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**Effect of repeated dosing of Delta 9-  
Tetrahydrocannabinol, the major psychoactive  
ingredient of marijuana, on memory in mice**

**Niyuhire Floride**

**PMCH 691 – Research Project**

**Virginia commonwealth University / School of Medicine**

**Medical College of Virginia Campus**

**Department of Preventive Medicine and Community Health**

**Master of Public Health Program**

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Continuing Medical Education**

**Preceptor: Aron H. Lichtman, Ph.D, Associate Professor,  
Pharmacology and Toxicology**

**April 23<sup>rd</sup>, 2004**

This MPH Research Project is submitted as partial fulfillment of the requirements for a Master of Public Health degree from the Virginia Commonwealth University/ Medical College of Virginia School of Medicine. I agree that the Master of Public Health Program will make it available for circulation in accordance with the program's policies and regulations pertaining to documents of this type. I also understand that I must receive approval from my Faculty Advisor in order to copy form or publish this document, or submit to a funding agency. I understand that any copying from or publication of this document for potential financial gain is not allowed unless permission is granted by my Faculty Advisor or (in the absence of my Faculty Advisor) the Director of the MPH Program

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Signature

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Date



**DESCRIPTION OF METHODS (continued)**

*Describe methods to be used for data analysis:*

One way ANOVA analysis will be used for the comparison among groups and a t-test for the comparison between two groups.

**ANTICIPATED RESULTS:**

No tolerance develops to the disruptive effects of THC.

**SIGNIFICANCE OF PROJECT TO PUBLIC HEALTH:**

Assess the safety of potential marijuana derived medication.

Help public health officials educating, especially the youth, about the potential danger of using marijuana.

**PROPOSED SCHEDULE:** Start Date: Nov 2003 End Date: March 2004

**INDICATE WHICH OF THE FOLLOWING AREAS OF PUBLIC HEALTH KNOWLEDGE WILL BE DEMONSTRATED:**

1. Biostatistics – collection, storage, retrieval, analysis and interpretation of health data; design and analysis of health-related surveys and experiments; and concepts and practice of statistical data analysis. \*yes no (if yes, briefly describe):

This experimental study will collect, store, analyze, and interpret data relevant to human consumption of marijuana or marijuana-derived products.

2. Epidemiology – distributions and determinants of disease, disabilities and death in human populations; the characteristics and dynamics of human populations; and the natural history of disease and the biologic basis of health. yes no (if yes, briefly describe):
3. Environmental Health Sciences – environmental factors including biological, physical and chemical factors which affect the health of a community. \*yes no (if yes, briefly describe):

The present study will explore the potential effects of chronic marijuana or marijuana derived products.

4. Health Services Administration – planning, organization, administration, management, evaluation and policy analysis of health programs. yes no (if yes, briefly describe):
5. Social/Behavioral Sciences – concepts and methods of social and behavioral sciences relevant to the identification and the solution of public health problems. yes no (if yes, briefly describe):

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## Abstract

**Purpose:** Marijuana is the most widely used illicit drug in the United States. However, marijuana and cannabinoid derivatives have potential therapeutic uses. Studies in cannabis users have yielded contradictory results with regard to long-term effects on cognitive functions. There is no prospective study assessing this issue, and such studies may raise ethical issues in humans, whereas mice have been shown to exhibit similar cannabinoid-mediated behaviors as humans. The purpose of this study was to assess the consequences of chronic administration of  $\Delta^9$ -THC, the major psychoactive component of marijuana, in a mouse memory model.

**Methods:** In Experiment 1, the dose-response relationship of  $\Delta^9$ -THC was assessed in the object recognition task, a well-documented rodent memory model. In Experiment 2, mice were treated repeatedly with either escalating doses of  $\Delta^9$ -THC or vehicle for one week, and then challenged with the drug to assess whether tolerance had developed.

**Results:** Acute  $\Delta^9$ -THC dose-dependently interfered with memory as assessed in the object recognition task ( $ED_{50}$  95% C.I. = 0.5 (0.1 to 1.7) mg/kg). No tolerance to the memory disruptive effects of 1 mg/kg  $\Delta^9$ -THC was evident after chronic treatment.

**Conclusions:** Considerably low doses of  $\Delta^9$ -THC impaired memory. The failure of chronic  $\Delta^9$ -THC to produce tolerance in this model was surprising considering that a similar dosing regimen has been reported to produce tolerance in non-mnemonic behaviors. The results suggest that memory is particularly sensitive to the disruptive effects of  $\Delta^9$ -THC and chronic cannabis use is likely to elicit persistent impairment of cognitive function. Caution should be applied in advocating chronic use of medicinal cannabinoids. Potential solutions lie in reinforcing education on the harm caused by cannabis use and availability of alternative solution to cannabis users, especially among youth that have shown to be more vulnerable to this drug.

## **Introduction**

Marijuana, also known as *Cannabis sativa*, is the most widely abused illicit drug in the United States (US). The 2002 National Survey on Drug Use and Health (NSDUH) reported that 14.6 million people have used marijuana in the month preceding the survey (1). The same survey reported an estimated 2.6 million new marijuana users for that year. A contributing factor to its widespread use is that marijuana is generally perceived as less harmful than other drugs and is fairly easy to obtain. This same belief prevails among US youth. In the National Vital Statistics report for 2001, 22.4% of high school seniors in the US reported to have smoked marijuana in the month preceding the survey (2). According to the NSDUH, 20.6 percent of youths aged 12 to 17 and 53.8 percent of young adults aged 18 to 25 had used marijuana at least once, with 55.0 percent of youths reporting that it was fairly or very easy to obtain marijuana (1). In 2003, 17.5, 36.4 and 46.1 percent of respective 8<sup>th</sup>, 10<sup>th</sup> and 12<sup>th</sup> graders in the US reported to have used marijuana at least once, and 44.8%, 73.9% and 87% of respective 8<sup>th</sup>, 10<sup>th</sup> and 12<sup>th</sup> graders reported this drug as being accessible (3).

Despite the high rate of youth abusing marijuana, it is now established that acute intoxication with marijuana disrupts short-term memory and problem solving, distorts perception, and impairs coordination in humans as well in laboratory animals (4). Since marijuana use can impair thinking and judgment, its users neglect to have safe sex, which may expose them to infection to the Human Immunodeficiency Virus (HIV), or other sexually transmitted diseases. In fact, marijuana use had been associated with increased

unprotected sex among adolescent, exposing them to sexually transmitted diseases and AIDS (5,6).

On the other hand, marijuana and cannabinoid derivatives have potential therapeutic uses. Recent studies suggest that cannabinoids are likely to have a natural role in pain modulation, control of movement, cognition, and memory (7). The office of National Drug Control Policy, under public pressure to allow the medical use of marijuana, funded a study by the Institute of Medicine (IOM) to evaluate the scientific evidence for benefits and risks of using marijuana as a medicine (7). The IOM concluded that cannabinoids would be moderately well suited for certain conditions, such as chemotherapy-induced nausea and vomiting and poor appetite in AIDS wasting syndrome; that cannabinoids may provide useful adjuncts to existing medications. However, the IOM report concluded that several problems need to be addressed before marijuana plant could be legally prescribed as a medication. Today, marijuana remains a scheduled 1 substance, under the Controlled Substance Act (8), and there is no currently accepted medical use of crude marijuana. However, delta-9-tetrahydrocannabinol ( $\Delta^9$ -THC), the psychoactive constituent of marijuana, has therapeutic indications approved by the Food and Drugs Administration. Oral  $\Delta^9$ -THC (dronabinol) is an approved medication to treat nausea associated with cancer chemotherapy and as an appetite-stimulator for patients with wasting related to AIDS or severe cancer-related anorexia (9). Cannabinoids have other potential therapeutic uses for a variety of disorders, including pain, anxiety, eating disorders, movement disorders, glaucoma and cardiovascular diseases (7, 10).

The field of cannabinoid biology has made considerable progress over the last two decades. Two types of cannabinoid receptors have been isolated and cloned, the CB<sub>1</sub> receptor, which is located predominantly in the central nervous system, and the CB<sub>2</sub> receptor, which is associated with the immune system in the periphery. High concentrations of CB<sub>1</sub> receptors are located in the cerebral cortex, motor system, limbic system and hippocampus (7, 11). In addition, two endocannabinoids, anandamide and 2-arachidonoyl glycerol have been discovered (12). The CB<sub>1</sub> receptor is thought to be responsible for the majority of the effects in the central nervous system (CNS). CB<sub>1</sub> receptors are frequently located on presynaptic terminals where they modulate the release of a variety of neurotransmitters (13,14). Activation of these receptors results in inhibition of both excitatory and inhibitory neurotransmitter release (15,16) through a depolarization-induced suppression of the inhibition when the presynaptic neurotransmission is inhibitory, such as GABA, and depolarization-induced suppression of excitation when the neurotransmitter is excitatory, such as glutamine (13,14). Cannabinoids may disrupt short-term memory by interfering with the encoding of events in the hippocampus during memory processing (11,17). The availability of SR141617A, a specific CB<sub>1</sub> receptor antagonist, has confirmed cannabinoid-mediated effects on a variety of function including cognitive function in animal (18) and human studies (19).

Oral  $\Delta^9$ -THC and smoked marijuana have been shown to produce similar effects in human subjects (42,43). Studies suggest that repeated exposure to  $\Delta^9$ -THC or smoked marijuana produces dependence and tolerance to at least some of the effects of cannabis in humans (4,20,21). Several withdrawal symptoms associated with abstinence following prolonged marijuana use include hyperirritability, tremors, sweating, auditory and visual

hallucinations, euphoria, anxiety, negativism, insomnia or abnormal sleep patterns, and GI distress. Studies in animals have shown that chronic administration of  $\Delta^9$ -THC produces cannabinoid receptor desensitization and down-regulation (22,23,24), as well as tolerance to cannabinoid-mediated hypoactivity, catalepsy, antinociception, and hypothermia (22,25). However, the cognitive effects of long-term cannabis use are insufficiently understood. Previous animal studies that assessed tolerance of the cognitive functions to the disruptive effects of  $\Delta^9$ -THC have produced contradictory results (4). Deadwyler et al. reported a complete adaptation to the disruptive effects of  $\Delta^9$ -THC on a spatial discrimination version of a delayed-match-to-sample, short-term memory, in rats chronically treated with this drug (26). Whereas others studies didn't report tolerance to the disruptive effects of  $\Delta^9$ -THC on cognitive function (27,28). It has been suggested that cognitive impairments in long-term cannabis users might be reversible after abstinence or cessation of use (30,31). Yet, there have been reports that prenatal exposure to marijuana may interfere with tasks that require visual memory, analysis and integration (32,33); that cannabinoid induces changes in gene expression (34); that marijuana use in early adolescence was associated with poor school performance, being suspended or expelled from school, exposure to drugs and violence, lower work aspirations, being fired from a job, collecting welfare, being and an unmarried parent in the young adulthood (35,36,37). The long-term neurocognitive effects of marijuana may be influenced by both the early-onset cannabis use and the dose of marijuana consumed (36,38,39).

## **Objective**

The primary objective of this study is to determine whether acute administration of  $\Delta^9$ -THC impairs working memory in the object recognition task in naïve mice and in mice that are tolerant to  $\Delta^9$ -THC. The object recognition task was chosen over other tasks because its design allowed the avoidance of potential conflicts associated with the mice learning the task while receiving repeated doses of the drug.

With the increased interest in potential therapeutic uses of marijuana and marijuana derivatives, the mounting pressure to allow medical marijuana use and the high rate of youth abusing this drug, understanding the possible detrimental effects of chronic marijuana on learning and memory is of critical importance.

The results of this study will contribute to the understanding of the effects of long-term use of marijuana and marijuana derivatives on learning and memory. Additionally, the results reported here may provide some insight as to whether these products can be safely used in therapeutics. Finally, the outcome of this study may be helpful in developing public health policies aimed to protect both youth and adults from the potential short- and long-term harm of marijuana use.

## **Hypothesis**

Tolerance to cannabinoid-induced disruption on memory in mice develops after chronic dosing with  $\Delta^9$ -THC.

## **Materials and methods**

### **Subjects**

Male ICR mice, obtained from Harlan Sprague Dawley, Inc, Indianapolis, IN, were used for the purpose of this experiment. They were housed in a temperature-controlled room (20-22°C) with a 12:12 light/dark cycle. The mice had access to food and water, and ad libitum in their home cage. The Institutional Animal Care and Use Committee at Virginia Commonwealth University approved all experiment procedures.

### **Drug**

$\Delta^9$ -THC was provided by the National Institute on Drug Abuse (Bethesda, MD.)

$\Delta^9$ -THC was dissolved in a vehicle consisting of a 1:1:18 solution of ethanol/emulphor/saline. The acute treatment was given intraperitoneally (i.p.) and the chronic treatment was given subcutaneously (s.c.). The injection volume was 0.1ml/kg.

### **Object recognition task**

#### **Acute experiment**

The object recognition task is a well-characterized paradigm for assessing memory that makes use of a mouse's natural tendency to investigate novel aspects of their environment (40,41). All the experiments were conducted in a quiet room. The experiment was conducted on two consecutive days. On day one, the mice received an acclimation session in which they were allowed one hour to acclimate to the laboratory and to the Plexiglas boxes (25 x 46 x 20 cm). A transparent sheet of Plexiglas, with three small aeration holes (1cm of diameter), covered the box to prevent mice from escaping. On day two, the mice were allowed one hour to acclimate to the observation room.

Twenty minutes prior to the exposure to an object, the mice were given an i.p. injection of a vehicle, or  $\Delta^9$ -THC (0.1, 0.3, 1, or 3 mg/kg). Two types of objects were used throughout the experiment: a glass water-stopper and a metallic door pull. Following each session, the objects were washed with alcohol and rinsed with water before being introduced in the box. The mouse was placed in a clean Plexiglas box and one of the objects was introduced in the box for a period of 20 minutes. The presentation of objects was counter-balanced, such that half the mice were exposed to the water stopper and the other half were exposed to the door pull. After the 20 minutes exposure, the object was taken out, while the mouse remained in the box.

Five minutes later, both the familiar (object previously exposed to the mice) and a novel object were placed in the box. The test sessions were videotaped for 5 minutes and the time each mouse investigated the familiar and the novel objects was scored. After the 5-minute session, both objects were taken out of the box and the mouse returned to its home cage.

Investigatory behavior was defined as when a mouse sniffed the object with its snout within 2 cm of the object or actively exploring the object. Mice that displaced any of the objects were excluded; and mice were required to spend at least 5 seconds exploring each object to be included in the analysis.

The mouse was considered to have formed a memory of the familiar object if it spent more time exploring the novel object than the familiar object. If the time spent on the familiar and the novel object was about the same, it was inferred that the mouse has forgotten the familiar object.



## **Chronic experiment**

Mice were treated for one week with subcutaneous injections of either a vehicle or an escalating dose of  $\Delta^9$ -THC: 10mg/kg of  $\Delta^9$ -THC twice a day for two days, 30mg/kg twice a day for two days, 60mg/kg twice a day for two days and a single 60mg/kg on the seventh day. On the seventh day, the mice were allowed to acclimate to the Plexiglas boxes as describe in the acute experiment and given the last dose of drug immediately after this acclimation session. Twenty-four hour after the last injection, the mice were assessed in the object recognition task as described above.

Twenty minutes before the exposure to an object, the mice were given an i.p. injection of a vehicle or 1 mg/kg of  $\Delta^9$ -THC, a dose that was found to impair memory in this task, but failed to affect motor behavior. The rest of the testing was conducted as described in the acute experiment.

## **Statistical analysis**

Sigma stat and sigma plot software were used for statistical analysis and graphs.

For the analysis of acute  $\Delta^9$ -THC effects in the object recognition task, a paired t test was conducted for each injection condition to assess whether the subjects spent a significantly different amount of time investigating the familiar and novel objects. For the analysis of the chronic treatment, a two-way ANOVA analysis was conducted on the difference in time spent exploring the new object minus the time spent on the old object, and significant results were further analyzed using the Tukey post hoc test. In order to determine the ED50 values, index recognition values were computed by determining the percentage of the time spent exploring the new object by the total time spent exploring

both the new and the familiar objects. An index of 50 indicated no difference between the time spent exploring the familiar and the new objects, and higher index scores indicated that the subjects spent more time investigating the novel object than the familiar object.

## Results

### Effects of acute $\Delta^9$ -THC

**Table 1** Mean +/-SEM time spent exploring the familiar and old objects (seconds)

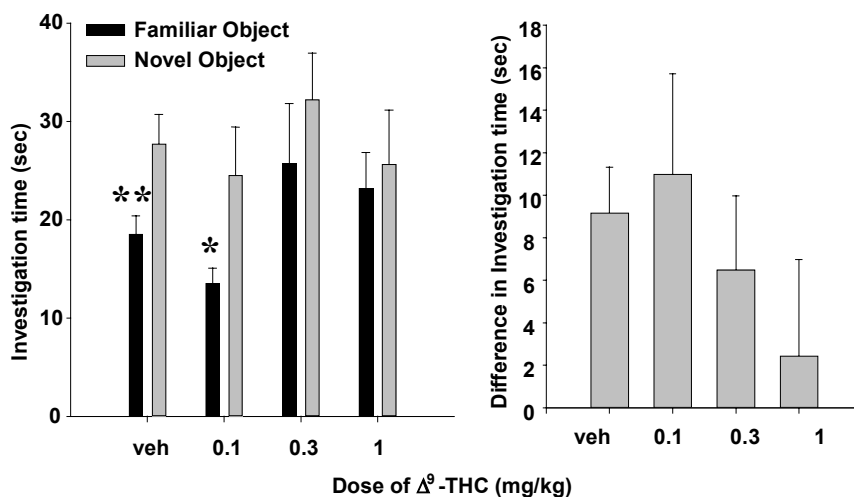
	Familiar object	New object
Vehicle (n=11)*	18.6+/-1.9	27.7+/-3.0
0.1mg/kg THC (n=9)*	13.6+/-1.6	24.6+/-4.9
0.3mg/kg THC (n=8)	25.8+/-6.1	32.3+/-4.7
1mg/kg THC (n=9)	23.2+/-3.6	25.7+/-5.5

Asterisks denote significant differences in time spent exploring the familiar and the novel object.

The acute effects of  $\Delta^9$ -THC on the object recognition task were assessed in mice treated with vehicle or  $\Delta^9$ -THC (0.1, 0.3, 1 and 3 mg/kg); the number of mice excluded in each group for failing to meet the criteria was respectively 4, 3, 6, 4, and 9. Because more than half of the mice treated with 3 mg/kg of  $\Delta^9$ -THC were excluded (9 mice out of 13), we suspected that mice treated with this dose were exhibiting motor impairment and were therefore excluded from further analysis.

The mean time and standard error values each group spent investigating the objects are presented in table 1.

Figure 1 Acute effects of  $\Delta^9$ -THC on object-recognition



**Figure 1** Effect of  $\Delta^9$ -THC on the investigation time of the familiar and the novel objects (left panel), and on the difference in investigation time (right panel) (Time spent on novel object minus time spent on familiar object) during the object recognition task. Each data point represents the mean time in that group of treatment (veh (n=11), 0.1 mg/kg (n=9), 0.3 mg/kg (n=8), 1 mg/kg (n=9)). Two asterisks denote a  $p < 0.001$  and one asterisk a  $p < 0.05$

As shown in figure 1, the mice treated with vehicle and 0.1 mg/kg of  $\Delta^9$  THC spent significantly more time exploring the new object than the familiar object ( $p < 0.001$  and  $p < 0.05$ , respectively). In contrast no significant differences were found in investigation time when the mice were treated with either 0.3 or 1 mg/kg  $\Delta^9$  THC ( $p = 0.10$  and  $p = 0.59$ , respectively), indicating that the mice had forgotten the familiar object. Although there was trend in decreased difference in time spent investigating the objects as the dose of  $\Delta^9$  THC increased, no significant differences were found.

The ED<sub>50</sub> (95% CI) of  $\Delta^9$ -THC in disrupting memory of the familiar object was 0.5 (0.1 to 1.7) mg/kg.

### Effects of chronic $\Delta^9$ -THC

**Table 2** Mean $\pm$ SEM time spent exploring the familiar and old objects (seconds)

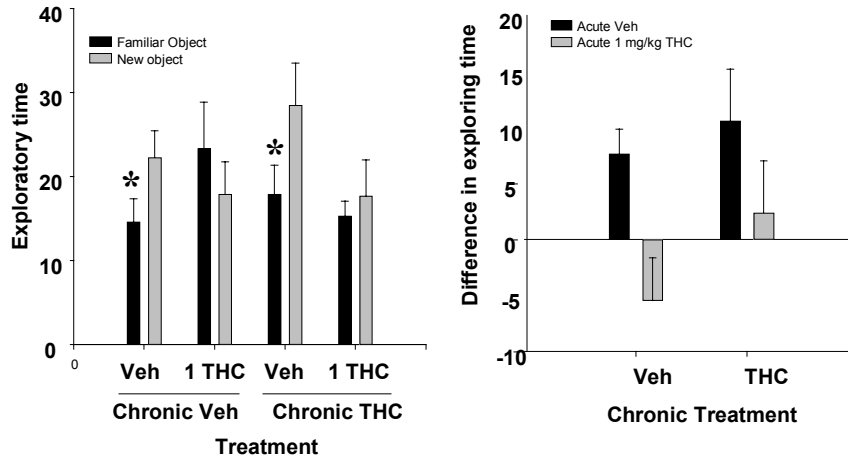
Chronic treatment	Acute treatment	Familiar Object	New object
Vehicle	Vehicle (n=12)*	14.6 $\pm$ 2.8	22.3 $\pm$ 3.2
Vehicle	THC (n=9)	23.3 $\pm$ 5.5	17.9 $\pm$ 3.9
THC	Vehicle (n=10)*	17.9 $\pm$ 3.5	28.5 $\pm$ 5.0
THC	THC (n=10)	15.3 $\pm$ 1.8	17.7 $\pm$ 4.3

Asterisks denote significant differences in time spent exploring the familiar and the novel object.

The effects of chronic treatment with  $\Delta^9$ -THC were assessed in mice chronically treated with a vehicle or escalating doses of  $\Delta^9$ -THC. In the mice chronically treated with vehicle, 3 mice and 6 mice, respectively, from the vehicle and  $\Delta^9$ -THC acute conditions were excluded for failing to meet criteria; and in the  $\Delta^9$ -THC chronically treated mice, 6 mice and 5 mice were excluded from the acute vehicle and  $\Delta^9$ -THC conditions, respectively.

The mean time with standard errors that each group spent exploring the familiar and the new objects is presented in table 2.

**Figure 2** Effects of 1 mg/kg  $\Delta^9$ -THC in mice treated chronically with  $\Delta^9$ -THC or veh



**Figure 2** Effect of acute  $\Delta^9$ -THC in mice chronically treated with  $\Delta^9$ -THC on investigation time of the familiar and the novel objects (left panel), and on the difference in investigation time (right panel) (Time spent on novel object minus time spent on familiar object) during the object recognition task. Each data point represents the mean time in that group of treatment (veh/veh (n=12), veh/THC (n=9), THC/Veh (n=10), THC/THC (n=9)). Asterisk denote a  $p < 0.05$

A Two-Way ANOVA analysis was conducted on the difference in time spent exploring the objects.  $\Delta^9$  THC chronic treatment did not significantly affect the object recognition task ( $F_{3,37} = 1.96$ ,  $p < 0.17$ ). Additionally, there was no significant interaction between acute and chronic treatment ( $F_{3,37} = 0.41$ ,  $p < 0.53$ ). On the other hand, there was an overall significant effect of acute treatment with 1 mg/kg of  $\Delta^9$  THC on the object recognition task ( $F_{3,37} = 7.67$ ,  $p < 0.009$ ).

Planned comparisons were conducted to compare the amount of time each group spent investigating the familiar object versus the novel object. As shown in figure 2, in the

chronic vehicle group, mice tested with acute vehicle spent significantly more time exploring the new object than the familiar object ( $p < 0.01$ ), whereas the mice tested with 1mg/kg of  $\Delta^9$  THC failed to show a significant difference in the time spent investigating the objects ( $p < 0.13$ ). In the chronic  $\Delta^9$ -THC group, the mice challenged with vehicle spent significantly more time exploring the new object than the familiar object ( $p < 0.05$ ), whereas challenge with 1 mg/kg of  $\Delta^9$ -THC resulted in no significant differences between the amount of time spent investigating each object ( $p < 0.62$ ).

## **Discussion**

In the present experiment, low doses of acute  $\Delta^9$ -THC impaired recognition of the familiar object in the object recognition task. These results are consistent with those of others studies using a variety of memory paradigms. Varvel et al. (18) has shown that  $\Delta^9$ -THC disrupts working memory version of the Morris water maze at a lower dose than that required to disrupt the reference memory or other behavioral effects such as hypomotility, antinociception, and hypothermia. Hampson et al (11) had shown the disruptive effects of  $\Delta^9$ -THC in rats on short-term memory during the performance of a delay nonmatch-to-sample task.  $\Delta^9$  THC has also been shown to produce impairment of cognitive functions in humans (42,43,44,45). The  $\Delta^9$ -THC content in marijuana (46) ranges on average between 1 and 5%, though the  $\Delta^9$  THC content of some cannabis strains are up to 12%. The plasma levels of  $\Delta^9$  THC shown to impair memory in human (42) are roughly comparable to plasma levels shown to induce memory impairment in this task (unpublished data from Litchman's laboratory).

In the present experiment, mice chronically treated with  $\Delta^9$  THC and challenged with a vehicle 24 hours later recognized the familiar object in the object recognition task. This finding suggests that the pharmacological effects of the drug had worn off. Moreover, these results indicate that the chronic regimen employed in the current study was insufficient to produce long-lasting cognitive deficits.

An unexpected finding of this experiment was that no tolerance to the memory disruptive effects of  $\Delta^9$  THC developed in the object recognition task. Specifically, mice chronically treated with  $\Delta^9$ -THC and challenged with a  $\Delta^9$ -THC 24 hours later failed to remember the familiar object. This finding suggests that, whereas tolerance to cannabinoid-induced motor impairment, hypothermia and analgesia has shown to develop quickly (25), the effects of chronic  $\Delta^9$  THC or other cannabinoids on cognition function appears to be resistant to tolerance, or at least tolerance to the cognitive function may develop slower than tolerance to others cannabinoid-mediated behaviors.

Consistent with the finding reported here, Nava et al (27) had reported a lack of tolerance to the disruptive effects of  $\Delta^9$  THC in another cognitive test, the rat alternation T-maze task. Additionally, this same study reported a lack of tolerance to the inhibition of extracellular acetylcholine. Inhibition of acetylcholine, a major neurotransmitter in the hippocampus, has been reported as one of the mechanism by which cannabinoids may disrupt memory (27,47). Further, some studies in chronic cannabis users had reported a lack of tolerance to the cognitive impairment effects of cannabinoids (38,39). Thus, individuals who chronically take cannabinoids, either for recreational or medical reasons, may continue to have disrupted memory while under the influence of the drug. Some studies have even reported that chronic cannabis users exhibited impaired cognition long

after  $\Delta^9$  THC effects would have worn off (30,31). Also consistent with the finding of this study, some studies in adolescents (35,36,37) have linked marijuana use in early adolescence to problems in young adulthood such as not graduating from high school, low work aspirations, being fired from a job, collecting welfare and being an unmarried parent. Suggesting that ended marijuana to some degree was interfering with social functioning. Even though, others studies did not found long-lasting cognitive impairment after abstinence or cessation of use in human subjects who had been chronically exposed to cannabinoids (48,49).

On the other hand, Deadwyler et al (26) reported a development of tolerance to the memory disruptive effects of  $\Delta^9$  THC in performance of a delayed-match-to-sample (DMTS) task in rats chronically treated with 10 mg/kg/day  $\Delta^9$  THC for up to 35 days. However, a confound in the Deadwyler study was that the rats exhibited impaired motor function, thus making it difficult to assess pure mnemonic function in the DMTS task. Nonetheless, the results of this study suggest at least that a longer period of treatment with cannabinoids may be sufficient to elicit tolerance to the disruptive effects of cannabis on memory.

One strength of the present study was that the object recognition task used to assess short-term memory in mice was very sensitive to drug-induced disruption, as low doses of  $\Delta^9$  THC were sufficient to impair memory, without any evidence of motor impairment. The fact that this task can essentially be conducted in one acclimation session and one test session simplified the experimental designed by avoiding potential conflicts associated with the mice learning the task while receiving repeated doses of drug.



However, in our chronic experiment, we only examined a single dose of drug at testing using a single dosing regimen. Therefore, future studies assessing tolerance should look at the entire dose-response relationship and examine different chronic treatment protocols. Nonetheless, the fact that the mice treated with escalating doses of  $\Delta^9$ -THC still exhibited memory impairment to a low dose of  $\Delta^9$ -THC suggests that tolerance did not occur or was minimal. The fact that the observation and the scoring of investigation time was conducted by the same individual, who was not blind to the drug, is also a limitation of this study.

## **Conclusions**

Acute  $\Delta^9$  THC produces memory impairment and tolerance may not develop to this effect of drug, or at least tolerance to the disruptive effects of  $\Delta^9$  THC may develop slower than that of other cannabinoid-mediated effects. These findings suggest that people who use cannabis regularly are likely to continue to exhibit impairment of cognitive function while under the influence of the drug.

Studies have reported that early onset use and quantity of cannabis consumed might play a determining role in the severity of harm caused by cannabis use. Potential solutions lie in reinforcing education on harm caused by cannabis use, which could decrease the number of new users, and developing alternative solutions for people who wish to quit, such as drugs for withdrawal symptoms or social support groups.

With regard to chronic use of medicinal cannabinoids, caution is needed, given the potential for the persistent disruption of cognitive function.

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