Exposure to marijuana smoke impairs memory retrieval in mice

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Abstract

Marijuana and its primary psychoactive component, delta-9-tetrahydrocannabinol (Δ⁹-THC), have long been known to disrupt cognition in humans. While Δ⁹-THC and other cannabinoids disrupt performance in a wide range of animal models of learning and memory, few studies have investigated the effects of smoked marijuana in these paradigms. Moreover, in preclinical studies, cannabinoids are generally administered before acquisition and because retention is generally evaluated soon afterwards, it is difficult to distinguish between processes related to acquisition and retrieval. In the present study, we investigated the specific effects of marijuana smoke and injected Δ⁹-THC on acquisition versus memory retrieval in a mouse repeated acquisition Morris water-maze task. In order to distinguish between these processes, subjects were administered Δ⁹-THC or exposed to marijuana smoke either 30 min before acquisition or 30 min before the retention test. Inhalation of marijuana smoke or injected Δ⁹-THC impaired the ability of the mice to learn the location of the hidden platform and to recall the platform location once learning had already taken place. In contrast, neither drug impaired performance in a cued task in which the platform was made visible. Finally, the CB₁ receptor antagonist N-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide HCl (rimonabant) blocked the memory disruptive effects of both Δ⁹-THC and marijuana. These data represent the first evidence demonstrating that marijuana impairs memory retrieval through a CB₁ receptor mechanism of action and independently of its effects on sensorimotor performance, motivation, and initial acquisition.
Introduction

Marijuana produces a constellation of effects in humans, including alterations in perception and mood, intoxication, euphoria, increased heart rate, physical dependence upon chronic use, and cognitive impairment (Pacher et al., 2006; Ranganathan and D'Souza, 2006). During the more than 40 years since Δ⁹-tetrahydrocannabinol (Δ⁹-THC) was first identified as marijuana’s primary psychoactive ingredient (Gaoni and Mechoulam, 1964), great strides have been made in understanding its actions in the brain. Δ⁹-THC and related chemicals, known as cannabinoids, produce their psychoactive effects by acting at the cannabinoid-1 (CB₁) receptor in brain areas associated with learning and memory and elsewhere (Herkenham, 1991). Particular interest has focused on the effects of cannabis on cognition, as both naturally occurring and synthetically derived cannabinoids disrupt performance in a variety of rodent spatial (Lichtman et al., 1995; Lichtman and Martin, 1996; Mishima et al., 2001; Nava et al., 2001) and operant (Heyser et al., 1993; Hampson and Deadwyler, 1999) models of learning and memory.

While most studies investigating the consequences of cannabinoids on learning and memory administer Δ⁹-THC or synthetic cannabinoids through various routes of injection, marijuana is typically smoked by human users. As marijuana contains over 60 cannabinoid components in addition to Δ⁹-THC, as well as several hundred noncannabinoid chemicals (Turner et al., 1980), it is plausible that these other constituents may elicit a distinct spectrum of pharmacological effects and/or alter the pharmacological effects of Δ⁹-THC. Additionally it is possible that the route of administration (injection vs. inhalation) could modify behavioral effects. However, virtually no animal studies have investigated the consequences of smoked marijuana on learning and memory in laboratory animals. Therefore, the primary aim of the present study was to investigate the consequences of exposure to marijuana smoke on
learning and memory, as assessed in mouse Morris water maze tasks. For comparison, we evaluated the effects of injected Δ⁹-THC in this same paradigm.

The Morris water maze has been a particularly useful tool to investigate both the physiological function of the endocannabinoid system (Varvel and Lichtman, 2002; Varvel et al., 2005a) and the impact of exogenous cannabinoid administration on learning and memory (Mishima et al., 2001; Varvel et al., 2001). Repeated injections of either Δ⁹-THC (da Silva and Takahashi, 2002) or the potent cannabinoid receptor agonist HU210 (Ferrari et al., 1999) before each acquisition session have been shown to disrupt the ability of rodents to learn the location of a hidden platform that remains in a fixed location. One limitation of these acquisition tasks is that they require several days of training and repeated drug administration, raising the possibility that drug tolerance could lead to an underestimation of acute cognitive-impairing effects. For example, the disruptive effects of Δ⁹-THC in a delayed non-match-to-sample procedure in rats were shown to undergo tolerance following repeated drug administration (Hampson et al., 2003). Using a repeated acquisition Morris water maze procedure, we have previously shown that Δ⁹-THC and other cannabinoid agonists produce CB₁ receptor mediated performance deficits when the location of the target was changed each session (Varvel et al., 2001; Varvel and Lichtman, 2002). However, these experiments were not designed to focus on any particular aspect of cognition, as the mice were administered the drugs 30 min before the acquisition trial and were assessed in the retrieval test only 30 s later. Accordingly, the second objective of the present study was to distinguish between the effects of cannabinoids (i.e., marijuana and Δ⁹-THC) on learning (i.e., acquisition) and memory retrieval.

To delineate between these two processes, we adapted a repeated acquisition Morris water-maze task. Specifically, the hidden platform was placed in a randomly selected position of the water maze before each session and the mice were given five acquisition trials to learn the platform location,
followed 60 min later by a 60 s probe trial in which the platform was removed from the tank to assess retrieval memory. The dose-response relationships of marijuana and $\Delta^9$-THC were evaluated independently on acquisition and on retrieval. The noncompetitive N-methyl-d-aspartate (NMDA) receptor antagonist, dizocilpine maleate (MK-801), served as a positive control. Finally, to determine whether the CB$_1$ cannabinoid receptor was involved, the mice were pretreated with rimonabant before exposure to marijuana smoke, or administration of $\Delta^9$-THC or MK-801.
Methods

Subjects

A total of 72 male C57BL/6J mice (Jackson Laboratories, Bar Harbor, ME.) served as subjects in these experiments. The mice were housed four per cage in a temperature-controlled (20-22°C) environment, with a 12-h light/dark cycle. Food and water were available ad libitum in their home cages. The Institutional Animal Care and Use Committee at Virginia Commonwealth University approved all experiments.

Drugs

Δ⁹-THC, marijuana (Cannabis sativa, containing 5.19% Δ⁹-THC, 0.37% cannabidiol, 0.23% cannabichromene, and 0.20% cannabigerol), ethanol-extracted marijuana (placebo material) and rimonabant (SR141716; N-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide HCl) were obtained from the National Institute on Drug Abuse (Bethesda, MD, USA). Dizocilpine maleate (MK-801) was purchased from Tocris Bioscience (Ellisville, MO). Δ⁹-THC and rimonabant were dissolved in a vehicle consisting of a 1:1:18 solution of ethanol/emulphor/saline. MK-801 was dissolved in a saline solution (0.9%). All injections were given through the i.p. route of administration. In the inhalation experiments, different quantities of marijuana were prepared by mixing marijuana with placebo material at the appropriate ratios, keeping the total weight of all samples at 200 mg.

Apparatus

The water maze consisted of a large galvanized steel pool (1.8 m diameter, 0.6 m height) half-filled with water (22°C), with the top of a white platform (10 cm diameter) submerged 1 cm below the water’s
surface. A sufficient amount of white paint (proline-latex flat) was added to make the water opaque and
to render the platform virtually invisible. Visual cues (i.e., cardboard cut-out letters and shapes) were
attached to the walls of the laboratory as well as attached to the sides of the tank (i.e., five sheets of
laminated paper with black and white geometric designs). An automated tracking system (Columbus
Instruments, Columbus, Ohio) analyzed the swim path of each subject, the path length and latency to
find the hidden platform; as well as the time spent in the specified target areas.

The smoke exposure system, developed and used in this laboratory, has been previously described
(Varvel et al., 2005b; Wilson et al., 2006). The smoke was drawn through a 27.5-cm length of Tygon
tubing to the manifold at a flow rate of 1.0 liter/min using a vacuum pump and flow regulator. The
amount of time required to burn a 200 mg marijuana sample was 1 to 2 min. A solenoid was used to
alternate the flow of smoke and fresh air to the animals every 8 s to mimic puffing. The subjects were
placed into holding tubes that fit snugly into a manifold, consisting of six ports for a nose-only exposure.
Tygon tubing, containing 0.5 g of glass wool fiber to sequester the smoke, was connected to the exhaust
of the manifold. If the substance ceased to burn at any time, it was lit again until completely consumed.

**Water Maze Procedures**

**Training**

Morris water maze training consisted of three phases - an acclimation session, fixed platform
training, and repeated acquisition training. Upon each exposure to the pool, the mice were gently
lowered into the water facing the wall. Before the first training day, the mice were given a single 300 s
acclimation session to the pool without the platform present. During initial training, a fixed platform
procedure was employed in which the subjects received eight days of acquisition training with the
platform remaining in a single location. Each training day consisted of four 120 s trials, with a 10 min
inter-trial interval. Each trial began from one of four start positions, balanced among and between mice. If a mouse failed to find the platform within the 120 s trial it was manually guided to it, and mice were allowed to remain on the platform for 30 s before removal from the tank. In between trials and at the end of each session, each mouse was placed in a heated cage containing dry paper towels.

After fixed platform training, the mice received 10-15 days of repeated acquisition training, in which the platform was placed in one of 32 possible locations in the tank before each session. Each subject received five two-min trials per day, from different and balanced start positions. The same four starting points described above were used (one position was used twice); no mouse was run from the same starting position on two consecutive trials. Positions immediately in front of the hidden platform, along the perimeter, or in the direct center were not used as starting points. Successful performance for each repeated acquisition session was defined as swimming to the platform in less than 30 s on two of the last three trials. In order to qualify for testing, mice were required to achieve this criterion on three of the previous four training sessions and maintain this level of performance throughout the study. Between each test session, the mice were given at least one training session in which they were required to swim to the platform in less than 30 s on two of the last three trials. Mice that failed five training days in a row were excluded from the study, even if they previously passed the criteria for testing.

Once mice had reached training criteria, an experiment was conducted to assess the effects of interposed delays on retrieval performance. Following completion of the fifth trial of an otherwise identical training session, the platform was removed from the tank and the subjects were given a 60 s probe trial after a variable delay (1 min or 1, 2, 6, or 18 h). Only those mice that achieved acquisition criteria for that day were tested for retrieval. The primary dependent measure of interest was the percentage of time that the animals spent in the target zone, an area immediately surrounding the target location (300% larger than the actual target, comprising approximately eight percent of the maze’s
surface area), compared to the amount of time spent in a control zone located directly opposite from the
target zone. The starting position for the retrieval task was along the side of tank halfway between the
two zones. Other measures that were recorded were the latency to target (i.e., the area in which the
platform was previously located), the path length to target, and average swim speed.

**Water maze testing**

In all drug tests, the probe trial was given 60 min after the fifth acquisition trial. A quasi-Latin
square design was employed in which the location of the hidden platform was counter-balanced in all
quadrants of the tank. No more than two subjects under each condition were assessed from any single
platform location and each subject was assessed from a different platform location for each treatment.
Mice were administered $\Delta^9$-THC (1, 3, and 10 mg/kg) or vehicle or exposed to smoke from marijuana
(50, 100, and 200 mg) or placebo either 30 min before repeated acquisition or 30 min before the probe
trial to assess learning and memory retrieval, respectively. As a positive control, we evaluated the effects
of an NMDA receptor antagonist, MK-801 (0.1 and 0.2 mg/kg), on memory retrieval. Additionally, we
examined the memory disruptive effects of marijuana, $\Delta^9$-THC, and MK-801 were CB$_1$ receptor
mediated by treating mice with rimonabant (3 mg/kg) 5 min before agonist administration. A minimum
washout period of 72 h and at least one successful training session was given between treatments and
mice received between one and five training sessions between test sessions.

All 72 mice learned the fixed platform location task; however, only 65% of these subjects achieved
criteria in the repeated acquisition task. Thus, a total of 47 mice were employed for the repeated
acquisition studies. A group of 14 mice was used in the memory decay, $\Delta^9$-THC dose-response on
memory retrieval, and rimonabant-$\Delta^9$-THC experiments. A second group ($n = 14$ mice) was used in the
$\Delta^9$-THC disruption of repeated acquisition experiment. A third group ($n = 12$ mice) was used to assess
the effects of marijuana and rimonabant in combination with marijuana on repeated acquisition and on
the memory retrieval. The fourth group (n = 7) was used to assess the effects of MK-801 and rimonabant
in combination with MK-801 on memory retrieval. In all the data presented throughout this manuscript,
the mice exhibited stable performance on training days or when tested with vehicle.

**Cued Water Maze Task**

The minimal dose of each drug that fully disrupted the retrieval task was evaluated in a cued Morris
water-maze task to identify potential sensorimotor or motivational deficits. In the cued version, the
platform was made visible by placing a black rubber stopper (height, 3 cm; radius, 1.5 cm), which
extended about 2 cm above the surface of the water, on top of the submerged platform. In order to
reduce the total number of mice needed for this study, mice that completed the repeated acquisition
testing were used for the cued task. During training, the subjects were given two 2-min trials per day
from different and balanced start positions. The latency and path length to the cued platform were
scored. Subjects that achieved the training criteria of swimming to the platform in less than 20 s on two
out of three consecutive days were administered drug and assessed in a single trial the following day.

**Statistical analysis**

Two-way ANOVAs were used to analyze the repeated acquisition experiments in which the factors
included drug treatment and trials. One-way repeated measure ANOVAs (in experiments in which all
the mice completed all drug conditions) and one-way ANOVAs (in experiments in which not all the
mice completed all drug conditions) were used to analyze the effects of treatment on time spent in the
target and the control target areas. Dunnett’s test was used for post-hoc comparisons in experiments in
which treatment groups were compared to a single control group (e.g., dose-response and repeated
acquisition experiments). The Tukey test was used for making multiple post-hoc comparisons in the rimonabant experiments. Planned comparisons were conducted between the target zone and control zone in the experiments assessing memory, and for all experiments in the cued platform task. For all analyses, differences were considered statistically significant at $p < 0.05$. The group data are presented as the mean ± SEM.

The ED$_{50}$ values for marijuana- and THC-induced disruption of acquisition and recall performance were determined by least squares linear regression followed by calculation of 95% confidence limits (Bliss, 1967). For both tests, the data were transformed to % maximal possible effect (%MPE), using the following formula: %MPE = 100*[(E$_{\text{max}}$ – Xi) / (E$_{\text{max}}$ – E$_{\text{min}}$)]. The dependent measure for the acquisition experiments was escape latency, and E$_{\text{max}}$ = control (i.e., placebo smoke or vehicle injection) performance on trial 1, E$_{\text{min}}$ = control performance on trial 5, and X$_i$ = trial 5 performance for each respective drug-treated mouse. The dependent measure for the retention experiments was percentage of time spent in the target zone, and E$_{\text{max}}$ = control performance, E$_{\text{min}}$ = predicted random performance (i.e. 8%, which reflects the percentage of the pool’s total surface area encompassed in the target zone), and X$_i$ = performance of each respective drug-treated mouse.
Results

Performance during the retrieval test is delay dependent

The path lengths and latency to swim to the hidden platform during repeated acquisition training are shown in Figure 1. Mice exhibited significant decreases in path length and latency to find the platform across the five acquisition trials, $F(4,140) = 16, p < 0.001$ and $F(4,140) = 17, p < 0.001$ respectively, signifying that they learned the location of the platform. The path lengths were significantly reduced on trials 3, 4, and 5 compared to trial 1 (Figure 1a). Similarly, the escape latencies were significantly decreased on trials 2, 3, 4, and 5 compared to trial 1 (Figure 1b). The average swim speed was $17.4 \pm 0.4$ cm/sec and there were no significant differences in swim speed across trials.

As shown in Figure 1c, the percentage of time spent in the target zone during the probe trial significantly decreased as a function of time delay, $F(4,39), p < 0.01$. In particular, mice spent less time in the target zone following the 6 and 18 h delay than in the 1 min delay condition, $p < 0.05$. In contrast, no significant effect was found for the time spent in the control zone, which did not differ from chance values (i.e., 8%). Subjects spent significantly more time in the target zone than in the control zone, following the 1 min, 1 h, 2 h, and 6 h delays.

Mice were challenged with MK-801 (0.1 and 0.2 mg/kg), which has already been established to impair Morris water maze acquisition in rats (Ramirez-Amaya et al., 2001), and assessed in the retrieval task. Subjects were administered the drugs 30 min after the repeated acquisition session and given a retrieval probe trial at 60 min. Whereas vehicle-treated subjects spent significantly more time swimming in the target zone than in the control zone during the retrieval test, MK-801 led to a significant decrease in the amount time spent swimming in the target zone, $F(2,12) = 6.9, p < 0.01$ (see Figure 1d). In particular, the 0.2 mg/kg dose of MK-801 led to a significant decrease in the amount of time spent in the target zone compared to the saline treatment, $p < 0.05$. Additionally, this dose of drug
significantly reduced the swim speed, F(2,12) = 4.3, p < 0.05 (data not shown). In contrast, the drug failed to elicit significant effects on the time spent in the control zone (p = 0.10). As can be seen by the representative swim traces in Figure 1e, control mice spent a considerable percentage of the probe trial swimming in the target zone and surrounding area, while the MK-801-treated mice displayed no bias to any region in their swim patterns.

**Marijuana and Δ⁹-THC disrupt repeated acquisition**

Two-way ANOVAs revealed significant statistical interactions between marijuana smoke and acquisition trial for both path length to platform, F(16,140) = 2.2, p < 0.01 (Figure 2a), and latency to platform, F(16,140) = 2.1, p < 0.05 (Figure 2b). Inhalation exposure to marijuana smoke failed to affect swim speed (p > 0.41; data not shown). Subsequent one-way repeated ANOVAs for each treatment condition indicated significant decreases in path length to target and escape latency data across the five acquisition trials under the naïve, placebo, and marijuana 50 or 100 mg conditions (p < 0.05), indicating that the mice learned the task. However, mice exposed to smoke from 200 mg marijuana failed to exhibit any improvement across the five acquisition trials for both the path length to target (p = 0.18) and escape latency (p = 0.18). The ED₅₀ (95% CI) value of marijuana was 80 (65 to 99) mg of marijuana, which contained 4.2 (3.4 to 5.1) mg of Δ⁹-THC before burning.

The effects of vehicle and Δ⁹-THC (1, 3, and 10 mg/kg) on repeated acquisition assessments are also shown in Figure 2. Two-way ANOVAs revealed that Δ⁹-THC dose-dependently disrupted acquisition, as reflected by significant main effects of drug on both path length to platform, F(3,52) = 7.7, p < 0.001 (Figure 2c), and the latency to platform F(3,52) = 7.8, p < 0.001 (Figure 2d). Subsequent one-way repeated ANOVAs for each treatment condition indicated that the path lengths to target were significantly longer (p < 0.05) after treatment with 3 or 10 mg/kg Δ⁹-THC than with vehicle. Similarly,
the latencies to target were significantly increased \((p < 0.05)\) following treatment with 10 mg/kg \(\Delta^9\)-THC compared to vehicle. Although there was a significant drug effect on the swim speed, \(F(3,52) = 5.8, p < 0.01\), none of the treatment groups differed from vehicle. This significant effect occurred because the mice swam significantly faster following 3 mg/kg \(\Delta^9\)-THC than following 10 mg/kg \(\Delta^9\)-THC (data not shown). The ED\(_{50}\) (95\% CI) of \(\Delta^9\)-THC was 4.4 (2.4 to 7.0) mg/kg.

**Marijuana and \(\Delta^9\)-THC disrupt retrieval**

The effects of exposure to smoke from marijuana (50, 100 and 200 mg) on retrieval are shown in Figure 3a. A one-way ANOVA revealed that marijuana smoke dose-dependently disrupted recall of the target zone location, \(F(4,24) = 5.1, p < 0.01\), exposure to marijuana (100 or 200 mg) smoke led to significant decreases in the amount of time spent in the target zone compared to exposure to placebo smoke \((p < 0.05)\). In contrast, exposure to marijuana smoke failed to affect the amount of time spent in the control zone \((p = 0.35)\). The ED\(_{50}\) (95\% CI) value of marijuana was 72 (47 to 110) mg, which contained 3.7 (2.4 to 5.7) mg of \(\Delta^9\)-THC before burning. Subjects in the naïve or placebo conditions exhibited significant recall of the platform location, as indicated by the observation that they spent significantly more time in the target zone than the control zone \((p < 0.05)\). Whereas mice exposed to smoke from the ethanol extracted marijuana (placebo) focused much of their swimming in the target zone region, the mice exposed to marijuana smoke swam throughout the tank with no bias to a particular region, as shown in Figure 3b.

Similarly, a one-way ANOVA revealed that \(\Delta^9\)-THC dose-dependently decreased the percentage of time that subjects spent in the target zone location, \(F(3,39) = 5.3, p < 0.01\), (Figure 3c). Again, vehicle-treated subjects spent significantly more time in the target zone than in the control zone during the memory retrieval test. In particular, the mice spent a significant decrease in the amount of time spent in
the target zone when treated with 10 mg/kg Δ⁹-THC compared to when treated with vehicle (p < 0.05). As shown in Figure 3d, the mice did display a bias to any region in their swim patterns when treated with Δ⁹-THC compared to the vehicle treatment. However, Δ⁹-THC failed to affect the amount of time spent in the control zone (p = 0.32). The ED₅₀ (95% CI) value of Δ⁹-THC was 2.5 (1.5 to 4.1) mg/kg.

**Assessment of sensorimotor and motivational deficits**

While the disruptive effects of Δ⁹-THC and marijuana during the retrieval test are consistent with the notion that cannabinoids impair memory, an alternative explanation is that the drugs disrupted performance-related factors, such as the ability to see the visual cues, impaired swimming ability, or decreased motivation to swim to the platform (Brandeis et al., 1989). In order to address these possibilities, the mice were given the minimal dose of each drug that disrupted the retrieval task and were then evaluated in a cued version in which the platform was made visible by placing a visible object on it. The effects of each drug are shown in figures 4a-c for the path length to the cued target, latency to cued target, and swim speed, respectively. In the cued Morris water-maze task, MK-801 (0.2 mg/kg) failed to elicit significant effects on either path length (p = 0.49) or latency (p = 0.11) to the platform, though it still significantly reduced swim speed (p < 0.001). Neither marijuana (100 mg) nor Δ⁹-THC (10 mg/kg) significantly affected the path length to platform, latency to platform, and swim speed measures.

**Evaluation of CB₁ receptor mechanism of action**

As can be seen in Figure 5a, mice treated with the vehicle-marijuana combination did not spend significantly more time in the target zone location than in the control zone location (p = 0.80). However, rimonabant pretreatment blocked the disruptive effects of marijuana on performance during
the retrieval test (p < 0.05, see Figure 5b for swim traces). Similarly, rimonabant blocked \( \Delta^9 \)-THC-induced disruption in the retrieval task, F(3,33) = 4.7, p < 0.01 (see Figure 5c). During the probe trial, subjects spent significantly more time in the target zone than in the control zone when treated with vehicle. However, there were no significant differences in time spent between the target and control zones when the mice were treated with \( \Delta^9 \)-THC. Rimonabant completely blocked these disruptive effects of \( \Delta^9 \)-THC during the retrieval test (p < 0.05; see Figure 5d for swim traces). The rimonabant-vehicle condition did not significantly differ from the vehicle-vehicle condition. In contrast, none of the treatments affected the percentage of time spent in the control zone, which again did not differ from chance levels (p = 0.21). Finally, rimonabant failed to reverse the disruptive effects of MK-801 (data not shown).
Discussion

For decades, marijuana has been the most widely abused illicit drug in the United States (Johnston, 2006). A common untoward effect of this drug is the impairment of both learning (acquisition of new information) and memory (retention and retrieval of previously learned information). While the effects of $\Delta^9$-THC in animal models of cognition have received considerable attention over the years, there have been few, if any, reports of cognitive disruptions induced by inhalation exposure to marijuana smoke in rodents. Moreover, most animal studies investigating the cannabinoid-induced memory impairment fail to distinguish between the disruptive effects of cannabinoids on acquisition and memory retrieval processes. The results of the present study are the first of which we are aware that evaluate acquisition and memory retrieval in mice that are either exposed to inhaled marijuana smoke or given an injection of its primary psychoactive constituent $\Delta^9$-THC. Direct comparisons between the effects of $\Delta^9$-THC and marijuana smoke are important because of suggestions that non-$\Delta^9$-THC constituents of marijuana may alter the pharmacological profile of $\Delta^9$-THC (Carlini et al., 1974; Zuardi et al., 1982; Russo and Guy, 2006), and because of possible differences due to the routes of administration.

In the present study, exposure to marijuana smoke before acquisition impaired the ability of mice to learn the position of a hidden platform. A subsequent experiment also demonstrated that exposure to marijuana smoke 30 min before the retention test impaired the ability of mice to return to the location where the platform had been previously located; suggesting impaired retrieval of recently learned spatial information, independent of effects on acquisition. Thus, two different aspects of cognition (acquisition and retrieval) were impaired by exposure to marijuana smoke. Additionally, this study provides further evidence that marijuana smoke produces disruptive effects on learning and memory independently of sensorimotor or motivational confounds, as performance of the cued version of the task was not
disrupted at a dose that disrupted memory. It should be emphasized that the total amount of $\Delta^9$-THC in
the plant material does not reflect the actual dose administered to each animal, as the majority of smoke
passes through the system and is trapped in the filter. Previous work from our laboratory comparing the
absorption of $\Delta^9$-THC in mice exposed to marijuana smoke has shown that levels of $\Delta^9$-THC in the brain
and plasma increased systematically with increased quantities of marijuana (Wilson et al., 2006). Brain
levels following exposure to smoke from 200 mg marijuana were roughly equivalent to those seen
following intravenous administration of 1 mg/kg $\Delta^9$-THC. Importantly, the effects of marijuana on
retrieval in the present experiment were blocked by pretreatment with rimonabant, demonstrating a CB$_1$
receptor mechanism of action.

Likewise, $\Delta^9$-THC disrupted both the acquisition and retrieval of recently learned spatial information
independent of effects on acquisition with no apparent sensorimotor or motivational confounds. The
potent impairment of acquisition is consistent with a number of previous demonstrations of $\Delta^9$-THC’s
ability to disrupt working memory in a variety of tasks, including the delayed alternation T-maze
(Jentsch et al., 1997; Nava et al., 2000) eight-arm radial maze (Lichtman and Martin, 1996), and
previous water-maze tasks (Varvel et al., 2001; da Silva and Takahashi, 2002; Fadda et al., 2004).
Interestingly, acquisition and retrieval were disrupted by similar doses of either marijuana or $\Delta^9$-THC.
Based on the calculated ED$_{50}$ values, each respective drug was equipotent in disrupting acquisition and
retrieval. In contrast, the retrieval of well-established (i.e. long-term) memories are relatively
impervious to $\Delta^9$-THC-induced impairment (Varvel et al., 2001; da Silva and Takahashi, 2002), though
the present results show that retrieval of recently learned information (i.e. short-term memory) is just as
sensitive to $\Delta^9$-THC-induced impairment as is acquisition. As with other cognitive effects of $\Delta^9$-THC,
this retrieval deficit appears to be mediated via CB$_1$ receptors as it was reversed by rimonabant pre-
treatment.
The experiments reported here required modification of the water-maze task to distinguish different aspects of repeated acquisition, or delayed match-to-place (DMTP) performance. Due to the novelty of these procedures, we performed a number of initial experiments to characterize performance of drug-naïve mice and to test their response to the NMDA antagonist MK-801, which produces well-known cognitive impairments, as a positive control. We have previously shown that mice well trained in a fixed-platform location task (four trials per day for eight days) can retain a memory of the platform location for a remarkably long duration of time, at least nine weeks (Varvel et al., 2005a). However, under the repeated acquisition procedure employed in the present study, performance in the memory retrieval task decays over a period of hours and is at chance levels by 18 hours after acquisition. These findings suggest that memory systems underlying retention in the repeated acquisition task are very different from those underlying retention when the fixed platform procedure is employed. The most obvious explanation accounting for the differences of cannabinoid-induced disruption of memory retrieval between these two procedures is that mice are given substantial training in the fixed platform procedure (i.e., 4 trials per day over eight days) compared with the repeated acquisition task procedure (i.e., 5 trials in which the platform is placed in a different location for each session). Accordingly, the mice trained in repeated acquisition task are unlikely to consolidate the daily platform location into long-term memory. This explanation is consistent with the hypothesis that ∆⁹-THC selectively impairs short-term memory as opposed to long-term memory processes.

The similarities between the effects of marijuana smoke and ∆⁹-THC administration support the hypothesis that the effects of marijuana on memory are predominantly mediated via its ∆⁹-THC content. Specifically, both treatments were equally effective in disrupting acquisition and retrieval, and rimonabant blocked the effects of both treatments. While the cannabidiol, cannabichromene, cannabigerol, and other non-∆⁹-THC cannabinoid constituents could conceivably contribute to
marijuana’s effects on cognition, it should be noted that only trace amounts of these compounds were in
the present sample. In a human study, altering the concentration of cannabidiol and cannabichromene in
marijuana failed to affect the study participants’ performance in cognitive tasks, neurophysiological
measures, and subjective effects (Ilan et al., 2005). On the other hand, in a rat water maze task
increasing the relative amount of cannabidiol was found to antagonize the memory-impairing effects of
Δ⁹-THC (Fadda et al., 2004), as well as blunt the anxiogenic effects of marijuana in humans (Zuardi et
al., 1982). Similarly, the marijuana constituent Δ⁹-tetrahydrocannabivarin elicited CB₁ receptor
antagonist effects in both in vitro (i.e., CP-55940-stimulated GTPγS binding and WIN-55,212-2’s
effects in the mouse isolated vasa deferentia) and in vivo (i.e., Δ⁹-THC-induced analgesia and
hypothermia) assays (Pertwee et al., 2007). However, the effects of other cannabinoids present in
marijuana at higher concentrations than naturally present in the plant material have yet to be
systematically evaluated on cognitive processes. Thus, further research examining the impact of other
marijuana constituents is warranted.

Taken together, the results of the present study demonstrate that marijuana smoke, as well as its
primary psychoactive constituent, Δ⁹-THC, impairs the retrieval of recent memory, independently of its
effects on initial learning, sensorimotor performance, or motivation. The effects of both treatments on
retrieval were mediated through the CB₁ cannabinoid receptor. Furthermore, the particular water-maze
procedures used in these experiments appear well suited to distinguish the effects of drugs on processes
related to acquisition versus memory retrieval, and for investigations of the mechanisms responsible for
marijuana-induced cognitive deficits.
References


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Footnotes

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Legends for Figures

Figure 1. Repeated acquisition in the Morris water maze. Each data point represents the average performance of each subject across five sessions. The path lengths (Panel A) and latencies (Panel B) to target significantly decreased across training trials. * Denotes significant difference from trial 1 (Dunnett’s test, p < 0.05). Panel C. The percentage time spent in the target and controls zones during probe trials across the different time delays. There was a significant decrement in the time spent in the target zone with increasing delays, but the percentage of time spent in the control zone remained at random chance, regardless of delay, N = 8 mice/group. * Denotes significant difference in percentage of time spent in target zone compared to the 1 min delay (Dunnett’s test, p < 0.05). Panel D. MK-801 given 30 min after acquisition, significantly disrupted memory retrieval of the target zone location, but failed to affect the percentage time in control zone (p < 0.10), N = 7 mice/group. * Denotes significant difference in percentage of time spent in target zone compared with control condition (p < 0.05). # (Panels C and D) Denotes significant difference in time spent in target zone compared to time spent in control zone (p < 0.05). The dashed line (Panels C and D) denotes the expected random percentage time spent in the target and control zones. All values are expressed as mean ± SEM. Panel E.

Representative sample of swim traces on the probe trial given 1 h after five acquisition trials. Mice treated with saline (top) spent much of their time swimming in the target zone, while mice treated with 0.2 mg/kg MK-801 (bottom) displayed no bias to any region. The target zone includes the shaded circle that represents the area where the platform was located during the acquisition trials, and opposite is the control zone, which includes the open circle.

Figure 2. Mice exposed to marijuana smoke 30 min before the first acquisition trial displayed poor acquisition in a dose-dependent fashion on both path length (Panel A) and latency (Panel B) to the
platform (p < 0.05, ANOVA for both measures). Similarly, mice given an injection of ∆9-THC 30 min before the first acquisition trial displayed poor acquisition in a dose-dependent fashion on both the path length (Panel C) and latency (Panel D) to the platform (p < 0.001, ANOVA for both measures). * Denotes significant difference from trial 1 (p < 0.05). All values are expressed as mean ± SEM, N = 7-9 mice per group for Panels A and B and N = 14 mice per group for Panels C and D.

Figure 3. **Panel A.** Inhaled marijuana smoke (mar) given 30 min after acquisition, dose-dependently disrupted memory retrieval of the target zone location (ANOVA, p < 0.01), but failed to affect the percentage time in control zone (p = 0.35). N = 5-6 mice/group. **Panel B.** Representative sample of swim traces of a mouse exposed to smoke from either a placebo (extracted) or marijuana (200 mg plant material). The target zone includes the shaded circle that represents the area where the platform was located during the acquisition trials, and opposite is the control zone, which includes the open circle. **Panel C.** ∆9-THC given 30 min after acquisition, dose-dependently disrupted memory retrieval of the target zone location (ANOVA, p<0.01), but failed to affect the percentage time in control zone (p < 0.32). N = 14 mice/group. **Panel D.** Representative sample of swim traces of a mouse given an injection of either a vehicle or ∆9-THC (10 mg/kg). The target zone includes the shaded circle that represents the area where the platform was located during the acquisition trials, and opposite is the control zone, which includes the open circle. * Denotes significant difference in percentage time spent in target zone compared with the control group (p < 0.05). # Denotes significant difference in the time spent in target zone compared to time spent in control zone (p < 0.05). *The dashed line* denotes the expected random percentage time spent in the target and control zones. All values are expressed as mean ± SEM.
Figure 4. The effects of MK-801 (0.2 mg/kg), $\Delta^9$-THC (10 mg/kg) and marijuana (100 mg) were compared to their respective controls (saline, vehicle and placebo) in the cued Morris water-maze task. There was no significant effect on either the path length (Panel A) or the latency (Panel B) to find the cued platform. Whereas neither $\Delta^9$-THC (10 mg/kg) nor marijuana (100 mg) affected swim speed, MK-801 (0.2 mg/kg) significantly reduced the swim speed (Panel C). * Denotes significant difference between the assessed drug and its respective control. All values are expressed as mean $\pm$ SEM, N = 7-9 mice/group.

Figure 5. The memory disruptive effects of both marijuana (Panels A and B) and $\Delta^9$-THC (Panels C and D) are CB$_1$ receptor mediated. Rimonabant (Rim; 3 mg/kg) completely blocked the disruptive effects of either marijuana (200 mg; Panel B) or $\Delta^9$-THC (10 mg/kg; Panel D) on the percentage of time spent in the target zone location. As shown in the representative swim trace samples, rimonabant (3 mg/kg) pretreatment restored effective search strategies during the probe test in marijuana (200 mg)-exposed (Panel B) or $\Delta^9$-THC (10 mg/kg)-injected mice (Panel D), as they spent much of their time swimming in the target zone, which is represented by the delimited zone that encompasses the former platform location (i.e. the shaded circle).* Denotes significant difference in percentage time spent in target zone compared with control condition (p < 0.05). # Denotes significant difference in percentage time spent in target zone compared to time spent in control zone (p < 0.05). The dashed line denotes the expected random percentage time spent in the target and control zones. All values are expressed as mean $\pm$ SEM, N = 12-13 mice/group.
Figure 1

A. Path Length (cm) vs. Acquisition Trial

B. Escape latency (s) vs. Acquisition Trial

C. % Time in Target zone and Control zone:
   - 1 min
   - 1 h
   - 2 h
   - 6 h
   - 18 h

D. % Time vs. MK801 (mg/kg)

E. Representative tracking of saline and MK801 treatments.