and Shapiro 2000), which in turn would disrupt the synchronization of pyramidal cell activity (Misner and Sullivan 1999), thereby interfering with associative functions.

Glutamate

Cannabinoids might produce their effects on learning and memory via modulation of glutamate release. The observation that CB1 agonists decrease evoked excitatory postsynaptic current in hippocampal neurons suggests that cannabinoids decrease the release of glutamate through a presynaptic mechanism (Pistis et al. 2001). Recent data also raise the presence of a novel cannabinoid receptor that may be involved in the modulation of glutamate release (Hajos et al. 2000, 2001; Katona et al. 2000).

Memories are believed to be formed by a process involving a rapidly formed and relatively long-lasting increase in the probability that postsynaptic neurons in the hippocampus will fire in response to neurotransmitters released from presynaptic neurons. The leading candidate neural substrates for this mechanism are long-term potentiation (LTP) and long-term depression (LTD) of CA3–CA1 synaptic transmission. LTP is a long-lasting enhancement of synaptic transmission in response to brief, high-frequency stimulation of presynaptic neurons. LTP is readily induced in hippocampal neurons (Martin and Shapiro 2000). LTD is a weakening of a synaptic transmission that lasts from hours to days. It results from either strong synaptic stimulation (cerebellum) or persistent weak synaptic stimulation (as in the hippocampus). Hippocampal LTD may be important for the clearing of old memory traces. Cannabinoid receptor activation inhibits both LTP and LTD induction in the hippocampus (Collins et al. 1994; Misner and Sullivan 1999; Nowicky et al. 1987; Stella et al. 1997; Sullivan 2000; Terranova et al. 1995; Van Sickle et al. 2005). In particular, activation of CB1 receptors blocks LTP of field potentials in the CA1 region and was found recently to inhibit hippocampal LTD of CA1 field potentials as well (Misner and Sullivan 1999).

Acetylcholine

CB1R activation also effects acetylcholine release in an inverted "U" dose response fashion (Acquas et al. 2000, 2001; Gessa et al. 1997, 1998; Nava et al. 2001; Rodriguez de Fonseca et al. 2005). Inhibition of acetylcholine release from cholinergic hippocampal neurons located in the septohippocampal pathway may provide another mechanism for the amnestic effects of cannabinoids.

Dopamine

CB1R receptor activation stimulates mesoprefrontal dopamine (DA) transmission (Chen et al. 1990; Diana et al. 1998; Jentsch et al. 1998; Pistis et al. 2001). Considering that supranormal stimulation of DA D1 receptors in the PFC was shown to impair working memory, the negative effects of cannabinoids on working memory and other cognitive processes might be related to the activation of DA transmission in the PFC. Alternatively, cannabinoids, by inhibiting GABA release from GABAergic interneurons, may also suppress one mechanism by which DA controls PFC neuronal excitability. This might lead to nonspecific activation of the PFC, which in turn may disrupt normal signal processing and result in poor integration of transcortical inputs (Pistis et al. 2001).

Future directions

There is a need to replicate much of the existing data in larger samples. Almost all the data on the effects of cannabinoids on memory in humans is from studies using Δ-9-THC or Δ-9-THC containing herbal cannabis. As discussed earlier, Δ-9-THC is a partial CB1 agonist. Future studies need to investigate the effects of full CB1 agonists and CB1antagonists. Furthermore, studies with putative selective agonists and antagonists of the novel non-CB1/CB2 receptors that are relevant to the cognitive effects of cannabinoids will be important in clarifying the contributions of CB receptor subtypes in the memory impairing effects of cannabinoids. Most studies have included frequent users or heavy users. Future studies should include nonusers, users, and abusers of cannabis to further clarify the effects of tolerance, dependence, and residual Δ-9-THC effects on memory. Most of the literature on cannabinoids is from studies employing verbal memory tasks. However, other forms of memory may be affected by cannabinoids. CB1 receptor transmission was shown to be involved in emotional learning phenomena (Marsicano et al. 2002; Varvel et al. 2005; Varvel and Lichtman 2002). Do cannabinoids impair emotional memory in humans? Related to this, preclinical findings showing the critical role of cannabinoids in forgetting needs to be investigated in humans.

Similarly, there is a need to characterize the neural circuitry of the memory-impairing effects of cannabinoids in humans using brain imaging techniques with good spatial (fMRI or PET) and temporal (EEG) resolution. For

example, do verbal recall impairments induced by cannabinoids correlate with reduced medial temporal blood oxygen level dependent (BOLD) response during encoding of word lists? The development of CB1 receptor imaging radioligands, which has been challenging until now (Dhawan et al. 2006), may provide the tools to establish the relationship between the memory-impairing effects of cannabinoids and changes in CB1 receptor occupancy. Such approaches may permit the translation of preclinical data in humans. In this regard, using assessments of memory that can be used in animals and humans would bridge the gap between the preclinical and clinical literature.

It will also be important to establish the contributions of other neurotransmitters, e.g., dopamine, glutamate, and GABA to the memory-impairing effects of cannabinoids in humans. This could be accomplished, with some limitations, by studying the interactive effects of cannabinoids and drugs acting at other neurotransmitter systems on memory. In this regard, there are distinct differences between the amnestic effects of cannabinoids and other amnestic drugs, e.g., alcohol, benzodiazepines, and NMDA receptor antagonists. For example, the latter three are associated with the phenomenon of retrograde facilitation, which has not been observed with cannabinoids. Thus, comparing cannabinoid effects with the memory impairing effects of better-studied drugs, e.g., scopolamine or ketamine, would help in determining the specificity of cannabinoid effects. Finally, further work is also necessary to determine the differential effects of cannabinoids on encoding, consolidation, and retrieval.

Conclusions

Data from the 1970s and more recent data have shown that exogenous cannabinoids impair several aspects of memory and endocannabinoids may be involved in modulating memory. While progress in the understanding of cannabinoid receptor function has renewed interest and stimulated significant clinical and preclinical research on the cognitive effects of cannabinoids, there is a need to bridge the gap between the preclinical and clinical data.

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The Psychotomimetic Effects of Intravenous Delta-9-Tetrahydrocannabinol in Healthy Individuals: Implications for Psychosis

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Recent advances in the understanding of brain cannabinoid receptor function have renewed interest in the association between cannabinoid compounds and psychosis. In a 3-day, double-blind, randomized, and counterbalanced study, the behavioral, cognitive, and endocrine effects of 0, 2.5, and 5 mg intravenous delta-9-tetrahydrocannabinol (Δ-9-THC) were characterized in 22 healthy individuals, who had been exposed to cannabis but had never been diagnosed with a cannabis abuse disorder. Prospective safety data at 1, 3, and 6 months poststudy was also collected. Δ-9-THC (1) produced schizophrenia-like positive and negative symptoms; (2) altered perception; (3) increased anxiety; (4) produced euphoria; (5) disrupted immediate and delayed word recall, sparing recognition recall; (6) impaired performance on tests of distractibility, verbal fluency, and working memory (7) did not impair orientation; (8) increased plasma cortisol. These data indicate that Δ-9-THC produces a broad range of transient symptoms, behaviors, and cognitive deficits in healthy individuals that resemble some aspects of endogenous psychoses. These data warrant further study of whether brain cannabinoid receptor function contributes to the pathophysiology of psychotic disorders.

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INTRODUCTION

'...acute psychotic reactions, generally lasting but a few hours, but occasionally as long as a week; the reaction seemed dose-related and its main features included paranoid ideation, illusions, hallucinations, delusions, depersonalization, confusion, restlessness and excitement.' in 'Du Haschisch et d l'alientation mentale' JJ Moreau de Tours (1845) (Moreau, 1973).

Until recently, the mechanism of action of cannabinoids remained an enigma. The cloning of brain cannabinoid receptors (CB-1 R), the identification of several endogenous ligands and second messenger systems, the development of selective CB-1 R antagonists, and other recent advances (reviewed in Freund *et al*, 2003; Pertwee, 1999b) have rekindled interest in the association between cannabinoids and psychosis. Since the report of Moreau de Tours (1973), several studies (reviewed in Johns, 2001) suggest an association between psychosis and the use of cannabinoid compounds such as cannabis. There is a paucity of laboratory-based data directly evaluating the psychotomimetic effects of cannabinoid compounds and in particular those of delta-9-tetrahdrocannabinol (Δ-9-THC), the principal active ingredient of cannabis.

The effects of cannabis are a composite of several (up to 80) cannabinoid compounds that may have effects that are synergistic with or antagonistic to Δ-9-THC effects (Hollister, 1988). The principal aim of this study was to characterize the dose-related psychotomimetic effects of Δ-9-THC, the principal active ingredient of cannabis, in carefully screened healthy individuals under double-blind, placebo-controlled laboratory conditions, using standardized behavioral and cognitive assessments.

MATERIALS AND METHODS

The study was conducted at the Neurobiological Studies Unit (VA Connecticut Healthcare System, West Haven, CT)

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with the approval of the Institutional Review Boards at VA Connecticut and Yale University, and the Protocol Review Committee of the Department of Psychiatry, Yale University.

Subjects were recruited from the community by advertisements and were paid for their participation in the study. Subjects were informed about the potential for psychosis, anxiety, and panic. After obtaining informed consent, subjects underwent a structured psychiatric interview for DSM-IIIR (Spitzer *et al*, 1990) and were carefully screened for any DSM-IV Axis I or Axis II lifetime psychiatric or substance abuse disorder (excluding nicotine) and family history of major Axis I disorder. The history provided by subjects was confirmed by a telephone interview conducted with an individual (spouse or family member) identified by the subject prior to screening. In order to avoid exposing cannabis-naïve individuals to a potentially addictive substance, only subjects who had been exposed to cannabis but did not meet lifetime criteria for a cannabis use disorder were included. Past month cannabis use was quantified using a time-line-follow-back approach. Finally, subjects underwent a general physical and neurologic examination, EKG, and laboratory tests (serum electrolytes, liver function tests, complete blood count with differential and urine toxicology). Subjects were instructed to refrain from caffeinated beverages, alcohol, and illicit drugs from 2 weeks prior to testing until study completion. Urine toxicology was conducted on the morning of each test day to rule out recent illicit drug use.

Subjects completed three test days during which they received 5 or 2.5 mg of Δ-9-THC, the principal active ingredient of cannabis, or vehicle (ethanol) by intravenous route in a randomized, counterbalanced order under double-blind conditions. Test days were separated by at least 1 week (>3 times the elimination half-life of Δ-9-THC) to minimize carryover effects (Wall *et al*, 1976). Two doses of Δ-9-THC were chosen to examine dose–response relationships and were based on previous studies with Δ-9-THC demonstrating feasibility and safety (Agurell *et al*, 1986; Volkow *et al*, 1991, 1996). The intravenous route of administration was chosen to reduce inter and intraindividual variability in plasma Δ-9-THC levels with the inhaled route (Azorlosa *et al*, 1992) and to mimic the time course of plasma Δ-9-THC levels associated with the clinical 'high' (Agurell *et al*, 1986; Lindgren *et al*, 1981; Ohlsson *et al*, 1980a). Most studies with Δ-9-THC employ an oral or inhalation (smoking) route of administration. Oral administration delays the onset of effects by 30–120 min, produces lower peak plasma levels, and prolongs the action of the Δ-9-THC compared to the inhaled or intravenous route (Lemberger *et al*, 1971; Ohlsson *et al*, 1980b). The intravenous and smoked routes share similar pharmaco kinetic profiles. The dosing paradigm was designed to achieve peak Δ-9-THC plasma levels comparable to those achieved by smoking standard cigarettes containing 1–3.5% Δ-9-THC (16–34 mg).

Δ-9-THC of 99.6% purity was provided by the NORAC Company, USA. Δ-9-THC was dissolved in 95% ethanol (Agurell *et al*, 1986) to yield a concentration of 2 mg/ml stock solution, which was then passed through a 0.22 μm polymer filter, subjected to sterility and pyrogenicity testing, and assayed by gas chromatography mass spectrometry to confirm its concentration and stored at −20°C for future use. For the control condition, an equivalent volume (≅2 ml) of ethanol (vehicle) was used, which would amount to a concentration of 0.0004% in an adult with average blood volume (4–5 l). Postinjection blood sampling at multiple time points failed to detect ethanol in a subsample of subjects. Subjects fasted overnight and reported to the test facility around 0800, where they were provided a standard breakfast. After obtaining two intravenous accesses at −90 min and baseline assessments at −60 min, subjects were administered Δ-9-THC intravenously over a 2-min period into a rapidly flowing saline infusion. Subjects were attended to by a research psychiatrist, a research nurse, and a research coordinator. Clear 'stopping rules' were determined *a priori* and rescue medication (lorazepam) was available if necessary.

At the end of the last test day an exit interview was conducted to determine if subjects had been adequately informed prior to study participation and for feedback about the study procedures. The study was amended to include prospective measures addressing safety. Subjects were recontacted at 1, 3, 6 months poststudy and asked to estimate their desire for cannabis, whether their cannabis use had changed, and whether they had noted any new medical or psychiatric problems.

Outcome Measures

Behavioral ratings were conducted at the −60, +10, +80, +200 min timepoints (timepoint zero denotes the beginning of the Δ-9-THC infusion). Since the peak intensity of Δ-9-THC effects were expected to occur between +10 and +80 timepoints and were expected to disrupt a subject's capacity to describe subjective effects, behavioral ratings were readministered 140 min after Δ-9-THC administration to capture Δ-9-THC effects retrospectively. Positive, negative, and general symptoms were assessed using the PANSS positive, negative, and general symptoms subscales of the Positive and Negative Symptom Scale (PANSS) (Kay *et al*, 1989). Perceptual alterations were measured using the Clinician Administered Dissociative Symptoms Scale (CADSS) (Bremner *et al*, 1998), a scale consisting of 19 self-report items and eight clinician-rated items (0 = not at all, 4 = extremely) that has been shown to be sensitive to the effects of other psychoactive drugs including ketamine (Krystal *et al*, 1994). Feeling states associated with cannabis intoxication were measured using five self-reported items of a visual analog scale items ('high', 'calm and relaxed', 'tired', 'anxious', 'panic') associated with cannabis effects (Haertzen, 1965, 1966). Subjects were asked to score the perceived intensity of these feeling states at that moment on a 100 mm line (0 = not at all, 100 = extremely).

At 30 min after receiving Δ-9-THC a cognitive test battery was administered. Learning and recall were measured using the Hopkins Verbal Learning Test (HVLT) (Brandt *et al*, 1992; Bylsma *et al*, 1991). The test consists of three consecutive trials of immediate free recall of a 12-item, semantically categorized list, followed 30 min later by testing of delayed free recall, cued recall, and recognition recall. Different but equivalent versions of the test were administered on the 3 test days. Vigilance and distractibility to visual stimuli were measured using a continuous

performance task (Gordon, 1986) in which subjects attended to numbers presented sequentially on a screen. The subject pushed a button to signal when a '1' was preceded by a '9'. The distractibility task was identical to the vigilance task with the exception that numbers were presented sequentially in three contiguous columns. Subjects were instructed to attend to the middle column and ignore the outer two columns. The verbal fluency task requires subjects to generate as many words as possible beginning with a specified letter during a 1-min interval (Corkin *et al*, 1964). Equivalent versions of this task were administered on the 3 test days using letters equated for frequency in English (Borkowski *et al*, 1967). Working memory was assessed using a computerized working memory task for shapes analogous to the Delayed Match to Sample task (Belger *et al*, 1998) and is known to activate prefrontal and hippocampal regions. Each trial consisted of an 'easy' or 'difficult' block classified on the basis of the complexity of shapes. In each block, subjects were presented 20 different shapes for 1 s each at intervals of 1 s on a computer screen and five shapes were repeated. Subjects were instructed to respond by pressing the spacebar when they identified a shape previously shown in that block. In total, 12 different versions of this task were available such that none of the shape stimuli were repeated across the 3 days of testing.

Vital signs were recorded at −60, +10, +50, +80, +140, +200 timepoints. At the −60, +10, +80, and +140 timepoints, blood was sampled from the i.v. line opposite to the one used for administering study drug, for prolactin and cortisol to provide a behaviorally independent measure of cannabinoid effects, and for levels of Δ-9-THC and its primary inactive metabolite 11-nor-delta-9-tetrahydrocannabinol-9-COOH. However, for Δ-9-THC and its main metabolite, only blood samples from the two active THC conditions were assayed. Immediately after collection, blood samples were put on ice, centrifuged, and the extracted plasma was alliquoted into vials for storage at −70°C until time of the assay. Prolactin and cortisol assays were run in duplicate pairs using radioimmunoassay kits to determine prolactin (Serono Diagnostics, Inc.) and cortisol (Baxter Travenol Diagnostics, Inc.) levels. Δ-9-THC and 11-nor-delta-9-tetrahydrocannabinol-9-COOH were measured by GC/MS according to a method by Shaw *et al* (1991). Assays have intra- and interassay RSD% of <10% at 1 ng/ml with 0.5 ng/ml as the lower limit of detection.

Statistical Analyses

All analyses were performed in SAS Version 8.2. The change from baseline data was assessed for normality prior to analysis using normal probability plots and Kolmogorov–Smirnov test statistics. The absence of variance during the placebo Δ-9-THC (vehicle) administration combined with highly skewed responses during the Δ-9-THC conditions precludes the application of typical ANOVA's or mixed models and that ordinal or nonparametric approaches are needed. Since none of the outcomes conformed to normality due to floor effects, a nonparametric analysis for repeated measures data was used (Brunner *et al*, 2002). PANSS subscale scores, VAS scores, CADSS clinician, and CADSS subject ratings were analyzed using the %LD_F2 SAS macro (Brunner *et al*, 2002) with dose (placebo, low, high) and time (P10, P80, P200) as between-subject factors. One of the advantages of our statistical approach is that it uses all available data on each subject including dropouts. THC analyses were performed in the same way restricting the dose levels to low and high. The dose by time interaction was tested first and relative effects plots were used to interpret significant interactions. Hopkins, working memory, verbal fluency, measures of distractibility and vigilance (CPT), and retrospective behavioral data were analyzed using the %LD_F1 macro. Hopkins immediate recall was analyzed using %LD_F2 with only dose as a between-subject factor. Relative effect plots were also used to interpret significant dose effects. The overall alpha level for each hypothesis was fixed at 0.05 level. Bonferroni correction was applied within but not across hypothesis. Thus, for the two subscales of the PANSS (positive symptoms and negative symptoms), a cutoff alpha level of $0.05/2 = 0.025$ was used to declare effects significant for PANSS positive and for PANSS negative symptoms.

RESULTS

A total of 38 healthy subjects were initially screened of whom eight were found ineligible and eight never initiated the study. In all, 22 subjects initiated at least one test day (Table 1) with three and four subjects dropping out after completing 1 and 2 test days, respectively. Cannabis use histories are reported in Table 2. None of the subjects had used cannabis for at least a week prior to testing and this was confirmed by urine toxicology. Data are reported either in figures or tables (means ± SEM), while statistical analyses are reported in the text. For parsimony only those retrospective data that conflict with data collected at other timepoints (+10 or +80) are reported in the text.

Behavioral Measures

Positive symptoms (PANSS). Δ-9-THC transiently increased scores of the PANSS positive symptoms subscale (dose ($\chi^2_{1,87} = 20.2$, $p < 0.0001$); time ($\chi^2_{1,99} = 20.95$, $p < 0.0001$); dose × time ($\chi^2_{3,27} = 8.30$, $p = 0.0001$)) (Figure 1). The increases in positive symptoms induced by Δ-9-THC peaked 10 min after drug administration, were modest and returned to baseline levels by the last timepoint. The quality of symptoms showed similarity to the positive symptoms reported by schizophrenia patients (Table 3) with some subjects losing insight momentarily.

Negative symptoms (PANSS). Δ-9-THC transiently increased scores of the PANSS negative symptoms subscale (dose ($\chi^2_{1,92} = 19.1$, $p < 0.0001$), time ($\chi^2_{1,54} = 19.45$, $p < 0.0001$); dose by time ($\chi^2_{3,13} = 7.27$, $p = 0.0005$)) (Figure 1). Subjects were rated as being less spontaneous, internally preoccupied, and displaying blunted affect.

Perceptual alterations (CADSS). Δ-9-THC transiently increased perceptual alterations as measured by the CADSS clinician-rated subscale (dose ($\chi^2_{1,97} = 12.58$, $p = 0.0000$); time ($\chi^2_{1,84} = 27.27$, $p = 0.0000$); dose by time ($\chi^2_{3,18} = 9.09$, $p = 0.0000$)). Subjects were rated as being 'spaced out,'

Table 1 Demographic Information

	n	Mean (SD)
Age (SD) years	All ($n=22$)	29 (11.6)
	Males ($n=14$)	30.4 ($\pm$11.8)
	Females ($n=8$)	26.8 ($\pm$11.6)
Education (SD) years	All	16.3 (1.9)
	Males	16.4 ($\pm$2)
	Females	16.1 ($\pm$1.9)
Handedness	Right	18
	Left	4
Race	Caucasian	15
	Indian	1
	African American	6
Weight	All	174.7 ($\pm$46.4)
	Males ($n=14$)	184.1 ($\pm$40.2)
	Females ($n=8$)	158.1 ($\pm$54.3)

Table 2 Cannabis Use History

# of exposures	***n***
Estimated lifetime cannabis exposures	
Less than 5 times	7
5–10 times	0
11–20 times	3
21–50 times	2
51–100 times	4
>100 times	6
Time	***n***
Last exposure to cannabis	
Past week	0
1 week–1 month	4
1–6 months	6
6 months–1 year	1
1–5 years	4
5–10 years	3
>10 years	4

seeming separated or detached from the test environment, had said or done something bizarre or needed redirection. Δ-9-THC also transiently increased perceptual alterations as measured by the CADSS subject-rated subscale (dose ($\chi^2_{1.69}=21.006$, $p=0.0000$); time ($\chi^2_{1.86}=44.11$, $p=0.0000$); dose by time ($\chi^2_{2.78}=7.38$, $p=0.0001$)) (Figure 2). Subjects reported having distorted time perception, external perception, feelings of unreality, and altered body perception.

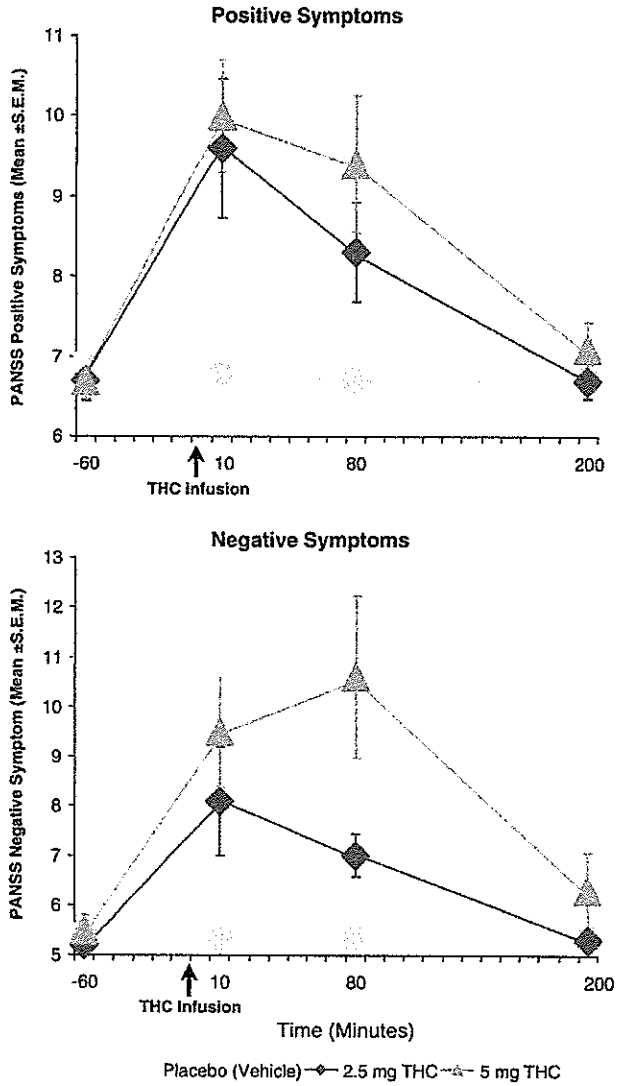

Figure 1 Effects of Δ-9-THC on the seven-item positive (left panel) and six-item negative (right panel) symptom subscales of the Positive and Negative Syndrome Scale (PANSS). The PANSS is used to measure the symptoms associated with schizophrenia. Scores for each item range from 0 (absent) to 7 (extremely). The ranges of scores on the positive and negative subscales are 0–49 and 0–42, respectively.

General symptoms (PANSS). Δ-9-THC transiently increased scores of the PANSS general symptoms subscale (dose ($\chi^2_{1,76}=3.3$, $p=0.043$), time ($\chi^2_{1,63}=37.51$, p<0.0001), and dose × time ($\chi^2_{3,32}=5.15$, $p=0.00095$)) that includes items for somatic concern, guilt feelings, tension, uncooperativeness, unusual thought content, poor attention, and preoccupation.

Feeling States

'High' (VAS). Δ-9-THC transiently increased VAS scores of 'high'(dose: ($\chi^2_{1.38}=31.56$, $p=0.0000$); time ($\chi^2_{1.79}=22.32$, $p=0.0000$); dose by time ($\chi^2_{2.02}=4.5$, $p=0.0108$)) (Figure 3).

'Anxious', 'calm and relaxed,' and 'panic' (VAS). Δ-9-THC transiently increased VAS scores of 'anxious' (dose ($\chi^2_{1.88}=11.44$, $p=0.00002$); time ($\chi^2_{1.27}=22.71$, $p=0.0000$);

Table 3 Subject Quotes

Subject quote	Symptom
'I thought you could read my mind, that's why I didn't answer'	Suspiciousness/paranoia with loss of insight
'I thought you all were trying to trick me by changing the rules of the tests to make me fail'	
'I thought you were turning the clock back to confuse me'	
'I could hear someone on typing on the computer ...and I thought you all were trying to program me'	
'I felt as if my mind was nude'	
'I thought you all were giving me THC thru the BP machine and the sheets'	
'I thought that this was real....I was convinced this wasn't an experiment'	Loss of insight
'I couldn't keep track of my thoughts... they'd suddenly disappear'	Conceptual disorganization, thought disorder, thought blocking, loosening of associations
'It seemed as if all the questions were coming to me at once... everything was happening in stacatto'	
'My thoughts were fragmented... the past present and future all seemed to be happening at once'	
'I felt I could see into the future...I thought I was God'	Grandiosity
'The AC that I couldn't hear before suddenly became deafening'	Inability to 'filter' out irrelevant background stimuli
'I thought I could hear the dripping of the i.v. and it was louder than your voice'	

dose by time ($\chi^2_{2.78} = 3.58$, $p = 0.0157$)). Consistent with an increase in anxiety, Δ-9-THC decreased VAS scores of 'calm and relaxed' (dose ($\chi^2_{2.00} = 1.66$, $p = 0.1899$); time ($\chi^2_{1.63} = 8.73$, $p = 0.00049$); dose by time ($\chi^2_{3.37} = 2.61$, $p = 0.042$)). However, Δ-9-THC effects on VAS 'panic' scores were not statistically significant (dose ($\chi^2_{1.63} = 0.72$, $p = 0.4612$); time ($\chi^2_{1.59} = 8.96$, $p = 0.00045$); dose by time ($\chi^2_{3.32} = 1.57$, $p = 0.1884$)).

'Tired'(VAS). Δ-9-THC effects on VAS 'tired' scores in analysis of primary timepoint data were significant for dose ($\chi^2_{1.97} = 11.53$, $p = 0.00001$) but not time ($\chi^2_{1.51} = 0.755$, $p = 0.435$) or the dose × time interaction ($\chi^2_{3.21} = 0.611$, $p = 0.62$). Analysis of the retrospective timepoint data revealed a significant dose effect ($\chi^2_{1.85} = 9.23$, $p = 0.0001$) of Δ-9-THC on increasing VAS 'tired' scores.

Neuropsychological Measures

Immediate recall, delayed recall and learning (Hopkins Verbal Learning Test) (Figure 4). Δ-9-THC significantly impaired immediate recall ((dose ($\chi^2_{1.31} = 12.32$, $p = 0.00011$); trial ($\chi^2_{1.73} = 64.51$, $p = 0.0000$)) in a dose-dependent manner across all three trials of immediate recall. However, its effects on learning were not statistically significant (dose by trial: $\chi^2_{3.28} = 1.58$, $p = 0.1875$). Δ-9-THC impaired delayed (+30 min) free recall (dose: $\chi^2_{1.69} = 6.55$, $p = 0.00266$) and delayed cued recall (dose: ($\chi^2_{1.97} = 4.06$, $p = 0.0177$) in a significant, dose-dependent manner. However, its effect on delayed recognition recall showed a trend towards significance (dose: ($\chi^2_{1.98} = 2.65$, $p = 0.07$). Finally, Δ-9-THC increased the number of false positives (dose: $\chi^2_{1.76} = 2.43$, $p = 0.095$) and intrusions (dose: $\chi^2_{1.88} = 2.85$, $p = 0.06$) with a trend towards significance.

Distractibility and vigilance. Δ-9-THC had no effect on omission (dose: $\chi^2_{1.99} = 0.46$, $p = 0.62$) or commission (dose: $\chi^2_{1.75} = 0.68$, $p = 0.487$) errors in the vigilance task. Δ-9-THC effects on latency trended towards significance ($\chi^2_{1.95} = 2.69$, $p = 0.068$). Δ-9-THC had significant dose effects on omission errors ($\chi^2_{1.73} = 4.70$, $p = 0.0126$) and latency ($\chi^2_{1.94} = 3.06$, $p = 0.048$) but not commission errors (dose: $\chi^2_{1.89} = 0.81$, $p = 0.44$) in the distractibility task (Table 4).

Verbal fluency. Δ-9-THC did not have any significant dose effects on the number of words generated in 1 min (dose: $\chi^2_{1.90} = 0.977$, $p = 0.373$), but trended towards increasing the number of perseverations (dose: $\chi^2_{1.94} = 2.61$, $p = 0.075$) (Table 4).

Working memory. Δ-9-THC significantly reduced the number of correct responses in the easy subtask (dose: $\chi^2_{1.91} = 4.22$, $p = 0.016$) without effecting reaction time (dose $\chi^2_{1.75} = 0.174$, $p = 0.8$). However, Δ-9-THC did not reduce the number of correct responses in the hard subtask (dose $\chi^2_{1.99} = 1.29$, $p = 0.275$), but trended towards increasing reaction time (dose $\chi^2_{1.87} = 2.47$, $p = 0.088$) (Table 4).

Neurochemical Effects

Cortisol and prolactin. Δ-9-THC had no significant effects on serum prolactin levels (dose (NS); time ($\chi^2_{1.84} = 20.4$, $p = 0.0000$); dose by time (NS)) (Figure 5), but significantly increased serum cortisol levels (dose ($\chi^2_{1.97} = 12.44$, $p = 0.0000$); time ($\chi^2_{1.81} = 4.01$, $p = 0.02164$), dose by time ($\chi^2_{2.52} = 5.3$, $p = 0.00236$)).

Δ-9-THC and 11-nor-delta-9-tetrahydrocannabinol-9-COOH levels. Blood samples were analyzed only on the

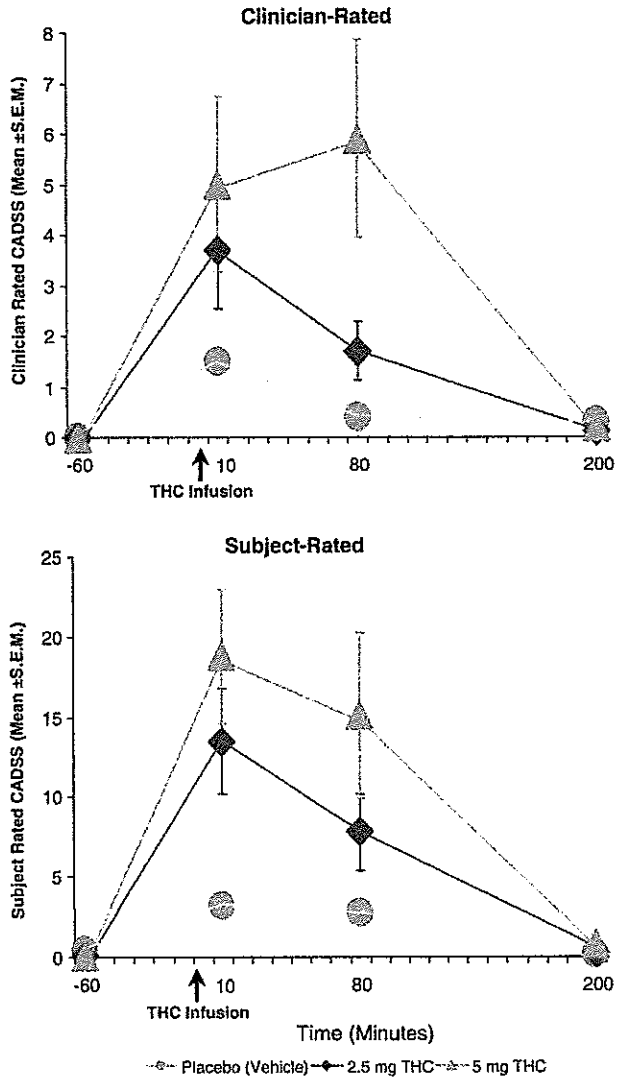

Figure 2 Effects of Δ-9-THC on the eight-item clinician-rated (left panel) and 19-item subject-rated (right panel) subscales of the Clinician Administered Dissociative Symptoms Scale CADSS). The CADSS is used to measure perceptual alterations. Scores for each item range from 0 (absent) to 4 (extremely). The ranges of scores on the clinician- and subject-rated subscales are 0–32 and 0–76, respectively.

two active test days. Plasma Δ-9-THC levels were highest at the +10 min timepoint (2.5 mg dose = 82 ng/dl (±87.4); 5 mg dose = 119.2 ng/dl (±166.5)), lower at the +80 timepoint and fell considerably by the +200 timepoint. However, differences between the two doses were not statistically significant (dose ($\chi^2_1 = 2.32$, $p = 0.1278$); time ($\chi^2_{1.43} = 69.36$, $p = 0.0000$); dose by time ($\chi^2_{1.63} = 1.86$, $p = 0.164$)). This is probably as a result of significant variability in the plasma Δ-9-THC concentrations observed. 11-nor-delta-9-tetrahydrocannabinol-9-COOH levels were highest at the +10 min timepoint (2.5 mg dose = 43.8 ng/dl (±26.1); 5 mg dose = 81.9 ng/dl (±47)) were lower at the +80 timepoint (2.5 mg dose = 28.6 ng/dl (±19.3); 5 mg dose = 49.5 ng/dl (±30.4)) and remained detectable at the +200 timepoint. Differences between the two doses were statistically significant (dose ($\chi^2_1 = 4.08$, $p = 0.043$); time ($\chi^2_{1.47} = 60.43$, $p = 0.0000$); dose by time ($\chi^2_{1.57} = 0.875$, $p = 0.394$)). Since plasma Δ-9-THC levels are out of phase (hysteresis) with behavioral changes (Cocchetto *et al*, 1981; Cone and Huestis, 1993; Huestis *et al*, 1992), no attempt was made to correlate plasma levels to behavioral, cognitive, or endocrine measures.

Safety Data

No serious adverse events (death, hospitalization, emergency room visit) occurred during the study. The reasons for dropouts included acute paranoia ($n = 1$), panic ($n = 1$), hypotension ($n = 2$), difficulty with venous access ($n = 1$), withdrawal of consent due to dislike of THC effects ($n = 3$), and scheduling difficulties or other non-study issues ($n = 1$). The one subject who experienced a significant, acute paranoid reaction associated with significant distress after receiving 5 mg THC was administered 2 mg lorazepam with good effect. Exit interviews conducted in a subsample of subjects revealed that subjects felt they had been adequately informed about the risks of the study during the consent process. Follow-up assessments (1, 3 and 6 months) failed to show the emergence of new psychiatric symptoms or any change on several measures of cannabis use (Table 5).

DISCUSSION

The principal finding of the study is that Δ-9-THC produced transient effects in healthy individuals including positive symptoms, negative symptoms, perceptual alterations, euphoria, anxiety, and deficits in working memory, recall, and the executive control of attention without altering general orientation. The positive symptoms induced by Δ-9-THC included suspiciousness, paranoid and grandiose delusions, conceptual disorganization, and illusions. It also produced depersonalization, derealization, distorted sensory perceptions, altered body perception, feelings of unreality and extreme slowing of time. Δ-9-THC produced negative symptoms including blunted affect, reduced rapport, lack of spontaneity, psychomotor retardation, and emotional withdrawal.

While cannabis has been reported to impair several aspects of cognitive functioning in a dose-related manner, deficits in verbal recall appear to be the most consistent finding in laboratory studies (Belmore and Miller, 1980; Chait and Zacny, 1992; Curran *et al*, 2002; Hart *et al*, 2001; Heishman *et al*, 1997, 1990; Hooker and Jones, 1987; Marks and MacAvoy, 1989; Miller and Branconnier, 1983; Miller *et al*, 1977). Our data are consistent with these effects. Δ-9-THC impaired verbal recall but not learning, suggesting that its effects are primarily on verbal working memory. While Δ-9-THC disrupted delayed recall, these effects appeared to be largely as a consequence of a disruption in immediate recall. The observation that recognition recall was least disrupted, suggests that Δ-9-THC impairs retrieval more than encoding.

In comparing the cognitive data from this study with more recent studies, several factors need to be considered including, but not limited to, the degree of current cannabis use (tolerance) and lifetime cannabis exposure of the study

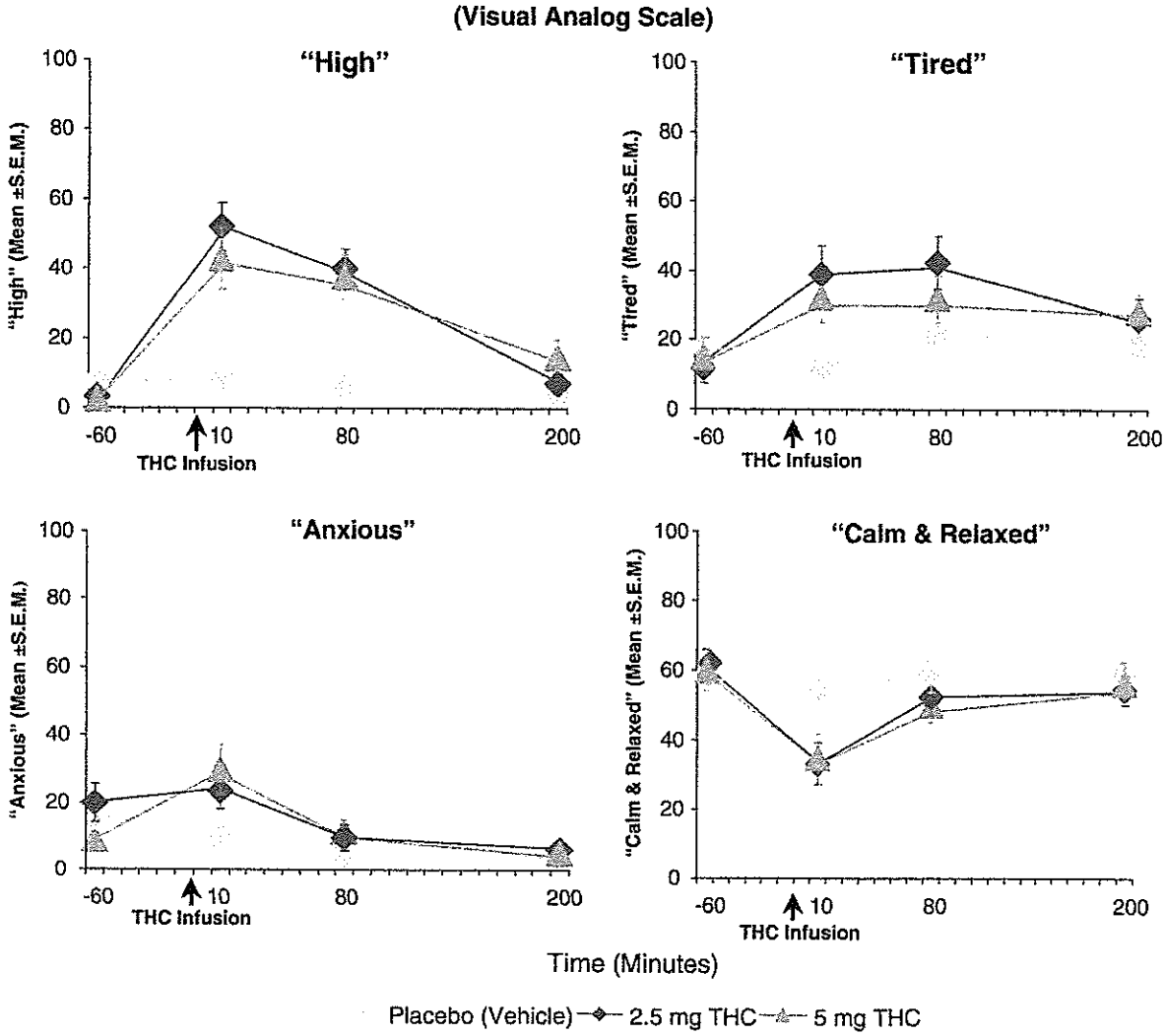

Figure 3 Effects of Δ-9-THC on feeling states associated with the cannabis response. Each feeling state was measured with a visual analog scale (0 = not at all to 100 = extremely).

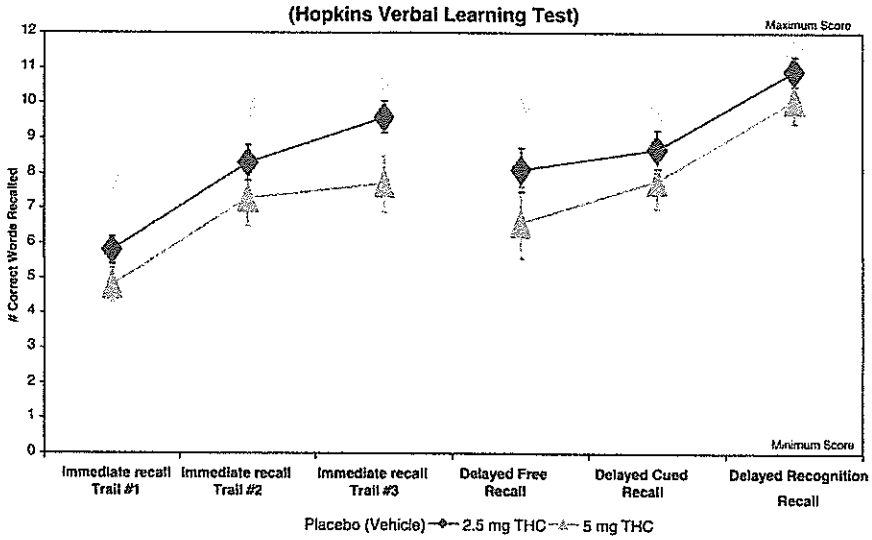

Figure 4 Effects of Δ-9-THC on learning, immediate free recall, delayed free recall, delayed cued and recognition recall measured by a 12-word learning task (Hopkins Verbal Learning Test).

Table 4 Δ-9-THC Effects on Neuropsychological Test Performance

Outcome measure	Placebo Δ-9-THC	2.5 mg Δ-9-THC	5 mg Δ-9-THC	Dose effect
Vigilance				
n	19	19	15	
Omission errors	1.1 (±2.1)	0.9 (±1.3)	2 (±4.9)	$\chi^2_{1,99}=0.46$, $p=0.62$
Commission errors	0.4 (±0.7)	0.4 (±0.6)	0.7 (±1)	$\chi^2_{1,75}=0.68$, $p=0.487$
Latency	42.4 (±7.4)	44.1(±8.4)	45.6 (±8.3)	$\chi^2_{1,95}=2.69$, $p=0.068$
Distractibility				
n	19	18	15	
Omission errors	2.5 (±3.7)	3.8 (±4.4)	6.6 (±8.4)	$\chi^2_{1,73}=4.70$, $p=0.0126$
Commission errors	0.9 (±2.3)	1.3 (±2.4)	5.7 (±17.1)	$\chi^2_{1,89}=0.81$, $p=0.44$
Latency	41.7 (±6.2)	44.8 (±6.7)	48.2 (±14.3)	$\chi^2_{1,94}=3.06$, $p=0.048$
Verbal fluency				
n	19	19	16	
# words generated	17.1 (±4.3)	17 (±5.3)	15.3 (±5.1)	$\chi^2_{1,90}=0.977$, $p=0.373$
Perseverations	0.4 (±0.8)	0.1 (±0.3)	0.4 (±0.5)	$\chi^2_{1,94}=2.61$, $p=0.075$
Working memory				
n	17	18	16	
Easy task correct	4.1 (±1.3)	3.1 (±1.5)	3.9 (±1.2)	$\chi^2_{1,91}=4.22$, $p=0.016$
Easy task reaction time	887.6 (±242.8)	908.8 (±240.3)	954.4 (±273.5)	$\chi^2_{1,75}=0.174$, $p=0.811$
n	18	18	15	
Hard task correct	3.5 (±1.2)	2.8 (±1.4)	2.9 (±1.9)	$\chi^2_{1,99}=1.29$, $p=0.275$
Hard task reaction time	977.8 (±311.3)	1077.3 (±266.9)	953.6 (±209.3)	$\chi^2_{1,87}=2.47$, $p=0.088$

sample, the dosing paradigm, the task characteristics and at what timepoint the tests were administered. In contrast to our study, Hart *et al* (2001) found minimal effects of Δ-9-THC on cognitive test performance; however, the subjects were cannabis dependent and were smoking an average of four cannabis joints per day for several years.

Curran *et al* (2002) studied subjects who had similar cannabis use histories to our subjects. However, relative to our study, Curran *et al* (2002) used oral Δ-9-THC which achieved much lower plasma levels, employed some different cognitive tasks, and administered those tasks at different timepoints in the Δ-9-THC dose–response curve. The effects of Δ-9-THC on immediate and delayed verbal recall are in agreement with the current study. However, whereas we found that Δ-9-THC impaired performance on a computerized visual working memory for shapes, Curran *et al* (2002) did not find an effect on a relatively simpler task of working memory, the serial sevens task. Our data are consistent with an extensive animal literature showing a robust effect of cannabinoids on working memory (reviewed in Lichtman *et al*, 2002). When the rapid visual processing task of sustained attention was made more demanding in our study similar to the task of Curran (2002), Δ-9-THC appeared to impair performance. Consistent with this, several subjects reported that after receiving Δ-9-THC, irrelevant sounds and visual patterns that were previously in the background, for example, the sound of the airconditioner or the pattern of the curtains, came to the foreground and was perceived as distracting. This might reflect a disruptive effect of Δ-9-THC on the 'filtering' of nonsalient information that has been observed in long-term cannabis users (Solowij *et al*, 1991).

Δ-9-THC produced these effects in healthy individuals carefully screened for any obvious risk factors for psychosis, including any DSM-IV Axis I diagnosis in first-degree relatives. The basis of why some subjects but not others experienced transient but significant psychotic symptoms is not clear, but is of considerable interest. Several large sample studies ($n=7000$–$50\,0000$) suggest that moderate (more than 20 times) lifetime exposure to cannabis is associated with a higher risk to develop schizophrenia later on (Andreasson *et al*, 1988; van Os *et al*, 2002; Zammit *et al*, 2002). Therefore, we examined the relationship between lifetime cannabis exposure (Table 2) and psychotomimetic effects of Δ-9-THC in this study. The sample was divided into two groups based on whether subjects had been exposed to cannabis more or less than 20 times in their lifetime. The difference in peak change in PANSS positive symptom subscale scores between the 5 mg Δ-9-THC and placebo condition was the outcome used. The two groups were not significantly different ($t_{15}=0.44$, $p=0.666$) in their response to Δ-9-THC effects on peak positive symptom scores. The lack of any obvious relationship between lifetime cannabis exposure (Table 2) and psychotomimetic response to Δ-9-THC are in contrast large epidemiological studies. One possible explanation for this

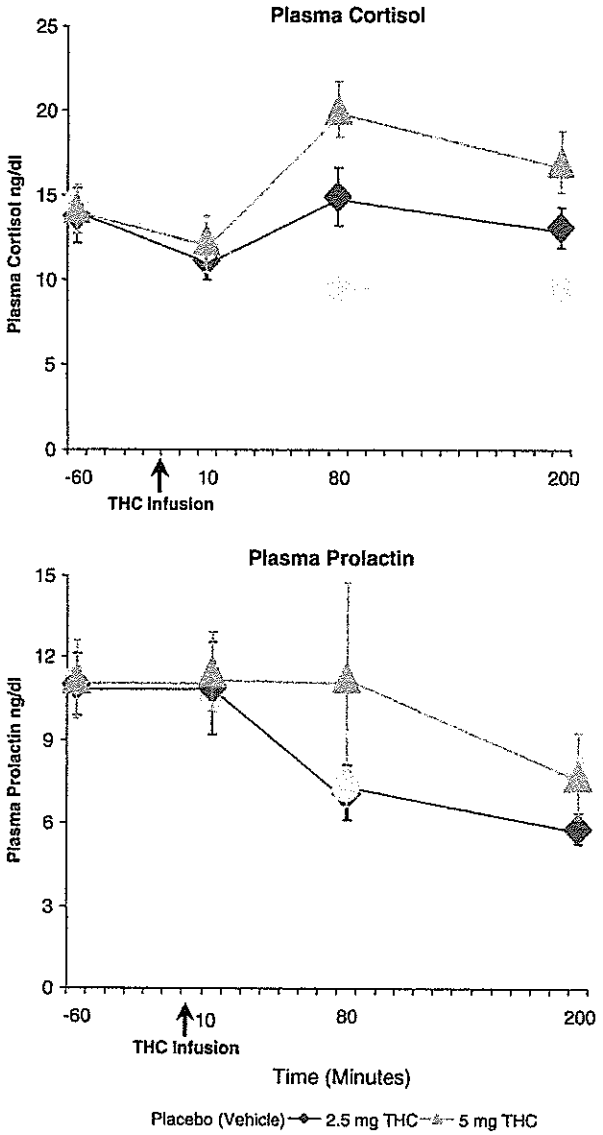

Figure 5 Effects of Δ-9-THC on plasma prolactin and cortisol levels.

contrast is that the small sample in our study may not have allowed the detection of a relationship between lifetime cannabis exposure and the psychotomimetic response to Δ-9-THC.

The constellation of symptoms produced by Δ-9-THC resembles several dimensions of endogenous psychotic disorders like schizophrenia. The findings of this study provide support for a cannabinoid 'model' psychosis (Beringer and Marx, 1932) just as dopaminergic (DA) stimulants (amphetamine), serotonergic agents (LSD and psylocibin), and glutamatergic antagonists (ketamine) have been studied as laboratory-based models of endogenous psychotic disorders (Adler *et al*, 1998; Angrist *et al*, 1974; Ellison, 1994; Krystal *et al*, 1994; Lieberman *et al*, 1987; Malhotra *et al*, 1996; Siomopoulos, 1975; Snyder, 1973; Vollenweider *et al*, 1998, 2000). In contrast to DA stimulants, Δ-9-THC like ketamine produced positive, negative, and cognitive symptoms of psychosis.

The findings of this study add to a growing body of literature from pharmacological (Jones, 1971; Leweke *et al*, 2000, 1999b; McGuire *et al*, 1995), epidemiological (Andreasson *et al*, 1987, 1988, 1989; Arseneault *et al*, 2002; McGuire *et al*, 1995; Zammit *et al*, 2002), genetic (Ujike *et al*, 2002), neurochemical (Leweke *et al*, 1999a), and post-mortem (Dean *et al*, 2001) approaches, suggesting that the consumption of cannabinoids (exogenous) and/or brain cannabinoid dysfunction (endogenous) may contribute to the pathophysiology of psychosis and/or schizophrenia (Emrich *et al*, 1997; Schneider *et al*, 1998). Clearly, further work is needed to test these hypotheses.

The Mechanism of the Psychotic Symptoms Induced by Δ-9-THC

The psychotropic effects of Δ-9-THC are mediated by partial agonist effects at CB-1 receptors (CB-1R) where it has modest affinity ($K_i = 35$–80 nmol) and low intrinsic activity (Compton *et al*, 1992; Gerard *et al*, 1991; Howlett *et al*, 2002; Matsuda *et al*, 1990). However, its hydroxy metabolite has higher affinity and potency. The primary effect of cannabinoids is the modulation of neurotransmitter release via activation of presynaptic CB-1Rs (reviewed in Belue *et al*, 1995; Freund *et al*, 2003; Pertwee, 1999a). CB-1Rs are distributed with high density in the cerebral cortex, particularly frontal regions, basal ganglia, hippocampus, anterior cingulate cortex, and cerebellum (Egertova and Elphick, 2000; Egertova *et al*, 1998; Elphick and Egertova, 2001; Glass *et al*, 1997; Herkenham *et al*, 1991, 1990), brain regions that are relevant to both the known effects of cannabinoids and also regions that have been implicated in the putative neural circuitry of psychosis.

The effect of CB-1R activation on increasing mesolimbic DA activity may provide one explanation for the positive psychotic symptoms induced by Δ-9-THC (Chen *et al*, 1990b, 1991; French, 1997; French *et al*, 1997; Melis *et al*, 2000; Pistis *et al*, 2002; Tanda *et al*, 1997). CB-1R agonists induce cfos in the NAc (Miyamoto *et al*, 1996) and A10 DA neurons within the ventral tegmentum (Patel and Hillard, 2003), and these effects are blocked by DA D2 receptor antagonists (Miyamoto *et al*, 1996) and CB-1R antagonists (Patel and Hillard, 2003; Porcella *et al*, 1998).

In the hippocampus, CB-1R are located primarily on cholecystokinin containing GABAergic interneurons (Hajos *et al*, 2000; Katona *et al*, 2000, 1999a, 1999b; Tsou *et al*, 1999). These GABAergic interneurons are believed to orchestrate fast synchronous oscillations in the gamma range, a critical process in synchronizing pyramidal cell activity (Hajos *et al*, 2000; Hoffman and Lupica, 2000). Gamma oscillations are synchronized over long distances in the brain and are hypothesized to 'bind' together sensory perceptions and to play a role in cognition (reviewed in Wilson and Nicoll, 2002). Abnormalities in gamma band synchronization have been reported in schizophrenia (Spencer *et al*, 2003). Activation of these presynaptic CB-1Rs reduces GABA release by interneurons (Sullivan, 1999; Katona *et al*, 1999a), which in turn would disrupt the synchronization of pyramidal cell activity (Wilson and Nicoll, 2002; Hoffman and Lupica, 2000), thereby interfering with associative functions, disrupting normal gating mechanisms, and eventually inducing psychotic symptoms.

Table 5 Prospective Follow-up of Safety

Visit #	*n*	No change	Increased	Decreased			
Do you think your exposure to THC in the laboratory has changed your cannabis use?							
1 month	10	8	0	2			
3 month	12	8	0	4			
6 month	12	10	0	2			
Visit #	***n***	**None at all**	**Slightly less**	**About the usual**	**Slightly more**	**Much more**	
Please estimate how intense your desire for cannabis has been since your last test day or questionnaire							
1 month	10	5	1	3	1[a]	0	
3 month	12	5	2	4	1[a]	0	
6 month	12	7	2	3	0	0	
Visit #	***N***	**Not at all**	**1 × /week**	**2–3 × /week**	**4–5 × /week**	**6–8 × /week**	**⩾9 × /week**
Since your last test day or questionnaire, how many times per week have you used cannabis?							
1 month	10	6	2	1	1	0	0
3 month	12	7	2	1	1	1	0
6 month	12	9	1	1	0	1	0
Visit #	***N***	**0–1**	**2–3**	**4–5**	**6–8**	**⩾9**	
Since your last test day or questionnaire, please estimate your daily cannabis use (in dime bags)?							
1 month	10	12	0	0	0	0	
3 month	11[b]	12	1	0	0	0	
6 month	11[b]	12	1	0	0	0	

[a]This subject declared that his cannabis use had increased as a result of moving back to his home country where cannabis was more accessible to him, and the social and legal consequences to cannabis use were minimal.
[b]One person did not answer this question.

The effects of CB-1R activation on hippocampal LTP and LTD may explain Δ-9-THC's amnestic effects. CB-1R activation blocks LTP of CA1 region field potentials (Nowicky *et al*, 1987; Collins *et al*, 1994, 1995; Terranova *et al*, 1995; Misner and Sullivan, 1999) and CB-1 receptor knockout mice have been reported to show enhanced LTP (Bohme *et al*, 2000).

CB-1R activation also effects acetylcholine (ACH) release in an inverted 'U' dose–response manner (Acquas *et al*, 2000, 2001; Gessa *et al*, 1998, 1997; Nava *et al*, 2001; Carta *et al*, 1998). Inhibition of acetylcholine release from cholinergic hippocampal neurons located in the septohippocampal pathway may provide another mechanism for the amnestic effects of cannabinoids.

CB-1R receptor activation stimulates mesoprefrontal DA transmission (Chen *et al*, 1990a; Diana *et al*, 1998; Jentsch *et al*, 1997; Pistis *et al*, 2001). Considering that supranormal stimulation of DA D1 receptors in the PFC has been shown to impair working memory, the negative effects of cannabinoids on working memory and other cognitive processes might be related to the activation of DA transmission in the PFC. Alternatively, cannabinoids, by inhibiting GABA release from GABAergic interneurons, may also suppress a mechanism by which DA controls PFC neuronal excitability. This might lead to nonspecific activation of the PFC, which in turn may disrupt normal signal processing and result in poor integration of transcortical inputs (Pistis *et al*, 2001). Cannabinoids have also been shown to influence glutamatergic synaptic transmission and plasticity in the PFC favoring LTD at the expense of LTP (Auclair *et al*, 2000).

Finally, animal studies have demonstrated that chronic exposure to cannabis in animals can induce behavioral sensitization to subsequent cannabinoid exposure (Cadoni *et al*, 2001; Rubino *et al*, 2001, 2003) and also to amphetamine (Gorriti *et al*, 1999; Lamarque *et al*, 2001; Miyamoto *et al*, 1995; Muschamp and Siviy, 2002). Sensitization has been implicated as a mechanism involved in psychosis (Laruelle, 2000; Duncan *et al*, 1999; Yui *et al*, 1999). It is tempting to speculate whether the behavioral sensitization induced by cannabinoids is a mechanism for the development of psychosis associated with chronic heavy cannabis use.

Neurobiology of the Endocrine Effects of Δ-9-THC

Consistent with the literature, Δ-9-THC increased plasma cortisol levels. Δ-9-THC increases ACTH and cortisol levels via CB-1 receptor activation within the paraventricular nuclei, and either directly or indirectly (via other

neurotransmitters) modulates CRH secretion (reviewed in Murphy *et al*, 1998). Δ-9-THC produces an early and brief increase followed by a predominantly inhibitory effect on prolactin release (reviewed in Murphy *et al*, 1998), that is mediated by CB-1R activation of tuberoinfundibular (TIDA) DA neurons. The lack of a significant inhibitory Δ-9-THC effect of on plasma prolactin in this study may be explained by the brief period of observation.

Limitations

The possibility that some of the results observed could be attributed to alcohol effects cannot be ruled out completely. However, this seems unlikely since (1) alcohol was undetectable in blood; (2) subjects did not report behavioral effects consistent with the alcohol; and (3) in a limited number of subjects who participated in other studies, cognitive test performance on the placebo THC test day (ethanol vehicle) was not different to their performance on the placebo condition (saline) of other studies that they participated in. Finally, other studies using alcohol vehicle did not report any interactions between alcohol and Δ-9-THC (Agurell *et al*, 1986; Lindgren *et al*, 1981; Ohlsson *et al*, 1980a).

The elimination half-life of Δ-9-THC has been reported to vary from 18 h to 4.3 days (Hunt and Jones, 1980; Johansson *et al*, 1989; Kelly, 1992; Sadler *et al*, 1984; Wall *et al*, 1976, 1983; Wall and Perez-Reyes, 1981). The mean interval between each test day was 10 days. It is possible that a test session could have been under the influence of a previous session/s. However, the absence of detectable-9-THC in both urine and plasma samples at the baseline timepoint of each test day, and (2) the lack of any order effect in the statistical analysis, do not support a carryover effect from one test session to another. Further, other recent studies (Curran *et al*, 2002) (Fant *et al*, 1998) suggest that deficits in performance on sensitive tests of cognition produced by Δ-9-THC do not persist beyond 24–48 h.

Several limitations of this study might compromise the generalizability of the findings to the risks of cannabis use. Subjects generally reported Δ-9-THC effects as dissimilar to their previous experience with cannabis. First, unlike the naturalistic setting, subjects were unable to 'titrate' the effects by controlling the dose or rate of administration. Second, the effects of cannabis are a composite of several (up to 80) cannabinoid compounds, terpenoids, and flavonoids that may modulate Δ-9-THC (Hollister, 1988) effects and have 'entourage' effects (Mechoulam and Ben-Shabat, 1999; Russo and McPartland, 2003). Cannabidiol (CBD), a major component of cannabis, has been shown to be a very low affinity, weak antagonist of CB-1R (Petitet *et al*, 1998). CBD and Δ-9-THC may have pharmacokinetic and pharmacodynamic interactions. Thus, CBD may offset some Δ-9-THC effects by its anxiolytic effects (Guimaraes *et al*, 1994; Zuardi *et al*, 1982), antipsychotic-like effects (Zuardi *et al*, 1995; Zuardi *et al*, 1991) and may block the conversion of Δ-9-THC to the more psychoactive 11-hydroxy-THC (Bornheim *et al*, 1995). However, the CBD content of cannabis varies greatly and some samples of cannabis have been reported to be devoid of CBD (Pitts *et al*, 1992). Fourth, the route of administration (intravenous) and rate of administration (2 min) in this study is not socially relevant and may have resulted in a faster delivery and higher levels of Δ-9-THC than what is typically achieved by recreational users. However, peak Δ-9-THC plasma concentrations with the 2.5 mg dose (82 ± 87.4 l ng/dl) and 5 mg (119.2 ± 166.5 ng/dl) were within the range of levels achieved by *ad libitum* smoking of a standard NIDA cigarette (70–163 ng/ml) containing 1–2.5% THC (16–34 mg) (Heishman *et al*, 1990; Lindgren *et al*, 1981; Ohlsson *et al*, 1980a). Of note is that the Δ-9-THC content of cannabis has increased (ElSohly *et al*, 2000) probably as a result of the cloning of high yield cannabis plants and advanced cultivation techniques. The average cannabis joint from the 1960s and 1970s contained about 10 mg of THC. In contrast, cannabis joints from the current era made out of skunkweed, netherweed, and other potent subspecies of cannabis sativa may be 10–20 times more potent (Gold, 1991; Solowij, 1998; WHO, 1997).

Finally, cannabis dependent individuals who might 'benefit' from cannabis were excluded from this study and individuals with negative responses to cannabis either did not volunteer or were excluded. Thus, this study may not represent individuals who have either the most positive or negative responses to cannabis.

In conclusion, Δ-9-THC produced a range of transient behavioral and cognitive effects in psychiatrically healthy individuals similar to those seen in schizophrenia and other endogenous psychoses. The findings of this study have implications for the toxicity of cannabinoid compounds and the pathophysiology of psychotic disorders.

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Cancerous toxins linked to cannabis extract

Date: September 26, 2017

Source: Portland State University

Summary: Researchers have found benzene and other potentially cancer-causing chemicals in the vapor produced by butane hash oil, a cannabis extract.

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FULL STORY

Researchers at Portland State University found benzene and other potentially cancer-causing chemicals in the vapor produced by butane hash oil, a cannabis extract.

Their study raises health concerns about dabbing, or vaporizing hash oil -- a practice that is growing in popularity, especially in states that have legalized medical or recreational marijuana.

Dabbing is already controversial. The practice consists of placing a small amount of cannabis extract -- a dab -- on a heated surface and inhaling the resulting vapor. The practice has raised concerns because it produces extremely high levels of cannabinoids -- the active ingredients in marijuana.

The process of making hash oil also is dangerous because it uses highly flammable and potentially explosive butane as a solvent to extract active ingredients from marijuana leaves and flowers. In July, two people in Portland, OR, died in an explosion and fire at a home where butane hash oil was being manufactured.

"Given the widespread legalization of marijuana in the USA, it is imperative to study the full toxicology of its consumption to guide future policy," said Rob Strongin, a Portland State professor who led the study. "The results of these studies clearly indicate that dabbing, while considered a form of vaporization, may in fact deliver significant amounts of toxins."

Strongin and his team analyzed the chemical profile of terpenes – the fragrant oils in marijuana and other plants -- by vaporizing them in much the same way as a user would vaporize hash oil.

Terpenes are also used in e-cigarette liquids. Previous experiments by Strongin and his colleagues at Portland State found toxic chemicals in e-cigarette vapor when the devices were used at high temperature settings.

The dabbing experiments produced benzene -- a known carcinogen -- at levels many times higher than the ambient air, Strongin said. It also produced high levels of methacrolein, a chemical similar to acrolein, another carcinogen.

Their findings were published in the Sept. 22 issue of *ACS Omega*, a journal of the American Chemical Society.

Story Source:

Materials provided by **Portland State University**. Original written by John Kirkland. *Note: Content may be edited for style and length.*

Journal Reference:

1. Jiries Meehan-Atrash, Wentai Luo, Robert M. Strongin. **Toxicant Formation in Dabbing: The Terpene Story**. *ACS Omega*, 2017; 2 (9): 6112 DOI: 10.1021/acsomega.7b01130

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Evidence on the acute and residual neurocognitive effects of cannabis use in adolescents and adults: a systematic meta-review of meta-analyses

Laura Dellazizzo, Stéphane Potvin, Sabrina Giguère, Alexandre Dumais

First published: 19 January 2022
https://doi.org/10.1111/add.15764

Abstract

Background

Cannabis is among the most consumed psychoactive substances world-wide. Considering changing policy trends regarding the substance, it is crucial to understand more clearly its potential acute and residual adverse effects from a public health viewpoint. Cognitive function is one of the targeted areas with conflicting findings. This meta-review measured the magnitude of acute and residual effects of cannabis on cognition in adolescents and adults provided by meta-analyses and evaluated quality of evidence.

Methods

A systematic search was performed in PubMed, PsycINFO, Web of Science and Google Scholar. Meta-analyses were included if they quantitatively examined the performances of users from the general population on cognitive tasks.

Results

The search retrieved 10 eligible meta-analyses (71 effects sizes, $n = 43\,761$) with evidence ranging from low to moderate quality, which were categorized into domains of cognitive functions: executive functions ($k = 7$), learning and memory ($k = 5$), attention ($k = 4$), processing speed ($k = 5$), perceptual motor function ($k = 2$) and language ($k = 2$). Verbal learning and memory displayed the most robust evidence and were most impaired by acute cannabis intoxication that persisted after intoxication passed. Small-to-moderate acute and residual adverse effects were reported for executive functioning. Cannabis use led to small deficits in inhibitory processes and flexibility, whereas small-to-moderate deficits were reported for working memory and decision-making. Evidence regarding processing speed and attention has shown that cannabis administration induced small-to-moderate adverse

effects and residual neurocognitive deficits were observed in heavy cannabis-using youths. Results showed no significant difference between cannabis users and non-users on language, and small-to-moderate effects for simple motor skills.

Conclusion

Meta-analytical data on the acute effects of cannabis use on neurocognitive function have shown that cannabis intoxication leads to small to moderate deficits in several cognitive domains. These acute impairments accord with documented residual effects, suggesting that the detrimental effects of cannabis persist beyond acute intake.

Supporting Information

Filename	Description
add15764-sup-0001-SUPPEMENTARY MATERIAL GENERAL POP 17 10 2021 Revision 1.pdf PDF document, 394.8 KB	**Table S1.** Electronic search strategy for the meta-review conducted. **Table S2.** Details of the retrieved studies included in the meta-review. **Table S3.** PRISMA Checklist

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Vaping marijuana by teens doubles in last seven years, with potentially harmful consequences, study says

By Sandee LaMotte, CNN
Updated 11:02 AM ET, Mon October 25, 2021

(CNN) Marijuana vaping by school-aged youth doubled between 2013 and 2020, a new study found, with reported use within the last 30 days rising seven-fold during the same time period.

A psychologist's advice: How to talk to your kids about social media and drug abuse

The study, published Monday in JAMA Pediatrics, analyzed 17 studies conducted throughout Canada and the United States that involved nearly 200,000 adolescents. The study found that teens in their senior year of high school were most likely to be vaping marijuana compared to younger adolescents. In 2018, for example, one in three grade-12 students reported vaping weed.

In one of the studies, adolescents also reported a preference for vaping cannabis extracts over dried herbs to get the buzz they desired from THC. THC, or tetrahydrocannabinol, is the main psychoactive compound in cannabis, the one that produces the "high" users desire.

Today's "high" is much more intense than in the past, even that of a mere decade ago, according to the National Institute on Drug Abuse, or NIDA. Modern ultra-potent strains of weed can contain over 15% THC, compared to the 4% or so available in the 1990s.

Choosing vaping oils, extracts and resins over dried weed, called "dabbing," is a disturbing and potentially dangerous trend because vape extracts contain "3 to 5 times more THC than the plant itself," noted the NIDA.

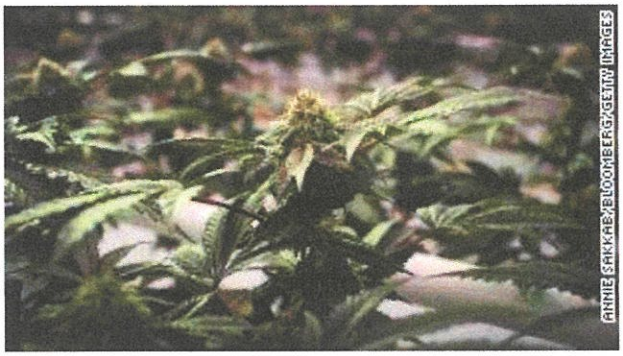
"The use of cannabis products with high THC (that are) easily achievable through vaping raises several potential problems," said study author Carmen Lim, a PhD candidate on Health and Behavioural Sciences at the University of Queensland in Australia via email.

"Not only it is linked to poorer cognitive development in adolescents, it could increase risk of dependence, other substance use and many other health, social, and behavioral problems later in life," Lim wrote.

Impact on teen brain

The use of marijuana by teens -- in any form -- is concerning because weed affects the adolescent brain differently, according to the US Centers for Disease Control and Prevention.

"The teen brain is actively developing and often will not be fully developed until the mid 20s," the CDC stated, adding that use during that time "can have permanent effects" such as poor coordination and damage to learning, memory, problem solving skills, and the ability to pay attention.



Marijuana abuse by youth with mood disorders linked to suicide attempts, self-harm and death, study finds

Use of weed by teens is linked to poor school performance and an increased likelihood of dropping out, the CDC stated. In addition, the CDC warns that teen use of marijuana has been "linked to a range of mental health problems in teens such as depression or anxiety," even psychosis.

Heavy use of marijuana by teens and young adults with mood disorders -- such as depression and bipolar disorder -- is linked to an increased risk of self-harm, suicide attempts and death, according to a study published in January.

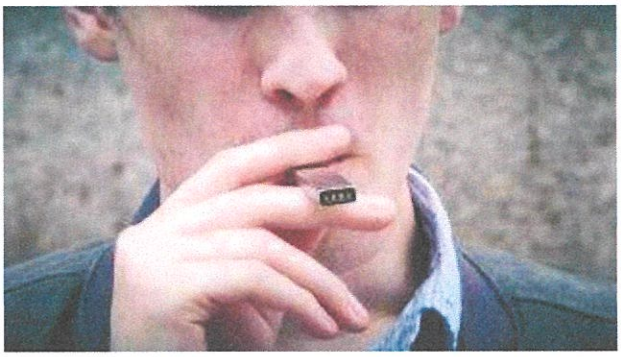
About one in six teens who use marijuana regularly become addicted, the CDC stated. A person is considered dependent on weed when they feel food cravings or a lack of appetite, irritability, restlessness and mood and sleep difficulties after quitting. Called cannabis use disorder, the problem is on the rise, especially in those who started using as teenagers.

"People who begin using marijuana before the age of 18 are four to seven times more likely to develop a marijuana use disorder than adults," the NIDA stated.

Vaping weed may be worse than vaping nicotine

A study published in March found teens were about twice as likely to report "wheezing or whistling" in the chest after vaping marijuana compared to when they smoked cigarettes or e-cigarettes.

"Without a doubt, cigarettes and e-cigarettes are unhealthy and not good for lungs. However, vaping marijuana appears even worse," study author Carol Boyd, professor emerita and codirector of the Center for the Study of Drugs, Alcohol, Smoking & Health at the University of Michigan in Ann Arbor told CNN in a prior interview..



Vaping marijuana linked to lung injury in teens, study says

"Since many teens who vape nicotine, also vape cannabis, I recommend parents treat all vaping as a risky behavior (just like alcohol or drug use)," Boyd said.

Vaping weed is associated with a dangerous, newly identified lung disease called EVALI, short for e-cigarette, or vaping, product use-associated lung injury. In most of the cases, young people were using vaping products that contain THC, the main psychoactive compound in marijuana.

"According to the CDC, 84% of the EVALI cases were associated with cannabis-containing products," Boyd told CNN.

As of February 2020, 68 deaths from EVALI have been confirmed in 29 states and the District of Columbia. The CDC believes EVALI may be linked to vitamin E acetate, a sticky oil substance often added to vaping products to either thicken or dilute the oil in cartridges.

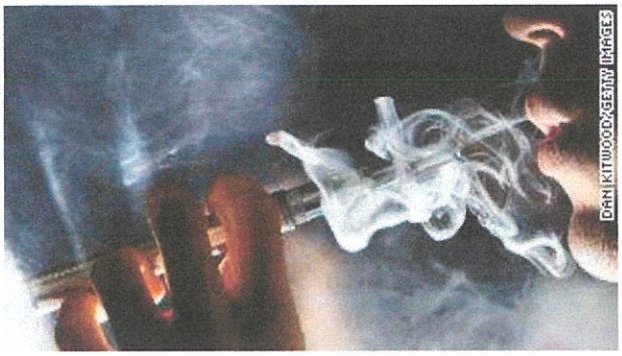
What can parents do?

Parent should be on the lookout for behavior that indicates their child is using marijuana, according to the American Academy of Child and Adolescent Psychiatry.

Red eyes and "getting the munchies" are obvious signs, but irritability, moodiness, forgetfulness and acting "silly or out of character" are also typical, the AACAP advised. Some teens may start to use words like "sparking up," "420," "dabbing" and "shatter," as well.

Be on the lookout for vaping paraphernalia. Not all e-cigarettes come in a cigarette-like package. Today's versions can look like an USB device or a small, refillable pod or case and be hard for a parent to spot.

"The discreet nature of e-cigarettes makes it possible for adolescents to conceal and experiment with drugs such as cannabis," Llm wrote.



Parents are less aware when their kids vape than when they smoke, study says

If you suspect your child may be using, be aware that many teens believe that using weed is safer than drinking alcohol or using other drugs. Prepare yourself for the conversation by knowing "the myths and the facts" about weed, the AACAP said.

"For example, teenagers may say, 'it is harmless because it is natural,' 'it is not addictive,' or 'it does not affect my thinking or my grades,'" the AACAP warned. Or they may say it's OK because people use it "for medical purposes."

Facts about the reality of marijuana use and other tips for parents can be found on the National Institute for Drug Abuse website, the Partnership to End Addiction, and Healthy Children.org, the website of the American Academy of Pediatrics.

Original Investigation

October 25, 2021

Prevalence of Adolescent Cannabis Vaping A Systematic Review and Meta-analysis of US and Canadian Studies

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Author Affiliations

JAMA Pediatr. 2022;176(1):42-51. doi:10.1001/jamapediatrics.2021.4102

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Key Points

Question What is the prevalence of adolescent cannabis vaping in the US and Canada?

Findings This systematic review and meta-analysis reviewed 17 unique studies from the US and Canada, with a total of 198 845 adolescents, and found that the lifetime prevalence of cannabis vaping doubled from 2013 to 2020 (6.1% to 13.6%), past 12-month use doubled from 2017 to 2020 (7.2% to 13.2%), and the 30-day prevalence of cannabis vaping increased 7-fold from 2013 to 2020 (1.6% to 8.4%). Preference for cannabis products may be shifting from dried herb to cannabis oil.

Meaning The findings of this study suggest that more effective prevention and response measures are required to mitigate the increasing prevalence of cannabis vaping among adolescents.

Abstract

Importance Vaping products were initially designed to deliver nicotine as a tobacco cigarette substitute (eg, electronic cigarettes) but are now frequently used to deliver

psychoactive substances, such as cannabis and its derivatives. Large, nationally representative surveys, such as Monitoring the Future, found that approximately 1 in 3 grade-12 students vaped cannabis in 2018 alone.

Objective To summarize the findings of epidemiological studies that reported the global prevalence of cannabis vaping in adolescents by survey year and school grades.

Data Sources PubMed, PsycINFO, Scopus, and Web of Science were searched systematically on August 19, 2020, for studies published globally between January 1, 2003, and August 19, 2020.

Study Selection Publications that reported the prevalence of cannabis vaping in adolescents in the general population were included.

Data Extraction and Synthesis Study characteristics and prevalence estimates were extracted from each article. Random-effects meta-analysis based on the DerSimonian and Laird method and meta-regression were performed on lifetime, 12-month, and 30-day prevalence estimates. Meta-regression was also conducted using survey year and school grades as moderators.

Main Outcomes and Measures Prevalence of cannabis vaping.

Results Seventeen studies met the eligibility criteria (n = 198 845 adolescents). Although no restrictions were imposed on study location, all 17 studies were from the US and Canada. Across all school grades, the pooled prevalence increased for lifetime use (6.1% in 2013-2016 to 13.6% in 2019-2020), use in the past 12 months (7.2% in 2017-2018 to 13.2% in 2019-2020), and use in the past 30 days (1.6% in 2013-2016 to 8.4% in 2019-2020). Heterogeneity across studies was large. The limited evidence from studies using similar survey and study designs suggested that adolescents' preference for cannabis products other than dried herbs, which usually contain higher Δ9-tetrahydrocannabinol levels, may have shifted over time.

Conclusions and Relevance The findings of this study suggest that the prevalence of cannabis vaping has increased among adolescents in the US and Canada and that more effective preventive and response measures are required.

Trial Registration PROSPERO Identifier: CRD42020219644

Vaping marijuana linked to lung injury in teens, study says

By Sandee LaMotte, CNN
Updated 12:13 PM ET, Wed March 3, 2021

(CNN) Teens are about twice as likely to report "wheezing or whistling" in the chest after vaping marijuana than after smoking cigarettes or using e-cigarettes, a new study has found.

"This surprised us, we thought we would find more negative respiratory symptoms in both cigarettes and e-cigarettes users," said study author Carol Boyd, co-director of the Center for the Study of Drugs, Alcohol, Smoking & Health at the University of Michigan in Ann Arbor.

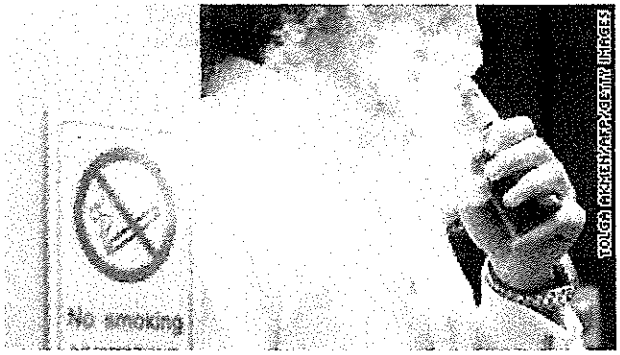
Young adults who vape cannabis are more likely to experience cough, bronchitis and wheezing, study finds

"Without a doubt, cigarettes and e-cigarettes are unhealthy and not good for lungs. However, vaping marijuana appears even worse," she said.

"Since many teens who vape nicotine, also vape cannabis, I recommend parents treat all vaping as a risky behavior (just like alcohol or drug use)," Boyd said via email.

Vaping weed linked to new, deadly lung disease

Vaping weed is associated with a dangerous, newly identified lung disease called EVALI, short for e-cigarette, or vaping, product use-associated lung injury.



Vaping-related lung injuries now in all states but one, new CDC numbers show

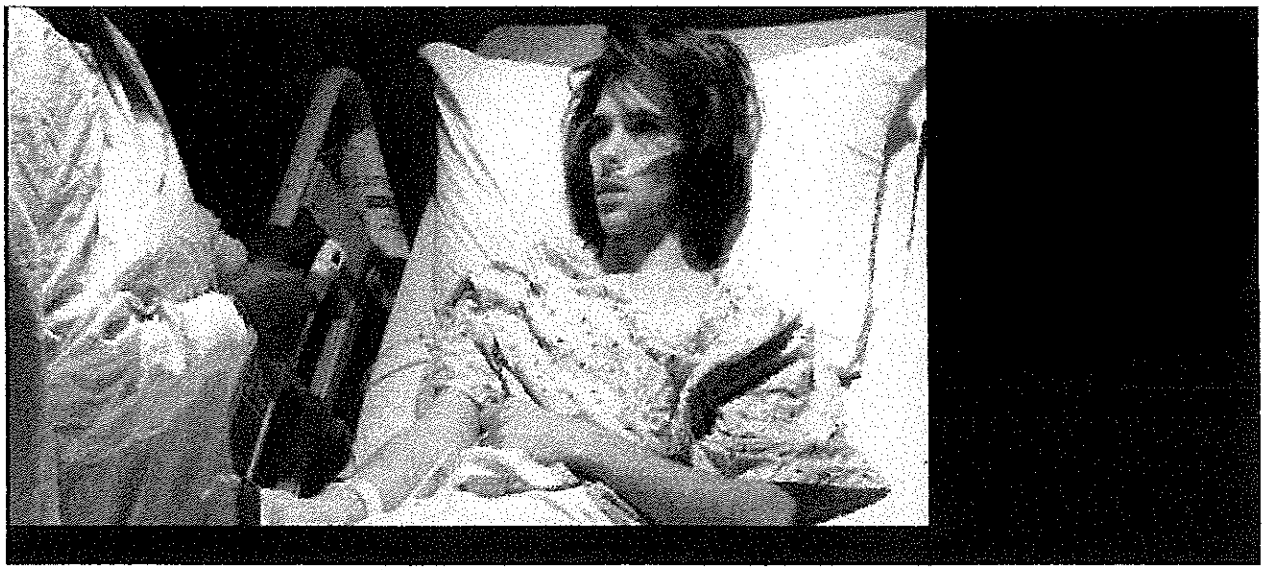
The disease was first identified by the US Centers for Disease Control and Prevention in August 2019, when otherwise healthy young people began being hospitalized for severe, sometimes fatal, lung infections across the country.

A link between the deadly new condition and vaping was soon found, with a major role being played by vitamin E acetate, a sticky oil substance often added to vaping products to either thicken or dilute the oil in cartridges.

That was especially common in vaping products that contain THC, the main psychoactive compound in marijuana.

"According to the CDC, 84% of the EVALI cases were associated with cannabis-containing products," Boyd said.

As of February 2020, 68 deaths from EVALI have been confirmed in 29 states and the District of Columbia.



This teenager almost died from a vaping-related illness 06:36

Five respiratory concerns

The new study, published Wednesday in the Journal of Adolescent Health, analyzed data collected over a two-year period by the Population Assessment of Tobacco and Health

study. It's a national longitudinal study of the health impact of tobacco use administered by the National Institutes of Health and the US Food and Drug Administration.

A fourth wave of the PATH study asked nearly 15,000 teens between the ages of 12 and 17 to describe their last 30-day cigarette, e-cigarette and weed use, as well as the total time they had spent vaping marijuana over their "lifetime."

Lawmakers urge the FDA to temporarily clear e-cigarettes from market amid Covid pandemic. Here's why

Each teen was also asked if they had any of these five symptoms over the last year: wheezing or whistling in the chest; disturbed sleep due to wheezing; limited speech due to wheezing; wheezing during or after exercise and experiencing a dry cough at night that was not due to a cold or chest infection.

After analyzing the data, Boyd and her team found "adolescents' lifetime cannabis vaping" use was associated with all five negative respiratory symptoms.

"This was not true for cigarette or e-cigarette use," Boyd said.

The study was limited by the original questions asked in the PATH study, which did not allow the researchers to fully explore vaping cannabis over time. A household survey, the longitudinal study also excluded adolescents residing in institutions who "may have higher rates of cannabis use," Boyd said.

Despite those limitations, "the current study had a large national sample and we found a robust association between lifetime cannabis use with ENDS (electronic nicotine delivery systems) and respiratory symptoms during a critical stage of development among youth," Boyd said.

Would these health concerns also apply to adults who vape weed? The study was not designed to test that, Boyd said, but "vaping THC/CBD is a relatively new behavior, and thus, not many individuals over the age of 25 years were vaping cannabis as teens. We have too few data to make an assessment."

That doesn't mean that vaping is a safe behavior, Boyd stressed.

"I often am approached by both parents and teens who believe vaping cannabis is 'OK' and better than smoking (a joint, blunt, dobie, etc.). And so, they ask, 'Vaping is safe—right?'

"My reaction: 'You are fooling yourself. We know that inhaling hot tobacco/cannabis smoke into your lungs is unhealthy and can cause bronchitis or life-threatening breathing problems.

"And yet, you seem to believe that heating chemicals (including carcinogens) into a vapor and inhaling them is healthy? My answer is, 'No, it is not a healthy behavior.' "

Original Investigation

January 19, 2021

Association of Cannabis Use With Self-harm and Mortality Risk Among Youths With Mood Disorders

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Author Affiliations Article Information

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Key Points

Question Is cannabis use disorder associated with heightened risk of self-harm, suicide, and mortality among youths with mood disorders?

Findings This population-based cohort study of Medicaid-enrolled youths with mood disorders found that the presence of cannabis use disorder was significantly associated with an increased risk of nonfatal self-harm, all-cause mortality, and death by unintentional overdose and homicide.

Meaning Cannabis use disorder is common among adolescents and young adults with mood disorders and is associated with an elevated risk of self-harm, overall mortality, and death by unintentional overdose and homicide in this already vulnerable population.

Abstract

Importance Cannabis use and cannabis use disorder (CUD) are common among youths and young adults with mood disorders, but the association of CUD with self-harm, suicide, and overall mortality risk is poorly understood in this already vulnerable population.

Objective To examine associations of CUD with self-harm, suicide, and overall mortality risk in youths with mood disorders.

Design, Setting, and Participants A population-based retrospective cohort study was performed using Ohio Medicaid claims data linked with death certificate data. The analysis included 204 780 youths (aged 10-24 years) with a diagnosis of mood disorders between July 1, 2010, and December 31, 2017, who were followed up to 365 days from the index diagnostic claim until the end of enrollment, the self-harm event, or death. Statistical analysis was performed from April 4 to July 17, 2020.

Exposure Physician-diagnosed CUD defined using outpatient and inpatient claims from 180 days prior to the index mood disorder diagnostic claim through the 365-day follow-up period.

Main Outcomes and Measures Nonfatal self-harm, all-cause mortality, and deaths by suicide, unintentional overdose, motor vehicle crashes, and homicide. Marginal structural models using inverse probability weights examined associations between CUD and outcomes.

Results This study included 204 780 youths (133 081 female participants [65.0%]; mean [SD] age at the time of mood disorder diagnosis, 17.2 [4.10] years). Cannabis use disorder was documented for 10.3% of youths with mood disorders (n = 21 040) and was significantly associated with older age (14-18 years vs 10-13 years: adjusted risk ratio [ARR], 9.35; 95% CI, 8.57-10.19; and 19-24 years vs 10-13 years: ARR, 11.22; 95% CI, 10.27-12.26), male sex (ARR, 1.79; 95% CI, 1.74-1.84), Black race (ARR, 1.39; 95% CI, 1.35-1.44), bipolar or other mood disorders (bipolar disorders: ARR, 1.24; 95% CI, 1.21-1.29; other mood disorders: ARR, 1.20; 95% CI, 1.15-1.25), prior history of self-harm (ARR, 1.66; 95% CI, 1.52-1.82), previous mental health outpatient visits (ARR, 1.26; 95% CI, 1.22-1.30), psychiatric hospitalizations (ARR, 1.66; 95% CI, 1.57-1.76), and mental health emergency department visits (ARR, 1.54; 95% CI, 1.47-1.61). Cannabis use disorder was significantly associated with nonfatal self-harm (adjusted hazard ratio [AHR], 3.28; 95% CI, 2.55-4.22) and all-cause mortality (AHR, 1.59; 95% CI, 1.13-2.24), including death by unintentional overdose (AHR, 2.40; 95% CI, 1.39-4.16) and homicide (AHR, 3.23; 95% CI, 1.22-8.59). Although CUD was associated with suicide in the unadjusted model, it was not significantly associated in adjusted models.

Conclusions and Relevance Cannabis use disorder is a common comorbidity and risk marker for self-harm, all-cause mortality, and death by unintentional overdose and homicide among youths with mood disorders. These findings should be considered as states contemplate legalizing medical and recreational marijuana, both of which are associated with increased CUD.

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Science News *from research organizations*

Teen brain volume changes with small amount of cannabis use, study finds

Date: January 14, 2019

Source: Larner College of Medicine at the University of Vermont

Summary: At a time when several states are moving to legalize recreational use of marijuana, new research shows that concerns about the drug's impact on teens may be warranted. The study shows that even a small amount of cannabis use by teenagers is linked to differences in their brains.

FULL STORY

At a time when several states are moving to legalize recreational use of marijuana, new research shows that concerns about the drug's impact on teens may be warranted. The study, published in The *Journal of Neuroscience*, shows that even a small amount of cannabis use by teenagers is linked to differences in their brains.

Senior author and University of Vermont (UVM) Professor of Psychiatry Hugh Garavan, Ph.D., and first author and former UVM postdoctoral fellow Catherine Orr, Ph.D., say this research is the first to find evidence that an increase in gray matter volume in certain parts of the adolescent brain is a likely consequence of low-level marijuana use.

Few studies have looked at the effects of the first few uses of a drug, says Garavan. Most researchers focus on heavy marijuana users later in life and compare them against non-users. These new findings identify an important new area of focus.

"Consuming just one or two joints seems to change gray matter volumes in these young adolescents," Garavan says.

The new study, part of a long-term European project known as IMAGEN, included 46 kids who reported having used cannabis once or twice by age 14. Their brains showed more gray matter volume in areas where cannabis binds, known as cannabinoid receptors, compared to the kids who didn't use the drug. The biggest differences in gray matter were in the amygdala, which is involved in fear and other emotion-related processes, and in the hippocampus, involved in memory development and spatial abilities.

Exploiting the advantages of the study's longitudinal data, the researchers ruled out the likelihood that the cannabis-using kids had pre-existing differences in gray matter thickness or that they had specific personality traits that might correlate with the difference in brain makeup.

"The implication is that this is potentially a consequence of cannabis use," Garavan says. "You're changing your brain with just one or two joints. Most people would likely assume that one or two joints would have no impact on the brain."

What the increased brain matter volume means is unclear.

Typically at that age, Garavan says, the adolescent brain undergoes a "pruning" process, where it gets thinner, rather than thicker as it refines its synaptic connections.

"One possibility is they've actually disrupted that pruning process," Garavan says of the marijuana-using kids.

Story Source:

Materials provided by **Larner College of Medicine at the University of Vermont.** Original written by Jennifer Nachbur. *Note: Content may be edited for style and length.*

Journal Reference:

1. Catherine Orr, Philip Spechler, Zhipeng Cao, Matthew Albaugh, Bader Chaarani, Scott Mackey, Deepak D'Souza, Nicholas Allgaier, Tobias Banaschewski, Arun L.W. Bokde, Uli Bromberg, Christian Büchel, Erin Burke Quinlan, Patricia Conrod, Sylvane Desrivières, Herta Flor, Vincent Frouin, Penny Gowland, Andreas Heinz, Bernd Ittermann, Jean-Luc Martinot, Marie-Laure Paillère Martinot, Frauke Nees, Dimitri Papadopoulos Orfanos, Tomáš Paus, Luise Poustka, Sabina Millenet, Juliane H. Fröhner, Rajiv Radhakrishnan, Michael N. Smolka, Henrik Walter, Robert Whelan, Gunter Schumann, Alexandra Potter, Hugh Garavan. **Grey Matter Volume Differences Associated with Extremely Low Levels of Cannabis Use in Adolescence**. *The Journal of Neuroscience*, 2019; 3375-17 DOI: 10.1523/JNEUROSCI.3375-17.2018

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Featured Content

from New Scientist

Leaded petrol may have lowered the IQ of over half the US population

Behavioral/Cognitive

Grey Matter Volume Differences Associated with Extremely Low Levels of Cannabis Use in Adolescence

Catherine Orr,[1,2] Philip Spechler,[1] Zhipeng Cao,[3,4] Matthew Albaugh,[1] Bader Chaarani,[1] Scott Mackey,[1] Deepak D'Souza,[5] Nicholas Allgaier,[1] Tobias Banaschewski,[6] Arun L.W. Bokde,[7] Uli Bromberg,[8] Christian Büchel,[8] Erin Burke Quinlan,[9] Patricia Conrod,[10,11,12] Sylvane Desrivières,[9] Herta Flor,[13,14] Vincent Frouin,[15] Penny Gowland,[16] Andreas Heinz,[17] Bernd Ittermann,[18] Jean-Luc Martinot,[19] Marie-Laure Paillère Martinot,[20] Frauke Nees,[6,13] Dimitri Papadopoulos Orfanos,[15] Tomáš Paus,[21] Luise Poustka[22,23] Sabina Millenet,[6] Juliane H. Fröhner,[24] Rajiv Radhakrishnan,[5] Michael N. Smolka,[24] Henrik Walter,[17] Robert Whelan,[3,4] Gunter Schumann,[9] Alexandra Potter,[1] and Hugh Garavan[1]

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Rates of cannabis use among adolescents are high, and are increasing concurrent with changes in the legal status of marijuana and societal attitudes regarding its use. Recreational cannabis use is understudied, especially in the adolescent period when neural maturation may make users particularly vulnerable to the effects of Δ-9-tetrahydrocannabinol (THC) on brain structure. In the current study, we used voxel-based morphometry to compare gray matter volume (GMV) in forty-six 14-year-old human adolescents (males and females) with just one or two instances of cannabis use and carefully matched THC-naive controls. We identified extensive regions in the bilateral medial temporal lobes as well as the bilateral posterior cingulate, lingual gyri, and cerebellum that showed greater GMV in the cannabis users. Analysis of longitudinal data confirmed that GMV differences were unlikely to precede cannabis use. GMV in the temporal regions was associated with contemporaneous performance on the Perceptual Reasoning Index and with future generalized anxiety symptoms in the cannabis users. The distribution of GMV effects mapped onto biomarkers of the endogenous cannabinoid system providing insight into possible mechanisms for these effects.

Key words: adolescent substance use; cannabis; cognition; marijuana; psychopathology; voxel-based morphometry

Significance Statement

Almost 35% of American 10th graders have reported using cannabis and existing research suggests that initiation of cannabis use in adolescence is associated with long-term neurocognitive effects. We understand very little about the earliest effects of cannabis use, however, because most research is conducted in adults with a heavy pattern of lifetime use. This study presents evidence suggesting structural brain and cognitive effects of just one or two instances of cannabis use in adolescence. Converging evidence suggests a role for the endocannabinoid system in these effects. This research is particularly timely as the legal status of cannabis is changing in many jurisdictions and the perceived risk by youth associated with smoking cannabis has declined in recent years.

Introduction

Preclinical evidence has consistently demonstrated a causal relationship between cannabis exposure and changes to brain morphology (for review, see Panlilio and Justinova, 2018). The human evidence, however, has been variable reporting both increases and decreases in brain volumes (Ashtari et al., 2011; Cousijn et al., 2012; Gilman et al., 2014), no volume differences (Jager et al., 2007; Weiland et al., 2015; Gillespie et al., 2018), and modest effect sizes (Weiland et al., 2015). Factors including the age of cannabis use initiation, comorbid substance use, and levels of use are believed to contribute to variability in the human findings (Curran et al., 2016).

Most neuroimaging research is conducted in adults with a heavy, chronic pattern of cannabis use and does not reflect most people's experience, which is recreational (SAMHSA, 2014). Dose-dependent associations with brain volumes have been reliably identified in preclinical studies (for review, see Lorenzetti et al., 2010) with some evidence of the same in humans (Battistella et al., 2014; French et al., 2015), suggesting consequences of lower levels of use. One study has reported differences in gray-matter density and shape of the amygdala and nucleus accumbens in recreational cannabis users (Gilman et al., 2014), but subsequent research has suggested that these findings may be associated with alcohol (Weiland et al., 2015) and nicotine (Gillespie et al., 2018) exposure in the cannabis users.

One mechanism by which cannabis may produce neurobiological changes is through the endogenous cannabinoid system (eCB). The amygdala, hippocampus, striatum, and cerebellum (Lorenzetti et al., 2016) are regions most frequently showing structural brain correlates of cannabis use and are also components of the eCB system (Burns et al., 2007); the preclinical literature suggests a causal role of this system in the effects of cannabis on brain morphology (Downer et al., 2001). The eCB system mediates maturation-related neural reorganization (Fernández-Ruiz et al., 2000), which may place adolescents at heightened vulnerability to structural brain effects of cannabis exposure as adolescence is a time of rapid neural maturation (Rubino and Parolaro, 2008). Consistent with this suggestion, those who commenced cannabis use in adolescence typically show greater structural brain differences than those who initiated use in adulthood (Battistella et al., 2014; Lubman et al., 2015). These findings may also have been influenced by the effects of other substances, however, as one study comparing adolescent daily cannabis users with controls matched for alcohol and nicotine use found no differences in subcortical gray-matter density or morphology (Weiland et al., 2015).

In the present study we identified participants with just one or two instance of cannabis use from a very large, population sample of adolescents (IMAGEN, $n = 2400$; Schumann et al., 2010) and control participants matched on a range of variables, including alcohol and nicotine consumption. We predicted that even extremely low levels of cannabis use would be associated with structural brain differences in regions previously implicated in cannabis use studies and in the eCB system: the amygdala, hippocampus, striatum, and cerebellum. We adopted a whole-brain, voxel-based morphometry (VBM) approach as it allows us to also test more extensive regions of the eCB system including the frontal cortex and posterior cingulate (Burns et al., 2007). We explored whether gray matter volume (GMV) predicted behavioral features previously associated with cannabis use and with the eCB system.

To test whether observed differences between cannabis users and controls may precede cannabis use, we also identified participants who were cannabis-naive at the time of imaging but went on to use cannabis 2 years later and matched controls who remained abstinent. Finally, to demonstrate association with the eCB system, we compared the spatial distribution of GMV effects with two biomarkers of the eCB system using CB_1 receptor availability taken from a previously published, independent sample (D'Souza et al., 2016) and the expression of the *CNR1* gene, which encodes this receptor, taken from the Allen Human Brain Atlas (Hawrylycz et al., 2012).

Materials and Methods

Standard operating procedures

Standard operating procedures for the IMAGEN project are available at https://imagen-europe.com/resources/standard-operating-procedures/ and contain details on ethics, recruitment, and assessment.

Participants

Data were acquired from a large sample of adolescents recruited through high schools in four European countries for the IMAGEN project (http://www.imagen-europe.com). Recruitment into the IMAGEN study was managed through eight sites and targeted adolescents for whom all four grandparents were the same nationality as the participant; as such, the sample is racially and ethnically homogenous. Raw, T1-weighted images were visually inspected for the presence of anatomical abnormalities or artifacts including head motion or reconstruction errors. After VBM processing, images were again inspected for any errors in tissue segmentation or normalization into MNI space. Images failing quality control for any reason were excluded.

Cohort 1. Forty-seven participants reported low levels of cannabis use at baseline (only 1 or 2 lifetime instances of use) and complete demo-

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Table 1. Demographic characteristics of Cohort 1, those 14 year olds reporting 1 or 2 instances of cannabis use ($n = 46$) and matched controls ($n = 46$)

Variable	Cannabis	Control	Statistic
Mean			
Age	14.60	14.51	$t_{(90)} = 1.06$
PDS	3.04	2.95	$t_{(90)} = 0.846$
VCI	108.33	108.20	$t_{(90)} = 0.042$
PRI	102.85	103.77	$t_{(90)} = 0.345$
SES	18.80	18.72	$t_{(90)} = 0.091$
Total GMV, mm^3	74,2955.12	72,8559.92	$t_{(90)} = 1.03$
Lifetime alcohol consumption	3.46	3.52	$t_{(90)} = 0.214$
Lifetime nicotine consumption	2.54	2.59	$t_{(90)} = 0.101$
Average age of first cannabis use	13.83 years		
Summary			
Sex	65% male	48% male	$U = 874$
Handedness	87% right handed	87% right handed	$U = 1058$
Site 1	3	2	$U = 1081$
Site 2	12	7	$U = 1173$
Site 3	4	1	$U = 1127$
Site 4	6	8	$U = 1012$
Site 5	7	8	$U = 1035$
Site 6	3	8	$U = 943$
Site 7	11	12	$U = 1035$
Site 8	0	0	
No. reporting cannabis use in the past 30 d (%)	10 (21.74)		
No. reporting cannabis use in the past 7 d (%)	6 (13.04)		

graphics to facilitate matching; one participant was excluded because of poor scan quality, leaving 46 adolescent cannabis using participants. The groups were matched on age, sex, handedness, pubertal development, intelligence quotient (IQ; verbal comprehension and perceptual reasoning index scores), socioeconomic status (SES), total GMV, alcohol use, and nicotine use across group. All participants denied any other illicit substance use, and none reported using the fictional control substance, relevin, supporting the integrity of the self-report metrics. Table 1 summarizes the demographic information. We also ensured that similar numbers of cannabis users and controls were selected from each site (Mann–Whitney U tests; Table 1) and confirmed that the proportion of cannabis users and controls did not differ by site using a Kruskal–Wallis test ($\chi^2_{(6)} = 5.919$, $p = 0.432$).

For a subset of the 14-year-old cannabis-using participants, data were available at 2 year follow-up for substance use, cognitive ability, and psychopathology at age 16 to allow us to assess the implications of cannabis-related GMV differences for future functioning in these domains. Table 2 summarizes the demographic information for this subset of participants.

Cohort 2. To determine whether group differences between cannabis users and matched controls may have preceded cannabis use, we also identified participants who were cannabis-naive at the age 14 baseline assessment but reported at least 10 instances of cannabis use by follow-up 2 years later. Sixty-nine participants who were cannabis-naive at baseline but with at least 10 instances of cannabis use by follow-up provided complete demographic data and all had GMV data that passed QC. Sixty-nine controls matched by group on the same demographic measures as above and who reported no cannabis use at baseline or follow-up were also identified. All participants denied any other illicit substance use at baseline and follow-up. Table 3 summarizes the demographic information for this sample of participants. We again ensured that similar numbers of cannabis users and controls were selected from each site (Mann–Whitney U tests; Table 3) and confirmed that the proportion of cannabis users and controls did not differ by site using a Kruskal–Wallis Test ($\chi^2_{(7)} = 4.633$, $p = 0.705$).

Table 2. Demographic characteristics for those members of Cohort 1 for whom specific substance use, psychopathology, and cognitive measures were available at 16 year old follow-up

	Substance use ($n = 31$)	Psychopathology ($n = 33$)	Delay discounting ($n = 31$)
Mean			
Age	14.60	14.60	14.58
PDS	3.04	3.04	3.03
VCI	110.19	110.31	110.46
PRI	103.91	103.36	103.87
SES	19.01	19.47	19.40
Total GMV, mm^3	74,2793.69	74,1208.83	74,2428.43
Lifetime alcohol consumption	3.61	3.64	3.61
Lifetime nicotine consumption	2.48	2.45	2.39
Summary			
Sex, %	61 male	61 male	61 male
Handedness, %	90 right handed	88 right handed	87 right handed

Table 3. Demographic characteristics of Cohort 2, those 16 year olds who were abstinent for cannabis use at baseline (age 14) but reported 10 or more instances of cannabis use by age 16 ($n = 69$) and matched controls ($n = 69$)

Variable	Cannabis	Control	Statistic
Mean			
Age	14.43	14.50	$t_{(136)} = 0.944$
PDS	2.80	2.79	$t_{(136)} = 0.290$
VCI	112.48	110.29	$t_{(136)} = 0.859$
PRI	109.16	108.26	$t_{(136)} = 0.367$
SES	17.97	17.42	$t_{(136)} = 0.835$
Total GMV, mm^3	75,5082.71	74,7752.65	$t_{(136)} = 0.647$
Lifetime alcohol consumption	2.33	2.29	$t_{(136)} = 0.166$
Lifetime nicotine consumption	1.33	1.16	$t_{(136)} = 0.577$
Average age of first cannabis use, y	14.97		
Summary			
Sex	74% male	70% male	$U = 2277$
Handedness	93% right handed	91% right handed	$U = 2346$
Site 1	3	7	$U = 2242.5$
Site 2	11	9	$U = 2449.5$
Site 3	4	3	$U = 2415$
Site 4	8	6	$U = 2449.5$
Site 5	11	10	$U = 2415$
Site 6	8	13	$U = 2208$
Site 7	15	10	$U = 2553$
Site 8	9	11	$U = 2311.5$

For both cohorts, the control subjects were selected from a larger pool of IMAGEN participants with T1 images that passed QC and who reported no illicit substance use. This selection was done using Python scripts written in our laboratory to randomly select subjects and compare them with the sample of cannabis users on nominated characteristics (in this case: age, sex, handedness, site (dummy coded as 8 binary variables), pubertal development, VCIQ, PRIQ, SES, total GMV, alcohol use, and nicotine use) without experimenter intervention.

Substance use measures

Substance use was assessed at baseline (age 14) and follow-up (age 16) via the European School Survey Project on Alcohol and Drugs (ESPAD; Hibell et al., 2004), a self-report questionnaire that measures use of alcohol, nicotine, cannabis, inhalants, tranquilizers, amphetamines, lysergic acid diethylamide (LSD), magic mushrooms, crack, cocaine, heroin, narcotics, methylenedioxymethamphetamine (MDMA), ketamine, γ-hydroxybutyric acid (GHB), anabolic steroids, and a fictional

control measure (relevin). Participants indicated how frequently they had used each of the substances in their lifetime, in the past 12 months, in the past 30 d, and in the past 7 d using a 7-point scale (0: never, 1: 1–2 times, 2: 3–5 times, 3: 6–9 times, 4: 10–19 times, 5: 20–39 times, and 6: 40 or more times); they also indicated the age at which they had first tried each of the substances.

Cohort 1 comprised those participants with an ESPAD of 1 for cannabis (i.e., 1–2 instances of cannabis use) and no reported use of any other illicit substances, and matched controls with no cannabis use and no use of any other illicit substances. We also extracted lifetime alcohol and nicotine use from the ESPAD to match the groups on these variables. To explore possible relationships between GMV and cannabis use metrics, we also extracted from the ESPAD age of first use, frequency of use in the past 30 d, and lifetime use by age 16 for those who reported cannabis use at baseline.

Cohort 2 comprised those participants with an ESPAD of 0 for cannabis at baseline, an ESPAD of 4, 5, or 6 for cannabis at follow-up (i.e., cannabis-naive at age 14 and with 10+ instances of cannabis use by age 16) and no reported use of any other illicit substances at either baseline or follow-up, and matched controls with no cannabis use and no use of any other illicit substances at either time point. We also extracted lifetime alcohol and nicotine use from the ESPAD to match the groups on these variables.

Demographic measures

Biological sex was determined by karyotype analysis (chromosome 23: XX = female, XY = male). Participants provided blood samples, which were shipped to the Institute of Psychiatry, London for genotyping with Illumina Human610-Quad Bead Chips (Illumina). DNA extraction was performed by a semiautomated process to ensure high quality and sufficient quantity (Schumann et al., 2010).

SES was indexed by a score that summed: Mother's Education Score, Father's Education Score, Family Stress Unemployment Score, Financial Difficulties Score, Home Inadequacy Score, Neighborhood Score, Financial Crisis Score, Mother Employed Score, and Father Employed Score from the parent report of the Development and Well-Being Assessment interview (DAWBA; Goodman et al., 2000; http://www.dawba.info).

Participants completed the Perceptual Reasoning, Matrix Reasoning, Similarities and Vocabulary subscales from the Wechsler intelligence scale for children WISC-IV(Wechsler, 1949) to generate Verbal Comprehension (VCIQ) and Perceptual Reasoning (PRIQ) indices.

Physical maturity was assessed using the Pubertal Development Scale (Petersen et al., 1988), a self-report measure of physical signs associated with the onset, progression, and completion of puberty.

Personality and temperament measures

Personality was assessed with the self-reported Substance Use Risk Profile Scale (SURPS; Woicik et al., 2009), the NEO Five Factor Inventory (NEO-FFI; Costa and McCrae, 1992), and the Temperament and Character Inventory (TCI; Cloninger et al., 1994). The SURPS produced summary measures for personality traits of hopelessness, anxiety sensitivity, impulsivity, and sensation-seeking. The NEO-FFI produced summary measures for five higher-order personality characteristics: neuroticism, conscientiousness, extraversion, agreeableness, and openness to experience. The TCI produced measures for exploratory excitability versus stoic rigidity, impulsiveness versus reflection, extravagance versus reserve, disorderliness versus regimentation, and a novelty-seeking summary statistic.

Cognitive measures

Delay discounting was assessed with the Monetary Choice Questionnaire (Kirby, 2009) that required participants to complete 27 two-alternative forced-choice items in which they indicated whether they would prefer a "smaller sooner" or a "larger later" reward (e.g., "Would you prefer €14 today or €25 in 19 d?"). The summary k statistic indexes the degree to which a participant discounts more temporally remote rewards.

Psychomotor speed and manual dexterity were assessed using the Perdue Pegboard (Tiffin, 1968). Participants were asked to place as many pins as possible in the small holes on the test board in 30 s. Participants completed three trials in each of three conditions: using only the dominant hand; only the non-dominant hand; and both hands.

Spatial working memory and decision-making were assessed using the Cambridge Neuropsychological Test Automated Battery (CANTAB; Robbins et al., 1994). We examined the number of memory failures made during a visual search task and the risk-taking summary statistic from a gambling task.

Psychopathology measures

Psychiatric symptoms of conduct disorder, oppositional defiant disorder, attention deficit/ hyperactivity disorder, generalized anxiety, depression, specific phobia, social phobia, agoraphobia, panic disorder, obsessive compulsive disorder, and eating disorders were assessed via the DAWBA, which was administered to participants and their parents at baseline and at follow-up. Computer generated band scores integrated reported symptoms and their impact with the approximate prevalence rates in an epidemiological sample for each disorder and reflect the likelihood that the participant would be diagnosed with the disorder in question (ranging from 0 to 5). Diagnostic criteria were based on the *Diagnostic Statistical Manual*, version 4.

Neuroanatomical MRI acquisition

MRI scanning was conducted at the eight IMAGEN assessment sites using 3T whole-body MRI systems (Siemens, 4 sites; Philips, 2 sites; General Electric, 1 site; Bruker, 1 site). A high-resolution, three-dimensional T1-weighted image was acquired using a magnetization prepared gradient echo sequence based on the ADNI protocol (http://adni.loni.usc.edu/methods/mri-tool/mri-analysis/), which specifies protocols designed to minimize differences in image contrast and signal-to-noise across scanner makes and models. Two additional quality control procedures were regularly implemented: (1) the American College of Radiology phantom was scanned every 2 months at each site and after every hardware and software upgrade to provide information about geometric distortions and signal uniformity related to hardware differences in radiofrequency coils and gradient systems, image contrast, and temporal stability; and (2) twice per year at each site and after any hardware or software upgrade, human volunteers were scanned to determine inter-site variability in raw MRI signal and tissue relaxation properties (Schumann et al., 2010).

Voxel-based morphometry

T1-weighted images were processed using the Statistical Parametric Mapping v8 (SPM8; http://www.fil.ion.ucl.ac.uk/spm/software/spm8/) VBM toolbox (http://dbm.neuro.uni-jena.de/vbm/) with default parameters incorporating the DARTEL toolbox implemented in MATLAB 7.0 (MathWorks). Image processing comprised iterative tissue segmentation and spatial normalization using both linear (12-parameter affine) and nonlinear transformations (Ashburner and Friston, 2000; Ashburner, 2007) without skull stripping. SPM8 default settings were used to be consistent with other VBM publications from the IMAGEN Consortium. To preserve information about absolute volume, the gray matter concentration images were modulated by multiplying by the linear and nonlinear components of the Jacobian determinants generated during spatial normalization. Thus, the dependent measure in the subsequent analysis was absolute gray matter volume. Voxel resolution after normalization was 1.5 × 1.5 × 1.5 mm. To make the residuals in later analyses conform more closely to a Gaussian distribution and to account for individual differences in brain anatomy, the modulated GM images were smoothed with an isotropic Gaussian kernel of 8 mm full-width at half-maximum.

Experimental design and statistical analyses

Whole-brain voxelwise analyses were conducted using the general linear model, implemented in AFNI (Cox, 1996). We tested for GMV differences at baseline between: (1) Cohort 1, those 46 participants who reported low levels of cannabis use at baseline and their matched controls; and (2) Cohort 2, those 69 participants who reported cannabis use by age 16 and their matched controls. Age, sex, handedness, and total GMV were included in the models as covariates of no interest. Imaging site was included as an additional covariate; given the cohort sizes and large num-

ber of covariates already used, additional measures inter-site imaging variance were not included in this analysis. Type 1 error was controlled using a combination of voxel-level significance and cluster extent: following Eklund et al. (2016), the updated AFNI program 3dTTest++ with the option –clustsim (https://afni.nimh.nih.gov/pub/dist/doc/program_help/3dttest++.html) was used to determine the cluster extent of contiguous significant voxels required to adequately correct for multiple comparisons. Within a gray matter mask, significant voxels ($p < 0.001$) were required to be part of a cluster of at least 600 voxels (2025 μl) to maintain familywise error at 5%. Anatomic regions implicated by these clusters were determined by the AAL Atlas. Given that the AAL atlas does not label the ventral striatum (VS), we used the Oxford-GSK-Imanova structural striatal atlas (Tziortzi et al., 2011) to separate the VS from the caudate and putamen.

We also conducted region-of-interest analyses in Cohort 2 in which we extracted GMV from the regions showing significant volume differences between baseline users and controls to confirm that GMV differences in these specific regions did not precede cannabis use. Note that these regions were defined by the analysis of Cohort 1 ($n = 46$), and then tested on an independent cohort (Cohort 2, $n = 69$).

A series of *post hoc* analyses were conducted to ensure that group differences in GMV between baseline users and controls could not be accounted for by any differences in cognitive ability, personality, or symptoms of psychopathology. Independent groups *t* tests were used to test for differences in the continuous variables and Mann–Whitney *U* tests were used to test for differences in the ordinal DAWBA band scores. We did not correct for multiple comparisons for these tests so as to have a liberal threshold for identifying any group differences. We then repeated the voxelwise GMV analyses with any behavioral variables that differed between the groups included as additional covariates.

We explored whether individual differences in GMV in those regions that differed between cannabis users and controls were associated with substance use factors (lifetime alcohol or nicotine consumption, recent cannabis use, or age of onset of cannabis use) in those participants reporting cannabis use at baseline. We also assessed whether GMV in regions that differed between cannabis users and controls were associated with individual differences in specific cognitive and psychopathological domains previously related to cannabis misuse in those participants reporting cannabis use. Spatial working memory, risk-taking, delay discounting, psychomotor speed, depression, generalized anxiety, and ADHD were assessed at baseline. For a subset of those participants reporting cannabis use at baseline, psychopathology ($n = 33$), delay discounting ($n = 31$), and substance misuse data ($n = 31$) were also available at follow-up 2 years later. We assessed whether regional GMV at baseline predicted symptoms of depression, generalized anxiety, or ADHD; delay discounting; or future cannabis use. For all *post hoc* analyses, regional GMV was normalized by total GMV.

Cannabinoid 1 receptor availability. To test for associations between the spatial distribution of group differences in GMV and a receptor for the eCB system, we used a map of CB_1 receptor availability generated from the healthy control participants in a previously published study (D'Souza et al., 2016). Maps of CB_1 receptor availability were generated using positron emission tomography and the reversible ligand [^{11}C]OMAR in 21 adult males aged 18–35 (D'Souza et al., 2016), the 21 individual participant maps were averaged to provide an estimate of CB_1 receptor availability at each voxel.

The map of the GMV comparison between cannabis users and controls was downsampled to the resolution of the PET map ($3 \times 3 \times 3$ mm^3 voxels) and Spearman correlations were conducted between the *t* statistic at each voxel and the average CB_1 receptor availability at the same site using the AFNI program 1dCorrelate. First, we tested all voxels within a gray matter mask; we then tested only those voxels within regions showing significant GMV differences between cannabis users and controls.

Gene expression. Associations between the spatial distribution of group differences in GMV and expression of the gene that encodes the CB_1R were tested with reference to the Allen Human Brain Atlas (Hawrylycz et al., 2012). Using the *alleninf* toolbox (Gorgolewski et al., 2014) we extracted normalized gene expression values for CNR1 (averaged within spherical ROIs with radii of 3 mm) from within a gray matter mask and then used random-label permutation to test for an association between CNR1 expression and the *t* statistic of GMV effects. Distributions of Spearman correlations between 50 randomly selected genes and the *t* statistics of GMV effects were obtained by 5000 bootstrap resamples and then merged to build a null model. The 95% confidence interval of this null distribution was calculated as the cutoff point against which the strength of the association between GMV effects and CNR1 gene expression was assessed. The list of randomly chosen genes, their expression at each sampling site, the expression of CNR1, and the GMV *t* statistic at each sampling site are available in Extended Data Tables 1-3, available at https://doi.org/10.1523/JNEUROSCI.3375-17.2018.t1-1; https://doi.org/10.1523/JNEUROSCI.3375-17.2018.t1-2; https://doi.org/10.1523/JNEUROSCI.3375-17.2018.t1-3.

Results

Cohort 1: group differences in GMV associated with low rates of cannabis use

Figure 1 illustrates extensive regions of greater GMV in those participants who reported low levels of cannabis use relative to matched controls. Bilateral medial temporal regions, including the hippocampus, the amygdala, and the striatum, and bilateral parietal regions were implicated, as were regions of the cerebellum and the left middle temporal gyrus (Table 4). Because of the relevance of the striatal subregions, especially the ventral striatum, for addiction and substance use, Table 5 details the number of voxels (and proportion of volume) implicated in each of the putamen, caudate, and ventral striatum as defined by the Oxford-GSK-Imanova structural striatal atlas (Tziortzi et al., 2011). Figure 2 illustrates the distribution of regional GMV, normalized by total GMV, for those regions at which GMV differed between cannabis users and controls.

Of all the variables describing cognitive ability, symptoms of psychopathology, and personality, only agoraphobia ($U = 868.00$, $p_{uncorr} = 0.038$) and the sensation seeking measure from the SURPS ($t_{(88)} = 2.824$, $p_{uncorr} = 0.006$) differed between the cannabis users and controls with the cannabis users reporting higher levels of both. When agoraphobia band score and sensation seeking were included in the voxelwise analysis as covariates, the three clusters reported in Figure 1 and Table 4 were still observed (albeit, with a small reduction in volume that may be accounted for by the reduction in power because of the addition of extra covariates). One additional cluster centered on the left inferior temporal gyrus (Table 6) was also revealed in this analysis as showing significantly greater GMV in the cannabis users than the controls.

Cohort 1: associations between GMV and contemporaneous behavioral measures

In light of the individual differences in normalized GMV effects in the cannabis using group, we conducted *post hoc* analyses to explore whether any of the demographic variables on which the groups were matched was associated with GMV in the ROIs for those adolescents reporting cannabis use. Age was not associated with normalized GMV in any of the identified ROIs; the difference between males and females in GMV in the bilateral parietal cluster approached but did not reach the corrected significance level ($t_{(44)} = 2.226$ $p_{uncorr} = 0.031$) with normalized GMV greater in males than females. When controlling for handedness, sex, and age, normalized GMV in the left and right temporal clusters ($r_{(41)} = -0.411$, $p_{corr} = 0.037$ and $r_{(41)} = -0.457$, $p_{corr} = 0.012$, respectively) were negatively associated with PRIQ such that greater relative volume in these regions was associated with re-

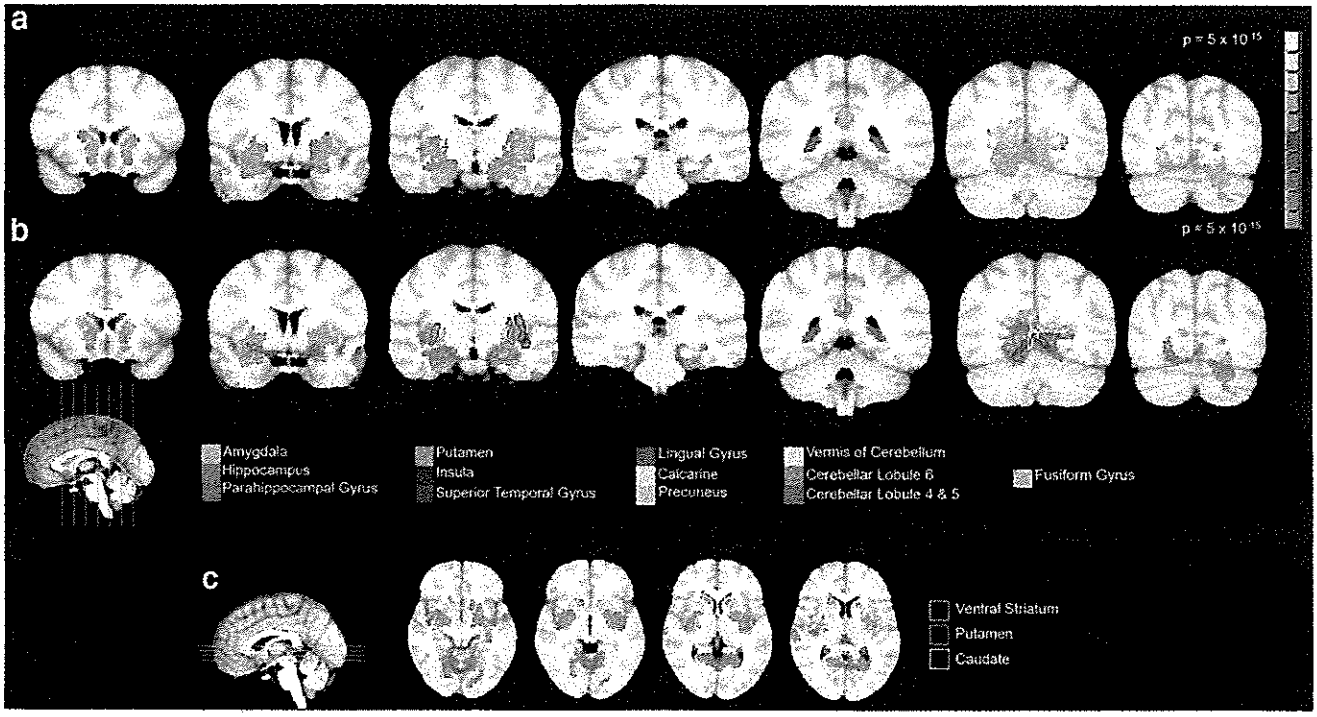

Figure 1. *a*, Those regions showing significantly greater GMV in 14-year-olds reporting one or two instances of cannabis use than in matched controls ($p_{FWE} < 0.05$). From left to right, slices are taken from anterior ($y = -18$) to posterior ($y = 72$) in 15 mm increments. The left hemisphere is to the right of the image. *b*, Outlines of anatomical regions (AAL atlas) superimposed on a binarized mask of the voxels showing significantly greater GMV in 14-year-olds reporting one or two instances of cannabis use than in matched controls ($p_{FWE} < 0.05$). For clarity, only those regions for which at least 10% of their volume was included in the significant clusters are represented. From left to right, slices are taken from anterior ($y = -18$) to posterior ($y = 72$) in 15 mm increments. The left hemisphere is to the right of the image. *c*, Outlines of striatal subregions (Oxford-GSK-Imanova structural striatal atlas; Tziortzi et al., 2011) superimposed on a binarized mask of the voxels showing significantly greater GMV in 14-year-olds reporting one or two instances of cannabis use than in matched controls ($p_{FWE} < 0.05$). From left to right, slices are taken from inferior ($z = -10$) to superior ($z = 8$) in 6 mm increments. The left hemisphere is to the right of the image.

duced PRIQ (Fig. 3). VCIQ, PDS, SES, alcohol use, and nicotine use were not associated with GMV in any of the identified ROIs. The cannabis use metrics (age of use or whether cannabis was used in the last month) were not associated with GMV.

Of the specific cognitive and psychological domains assessed at baseline, only psychomotor speed showed an association with GMV (Fig. 4): normalized GMV in the left temporal cluster showed a negative association with the number of pegs placed with the non-dominant hand ($r_{(39)} = -0.454$, $p_{corr} = 0.030$).

Cohort 2: associations between GMV and future cannabis use

There were no regions at which GMV differed between the future cannabis users and their matched controls. ROI analyses focused on those regions from Cohort 1 that differed between baseline users and matched controls also revealed no significant differences between future cannabis users and matched controls (Table 7).

Cohort 1: associations between GMV and future behavioral measures

A post hoc Mann–Whitney *U* test showed that baseline GMV in the right temporal cluster was significantly greater for those cannabis users who went on to have higher levels of generalized anxiety (DAWBA band scores of 1 or greater vs DAWBA band scores of 0: $U = 43$, $p_{corr} = 0.009$, Fig. 5). No other associations between regional GMV and cognition or psychopathology reached significance.

Cohort 1: spatial associations between GMV effects and CB_1 receptor availability

Comparison of the *t* statistic map of GMV differences between cannabis users and controls with the map of average CB_1 receptor availability in an independent sample (D'Souza et al., 2016) showed significant ($p < 0.05$) spatial association ($r_{(54,041)} = 0.1131$, 95% CI: 0.10468, 0.12152). Comparison of only those voxels showing a significant GMV difference between cannabis users and controls also showed a significant ($p < 0.05$) spatial association between the magnitude of GMV effects and CB_1 receptor availability ($r_{(1229)} = 0.0803$, 95% CI: 0.02537, 0.13444). This more conservative test illustrates that even within those regions showing a significant GMV difference between cannabis users and controls, the magnitude of the difference was associated with CB_1 receptor availability.

Cohort 1: spatial associations between GMV effects and CNR1 gene expression

Comparison of the *t* statistic map of GMV differences between cannabis users and controls with the map of CNR1 gene expression showed significant ($p < 0.05$) spatial association ($r_{(3685)} = 0.311$, 95% CI: 0.279, 0.341), while the null model showed no association with GMV (95% CI: −01930, 01977).

Discussion

We present evidence of GMV differences in adolescents associated with only one or two instances of cannabis use. Although novel, this work is consistent with reports of a dose–response effect of cannabis on behavioral and brain measures following

Table 4. Those regions showing significantly greater GMV in 14 year olds reporting 1 or 2 instances of cannabis use than in matched controls

Anatomical region (AAL)	No. of significant voxels	Anatomical region implicated, %
Cluster 1: left temporal (Vol. 4968 vox; 16,767 μl; $F_{(1,80)} = 8.88, p_{corr} = 0.008$; peak voxel −55, −2, −14)		
Frontal lobe		
Olfactory cortex	136	20.57
Gyrus rectus	48	2.33
Superior frontal gyrus (pars orbitalis)	29	1.34
Inferior frontal gyrus (pars orbitalis)	39	0.95
Temporal lobe		
Superior temporal gyrus	420	7.87
Middle temporal gyrus	164	1.38
Heschl's gyrus	3	0.55
Superior temporal pole	13	0.43
Rolandic operculum	5	0.21
Inferior temporal gyrus	5	0.07
Subcortical		
Amygdala	382	74.17
Hippocampus	777	35.63
Putamen	503	21.13
Pallidum	126	18.13
Insula	640	14.79
ParaHippocampal gyrus	202	8.56
Caudate	92	4.08
Cluster 2: Right temporal (Vol. 3710 vox; 12,491 μl); $F_{(1,80)} = 5.88, p_{corr} = 0.018$; peak voxel 30, −11, −27)		
Temporal lobe		
Heschl's gyrus	68	11.62
Superior temporal gyrus	50	0.67
Superior temporal pole	17	0.54
Subcortical		
Amygdala	439	73.91
Hippocampus	746	33.13
Pallidum	172	26.46
Putamen	564	22.20
Parahippocampal gyrus	410	15.61
Insula	185	4.39
Cluster 3: Bilateral Posterior (Vol. 4959 vox; 16,737 μl); $F_{(1,80)} = 14.32, p_{corr} = 8.0 \times 10^{-4}$; peak voxel −24, −59, 3)		
Temporal lobe		
Fusiform gyrus (L)	283	5.18
Fusiform gyrus (R)	114	1.91
Parietal lobe		
Posterior cingulate (R)	59	7.98
Posterior cingulate (L)	22	1.97
Precuneus (R)	268	3.45
Precuneus (L)	212	2.55
Occipital lobe		
Lingual gyrus (R)	1158	21.14
Lingual gyrus (L)	818	16.01
Calcarine (L)	269	5.16
Calcarine (R)	79	1.87
Cerebellum		
Cerebellar vermis (4 5)	258	17.61
Cerebellar lobule 4 5 (R)	308	14.62
Cerebellar lobule 6 (L)	332	8.20
Cerebellar lobule 6 (R)	265	6.18
Cerebellar lobule 4 5 (L)	156	5.80
Cerebellar vermis (6)	7	0.88
Crus cerebellum1 (L)	8	0.13

Table 5. The number of ventral striatum voxels (and percentage of total anatomical volume) showing significantly greater GMV in 14 year olds reporting 1 or 2 instances of cannabis use than in matched controls

	No. of significant voxels	Anatomical region implicated, %
Ventral striatum, left	131	30.32
Ventral striatum, right	226	54.72

heavier use (Lorenzetti et al., 2010; Silins et al., 2014). We identified significantly greater GMV in adolescents who reported only one or two instances of cannabis use relative to cannabis naive controls in large medial temporal clusters incorporating the amygdala, hippocampus, and striatum, extending into the left prefrontal cortex. Significantly greater GMV was also observed in the lingual gyri, posterior cingulate, and cerebellum. The regions identified in this whole-brain, VBM approach replicated previous findings of differences in volume (Yücel et al., 2008; Ashtari et al., 2011; Schacht et al., 2012) and shape (Gilman et al., 2014; Smith et al., 2014, 2015) associated with cannabis use in ROI studies and with the spatial distribution of the eCB system (Burns et al., 2007). Although cannabis use has been associated with reduced brain volumes, studies typically report on adults with heavy substance use histories (cf. Ashtari et al., 2011). Gilman et al. (2014), however, have reported gray-matter density increases in the amygdala and nucleus accumbens of young adult recreational users and Medina et al. (2007) observed hippocampal enlargement in cannabis using adolescents. Our results are also consistent with the Avon Longitudinal Study of Parents and Children (French et al., 2015), which showed a trend for greater cortical thickness in male adolescents with <5 instances of cannabis use relative to THC-naive controls.

Converging evidence suggests that these effects may be a consequence of cannabis exposure. GMV differences could not be explained by group differences in demographic, personality, psychopathology, or other substance use factors. Examination of THC-naive 14-year-olds who later used cannabis showed no GMV differences, even using a more liberal ROI test, suggesting that the differences do not precede cannabis use and are not because of unidentified factors in those predisposed to use. Finally, the spatial distribution of GMV effects was associated with the eCB system, suggesting cannabis exposure may cause these findings.

The preclinical literature presents a number of possible mechanisms by which low levels of cannabis exposure could result in greater GMV relative to THC-naive controls. Adolescent rats treated with cannabinoid agonist showed altered gliogenesis in regions including the striatum and greater preservation of oligodendroglia relative to control animals (Bortolato et al., 2014). Zebra finches treated with cannabinoid agonist showed greater dendritic spine densities (Gilbert and Soderstrom, 2011); critically, these effects were observed in late-prenatal but not adult animals. Of particular relevance to this study, a single dose of Δ9THC transiently abolished eCB-mediated long-term depression (LTD) in the nucleus accumbens and hippocampus of adolescent mice (Mato et al., 2004). Suspension of LTD may interrupt maturation-related neural pruning and preserve gray matter. Future studies should assess whether these processes operate in human adolescents and whether they produce persisting alterations in GMV.

These findings should be interpreted in light of the study's limitations. The IMAGEN sample is racially and ethnically homogenous so it remains to be determined whether the findings

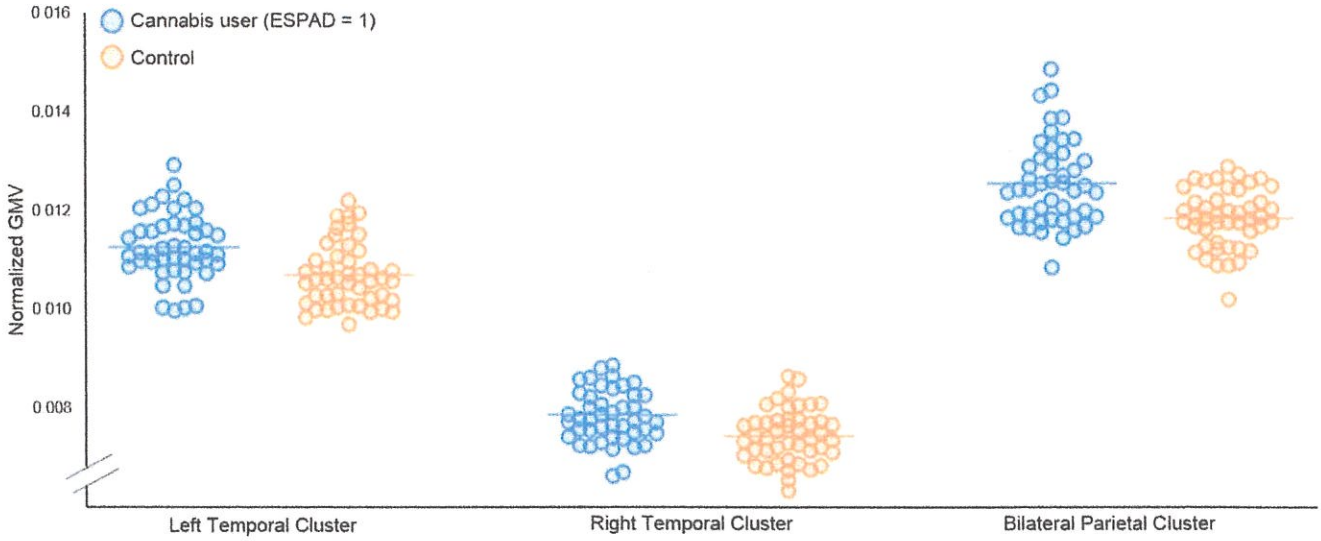

Figure 2. Distribution of Average GMV in the regions showing significantly different GMV between those 14-year-olds reporting one or two instances of cannabis use and matched controls.

Table 6. Those regions showing significantly greater GMV in 14 year olds reporting 1 or 2 instances of cannabis use than in matched controls, when controlling for agoraphobia and sensation seeking

Region	Vol	Peak voxel	
Left temporal	4836 vox (16,321 μl)	−55, −2, −14	$F_{(1,76)} = 8.018$, $p_{corr} = 0.011$
Right temporal	3425 vox (11,559 μl)	30, −11, −27	$F_{(1,76)} = 6.026$, $p_{corr} = 0.016$
Bilateral posterior/ inferior parietal	4907 vox (16,561 μl)	−24, −59, 3	$F_{(1,76)} = 12.718$, $p_{corr} = 0.002$
Left inferior temporal gyrus	603 vox (2,038 μl)	−50, −9, −42	$F_{(1,76)} = 12.755$, $p_{corr} = 0.002$

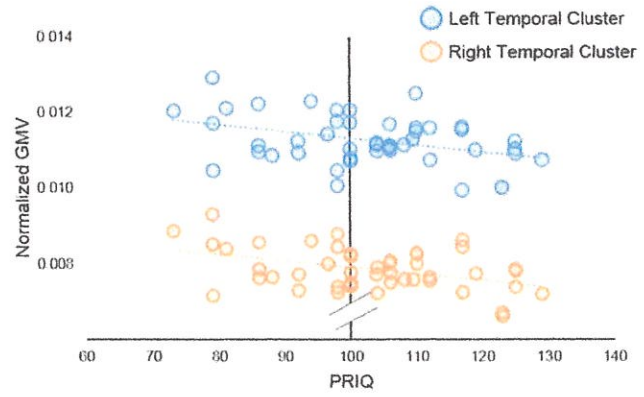

Figure 3. Inverse correlations were observed between PRIQ and normalized GMV in the left ($r_{(41)} = -0.411$, $p_{corr} = 0.037$) and right ($r_{(41)} = -0.457$, $p_{corr} = 0.012$) temporal clusters for those participants reporting one or two instances of cannabis use.

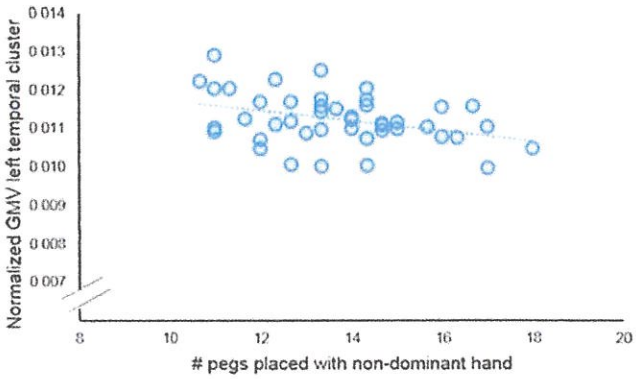

Figure 4. An inverse correlation was observed between normalized GMV in the left temporal cluster and contemporaneous pegboard performance in those participants reporting one or two instances of cannabis use ($r_{(39)} = -0.454$, $p_{corr} = 0.030$).

Table 7. No significant GMV differences were observed at baseline between those participants who were abstinent for cannabis use at age 14 but reported at least 10 instances of use by age 16 and matched controls (i.e., Cohort 2) in those regions defined in Cohort 1

Region	Vol	Peak voxel	
Left temporal	4968 vox (16,767 μl)	−55, −2, −14	$F_{(1,125)} = 3.026$, $p_{corr} = 0.252$
Right temporal	3710 vox (12,491 μl)	30, −11, −27	$F_{(1,125)} = 5.626$, $p_{corr} = 0.057$
Bilateral posterior/ inferior parietal	4959 vox (16,737 μl)	−24, −59, 3	$F_{(1,125)} = 0.021$, $p_{corr} = 1$

generalize to youth from more diverse backgrounds. Substance use was assessed using self-report and we do not have standard dose units of cannabis nor information on mode of use or a measure of drug metabolites. Combining images from different sites and imaging platforms remains controversial and is not completely controlled by including site as a covariate. Future studies should replicate the present results using images acquired at the same site on the same scanner or with equal numbers of cases and controls per scanner. We also note that the CNR1 gene expression (Hawrylycz et al., 2012) and CB_1 receptor density (D'Souza et al., 2016) maps were generated in independent samples of adults and may not accurately represent the eCB system in our sample of adolescents. Although we report significant spatial associations between GMV effects and both CNR1 gene expression and CB_1 receptor density, the effect sizes were small and any suggestion that these associations represent mechanisms for the effects we observe is speculative and requires further investigation.

We adopted a whole-brain, VBM approach to detect effects that were not limited by anatomical boundaries and to allow exploration of spatial relationships between GMV effects and the eCB system. There is evidence, however, that brain perfusion can influence VBM measures of local volume (Franklin et al., 2013, 2015; Ge et al., 2017; cf. Hawkins et al., 2018) so future studies should combine VBM with other measures of brain structure to

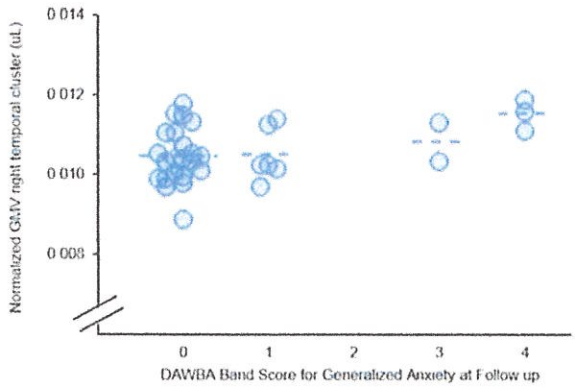

Figure 5. Associations between normalized GMV in the right temporal cluster at baseline and Generalized Anxiety Disorder DAWBA band scores at follow-up. For those participants reporting one or two instances of cannabis use at baseline, those with DAWBA band scores of zero at follow-up had significantly lower GMV at baseline than those with DAWBA band scores of 1 or greater at follow-up ($U = 43$, $p_{corr} = 0.009$).

provide confirmatory evidence. In particular, shape analysis has been shown to be sensitive to brain structural differences associated with cannabis use (Gilman et al., 2014; Smith et al., 2014, 2015; Weiland et al., 2015). Moreover, combining morphometry metrics allows for testing of associations between them, which can identify different relationships between shape deformations and local volume (Gilman et al., 2014) providing evidence of further differences between cannabis users and controls.

One source of variability in the human findings on brain structural correlates of cannabis use may be comorbid substance use (Weiland et al., 2015; Gillespie et al., 2018). Given recent evidence of different patterns of functional connectivity in groups using alcohol, nicotine, and cannabis alone and in combination (Vergara et al., 2018), it will be important to account for any possible interaction effects of cannabis with other psychoactive substances. This issue is particularly important considering the ways in which comorbid substance use has been addressed in two recent, widely cited studies. Gilman et al. (2014) covaried for alcohol and nicotine use and found gray-matter density increases and shape deformations associated with cannabis use. Weiland et al. (2015) matched groups on alcohol and nicotine use and reported no morphometric differences associated with cannabis use, concluding that previously reported differences associated with cannabis may instead be attributable to alcohol use. The participants in Weiland et al.'s (2015) study, however, were using alcohol and nicotine at higher levels than those in Gilman et al.'s (2014) study. It is possible that cannabis, alcohol, and nicotine have differential effects on brain morphometry; specifically, recreational cannabis use has been associated with volume increases, whereas alcohol has been associated with volume reductions. In the current study, we matched the groups on alcohol and nicotine use and, within the cannabis using group, neither alcohol nor nicotine use was associated with individual differences in GMV, suggesting that the GMV differences we report are associated with cannabis use.

We note individual differences in GMV effects: although regional GMV was greater at the group level for adolescents with low levels of cannabis exposure, the distributions showed a high degree of overlap such that many cannabis users had GMV equivalent to that of controls. None of the tested demographic, personality, or substance use factors stratified GMV in the cannabis users. We note evidence that an association between cannabis use and cortical thickness was stratified by genetic risk for schizophrenia (French et al., 2015) and that an association between cannabis use and hippocampal shape was stratified by dopamine-relevant genes (Batalla et al., 2018). Some adolescents may be vulnerable to GMV effects at extremely low levels of cannabis use and it will be critical to identify those at risk as these structural brain changes may be associated with individual risk for psychopathology and deleterious effects on mood and cognition.

Of the behavioral variables tested, only sensation seeking and agoraphobia differed between the cannabis users and controls and these factors were not related to GMV differences. In the cannabis using participants, GMV in the medial temporal clusters was associated with PRIQ and psychomotor speed such that greater GMV in these regions was associated with reduced performance. The finding that right medial temporal GMV predicted generalized anxiety symptoms at follow-up for those participants who had used cannabis should be interpreted with caution given the small sample size and that we were not able to identify factors that drove the individual differences in cannabis effects on GMV at baseline. These findings are notable, however, as panic and anxiety symptoms are frequently reported side effects by naive and occasional cannabis users (Hall and Solowij, 1998). We also note fMRI evidence of hypersensitivity of the amygdala to signals of threat in a partly overlapping sample of cannabis using adolescents (Spechler et al., 2015) and a relationship between adolescent cannabis use and future mood complaints (Wittchen et al., 2007), even with comparatively low levels of use (Cheung et al., 2010).

We have revealed greater GMV in adolescents with only one or two instances of cannabis use in regions rich in CB_1 receptors and CNR1 gene expression. Critically, we were able to control for a range of demographic and substance use effects, to confirm that these structural brain effects were not associated with comorbid psychopathology, and to demonstrate that these effects were unlikely to precede cannabis use. The pattern of results is characterized by individual differences in GMV effects in the cannabis users; these individual differences were associated with PRIQ and with vulnerability to future symptoms of generalized anxiety. Given the increasing levels of cannabis use among adolescents today, we suggest that studying the effects of recreational use early in life is an area of particular importance that should be addressed in the future by large scale, prospective studies.

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