Differential effects of delta$^9$-THC on learning in adolescent and adult rats

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Abstract

Marijuana use remains strikingly high among young users in the U.S., and yet few studies have assessed the effects of delta$^9$-tetrahydrocannabinol (THC) in adolescents compared to adults. This study measured the effects of THC on male adolescent and adult rats in the Morris water maze. In Experiment 1, adolescent (PD=30–32) and adult (PD=65–70) rats were treated acutely with 5.0 mg/kg THC or vehicle while trained on the spatial version of the water maze on five consecutive days. In Experiment 2, adolescent and adult rats were treated acutely with 2.5 or 10.0 mg/kg THC or vehicle while trained on either the spatial and non-spatial versions of the water maze. In Experiment 3, adolescent and adult rats were treated with 5.0 mg/kg THC or vehicle daily for 21 days, and were trained on the spatial and then the non-spatial versions of the water maze task four weeks later in the absence of THC. THC impaired both spatial and nonspatial learning more in adolescents than in adults at all doses tested. However, there were no long-lasting significant effects on either spatial or non-spatial learning in rats that had been previously exposed to THC for 21 days. This developmental sensitivity is analogous to the effects of ethanol, another commonly used recreational drug.

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1. Introduction

The use of marijuana remains strikingly high among adolescents in the U.S. In 1999, more than 2 million Americans used marijuana for the first time. Two-thirds of them were between the ages of 12 and 17 (DHHS, 2002). While 16% of the US population ages 18–25 reported past month use of marijuana in 2001, only 2.4% of the population over the age of 35 reported such use (DHHS, 2002). Although used predominantly by adolescents and young adults, there have been very few studies making direct comparisons of delta$^9$-tetrahydrocannabinol (THC) effects in juveniles or adolescents compared to adults in either humans or in animal models.

The scant evidence available suggests that THC should have robust effects on the adolescent brain. In humans, adolescence is generally considered to represent the second decade of life. Although there is considerable variance between adolescence models across species, postnatal days 30–50 are often used to model this developmental period in the male rat. The CB1 receptor, the receptor in the brain for the psychoactive constituents in marijuana, is present early in ontogeny in rats, and achieves adult levels in early adolescence (Belue et al., 1995; McLaughlin et al., 1994; Rodriguez de Fonseca et al., 1994). In fact, one study reported that CB1 receptor levels are maximal in early adolescence, and decreasing as the animal ages into adulthood (Belue et al., 1995). That study is particularly intriguing because it suggests that a post-adolescent pruning phenomenon, like that described for dopamine receptor, may occur (Seeman, 1999). Thus, it is possible that exposure to THC during adolescence may have important consequences for brain function. Indeed, studies in both humans and animal models suggest this possibility may be true. Chronic use of marijuana...
before the age of 16 in humans has been correlated with impairments in visual scanning reaction time (Ehrenreich et al., 1999). Even more striking, a recent study of chronic cannabinoid treatment (25 days) of adolescent rats showed that treatment with the agonist WIN 55212-2 during pubertal development causes persistent deficits in memory, sensory-motor gating and performance in a progressive ratio task that remain in adulthood, while the same treatment in adulthood does not have persistent effects (Schneider and Koch, 2003). These studies suggest that adolescent THC exposure could have enduring effects on normal cannabinoid receptor function involved in fundamental brain processes.

One of the most salient cognitive effects of THC is the impairment of learning and memory, a cognitive domain that is particularly important during adolescence given the academic and social demands experienced during that developmental period. THC and cannabinoid agonists have been shown to impair learning in a number of paradigms that reflect hippocampal function, including the spatial version of the Morris water maze (Da and Takahashi, 2002; Ferrari et al., 1999; Varvel et al., 2001). However, the amnesic effects of THC have not been systematically studied in animals at different stages of postnatal development. The present study examined the effects of both acute and chronic THC on spatial learning in adolescent and adult rats. We hypothesized that THC would impair learning more potently in male adolescent animals compared to male adults.

2. Methods

2.1. Animals

A total of 192 male Sprague–Dawley rats, half adolescent (postnatal [PD] 30–32) and half adult (PD 65–70) at the beginning of treatment, were purchased from Charles River Laboratories (Raleigh, NC) and housed in groups on a 12-h light–dark cycle with ad libitum access to food and water. At least four days were allowed to pass after delivery to the vivarium prior to the initiation of any experimental procedures, all of which were conducted in accordance with IACUC guidelines and were approved by the institution. “Principles of Laboratory Animal Care” were followed as well as pertinent U.S. laws. In all experiments, the body weights of the animals were monitored, and we observed no significant differences between animals in the control groups compared to the THC treatment groups within each age range.

2.2. THC treatment

Δ⁹-THC dissolved in ethanol, was obtained from NIDA and prepared in the vehicle containing 10% ethanol : emulphor [Rhodia; Cranbury, NJ] and 90% saline for a final concentration of 5 mg/ml. The 1 : 1 : 18 vehicle (ethanol : emulphor : saline) has a long history of use in many laboratories for the solubilization of cannabinoids. The ethanol concentration in the THC solution and in the vehicle is less than 10%, resulting in ethanol doses of approximately 0.05–0.15 g/kg.

2.2.1. Acute

Experiments 1 and 2 were designed to assess the acute effects of THC on spatial learning. In Experiment 1, 16 male adolescent rats and 16 male adult rats received intraperitoneal (i.p.) injections of either 5.0 mg/kg THC or an equal volume of the control vehicle solution. This dose was chosen for our initial study because it had been shown to cause memory impairments in other learning paradigms (Fadda et al., 2004). Thirty minutes after the injection, animals began training on the spatial task in the Morris water maze. Based on the results of the first experiment, Experiment 2 was designed to assess the age-dependent effects of a lower dose (2.5 mg/kg) and a higher dose (10.0 mg/kg) on spatial learning, as well as on non-spatial learning in the water maze. Sixty adolescent rats and 60 adult rats were randomly assigned to groups for training on either the spatial or the non-spatial task in the water maze. Thirty minutes prior to each training session, subjects received an i.p. injection of the control vehicle solution (n = 10), 2.5 mg/kg THC (n = 10), or 10.0 mg/kg THC (n = 10). Thus, animals received either drug or vehicle on every day in which maze training occurred.

2.2.2. Chronic

In Experiment 3, 20 male adolescent and 20 male adult rats were used to assess the long-lasting effects of chronic THC treatment on spatial learning. The rats were given i.p. injections of either the control vehicle solution (n = 10 in each age group) or 5.0 mg/kg THC (n = 10 in each age group) once daily for 21 days. This dose was chosen based on data from the acute study above, and previous, unpublished, data from our laboratory. Twenty-eight days after the last injection, the rats were trained, drug-free, on the spatial task in the Morris water maze. Forty-eight hours after the completion of the spatial task, the same rats were also trained on the non-spatial version of the water maze task. We used the 28-day post-treatment interval prior to behavioral testing in order to allow for full clearance of the THC and its metabolites, and to allow the animals treated as adolescents to age fully into adulthood.

2.3. Water maze

2.3.1. Spatial learning

The effects of THC on spatial learning were assessed using the Morris water maze. The water maze was a galvanized steel tank, 1.7 m in diameter, and 0.31 m in height. It was filled with water maintained at 22 °C. The water was made opaque using non-toxic, white tempera paint. Since the entire room supplied the visual cues, great care was taken not to disturb any objects in the room, and the experimenters maintained a consistent appearance and posture throughout the trials. The tank was divided into quadrants, and one quadrant was selected to be the goal quadrant for all animals throughout the spatial learning phase of the study. A Plexiglas platform (10 × 10 cm) was placed two cm below the surface of the water in the center of the goal quadrant. Each animal completed four trials per day on five consecutive days. Each trial began from one of four start locations. The order of start locations was the same for all rats during a given day, but the order was counterbalanced across
days. During each trial, the rat was placed in the water facing the wall of the maze. Subjects were given 60 s to locate the platform. If the platform was found, the subject was allowed to stay on top of it for a period of 10 s before being removed from the maze. If the subject failed to locate the platform, it was gently guided to the platform and allowed to remain on top of it for 10 s. In between trials, subjects were placed in a holding container for one minute. In all water maze experiments, the distance traveled prior to reaching the goal platform was used as the primary dependent measure.

2.3.2. Non-spatial learning

In this variation of the water maze task, the platform was raised above the surface of the water so that it was visible. In contrast to the spatial task, in which the platform location remained constant but the start positions varied, the non-spatial task changed the platform’s location from trial to trial while the start location remained constant. This procedure allowed the animal to see the platform and thus rely specifically on this visual cue, in various locations, for learning. Thus, this version of the water maze task required the animal to learn to go to the platform, rather than to learn to go to a specific location. Rats were given four trials per day for five days. The distance traveled prior to reaching the goal platform was used as the primary dependent measure.

2.4. Statistical analyses

A camera mounted above the maze and Poly-Track tracking software (San Diego Instruments; San Diego, CA) was used to capture and analyze the performance of each subject during each trial. The time to reach the goal platform and distance traveled were the primary dependent measures. Data were analyzed using two-way (age×dose) ANOVAs with repeated
Fig. 3. Representative swim traces from the second trial of Day 1 and the second trial of Day 5 in adolescent and adult animals treated with vehicle, 2.5 mg/kg THC, 10 mg/kg THC 30 min prior to behavioral testing. Each pair of swim traces from Day 1 and Day 5 is from the same animal. The black square represents the escape platform, and the number below each tracing is the number of centimeters traveled during the trial. Tracings were selected from animals whose performance was near to the mean for their treatment group, in all instances within one standard error.

Fig. 4. Mean (±SEM) distance swam to reach the visible goal platform on each of the five test days by adolescent (Panel a) and adult (Panel b) rats after acute exposure to vehicle (open circles), 2.5 mg/kg THC (closed squares), or 10.0 mg/kg THC (closed triangles) prior to water maze training. * Indicates significantly different from vehicle; *p* < 0.05. # Indicates both THC doses are significantly different from vehicle; *p* < 0.05. N=10 per group.
measures. Post-hoc, Fisher’s Least Significant Difference (LSD) tests were applied when appropriate.

3. Results

3.1. Acute treatment: experiment 1

This experiment was run initially to determine an effective dose for differentiating adolescent and adult animals. Due to differences in the performance of control animals between Experiments 1 and 2, these two sets of data could not be combined for the statistical analyses. Instead, data from Experiments 1 and 2 were analyzed separately. In both experiments, there was no effect of Age or Treatment on swim speed (data not shown). Across the five-day testing period, the performance of adult animals treated with THC improved rapidly, as did that of the adult control animals. In contrast, although the adolescent THC-treated rats also showed improved performance across days, they never reached the level of performance of their age-matched controls (Fig. 1a). In contrast to the significant effect of THC on spatial learning in adolescent animals ($F_{1,14} = 17.46, p = 0.001$), THC did not have a significant effect on spatial learning in adults (Fig. 1b), though a trend toward such an effect was present ($F_{1,19} = 3.75, p = 0.07$).

3.2. Acute treatment: experiment 2

Overall, THC impaired spatial learning across the five-day testing period ($F_{2,54} = 25.2, p < 0.001$). In addition, there was a significant Age × Treatment interaction among animals treated with 10.0 mg/kg THC ($F_{1,35} = 4.5, p = 0.04$). Post-hoc analyses revealed that THC impaired learning more in adolescent rats than in adults at both 2.5 mg/kg ($F_{1,18} = 6.16, p = 0.023$) and 10.0 mg/kg ($F_{1,18} = 10.17, p = 0.005$), although the performance of the adolescent and adult control rats did not differ significantly (Figs. 2 and 3).

Similarly, THC impaired non-spatial learning across the five-day testing period ($F_{2,48} = 18.95; p < 0.001$). There was no overall effect of Age on non-spatial learning. However, there was a significant Age × Treatment interaction ($F_{2,48} = 15.956, p < 0.001$). Post-hoc analyses indicated that, at both doses, THC impaired non-spatial learning more powerfully in adolescent, compared to adult rats (2.5 mg/kg — $F_{1,19} = 78.5, p < 0.009$; 10.0 mg/kg — $F_{1,19} = 295.2, p < 0.009$). There were also no differences in the performance of adolescent and adult rats given vehicle in either the spatial or non-spatial tasks.

3.3. Chronic treatment

Fig. 5 shows the performance in the spatial learning task after chronic pre-exposure to THC during either adolescence or adulthood. There were no overall effects of Age ($F_{1,31} = 0.13, p = 0.91$) or Treatment ($F_{1,31} = 0.95, p = 0.76$) on performance in the spatial task. There were also no overall effects of Age ($F_{1,32} = 2.54, p = 0.121$) or Treatment ($F_{1,32} = 1.11, p = 0.301$) in the non-spatial task (data not shown).

4. Discussion

The main finding of this study is that acute treatment with THC inhibited both spatial and non-spatial learning in the water maze more powerfully in male adolescent rats than in male adults. However, chronic THC treatment, either during adolescence or adulthood, had no effect on subsequent learning four weeks after the termination of THC exposure.

The effects of acute THC on learning are well known, but the developmental sensitivity to these effects has not been described much previously despite an emerging literature on adolescent psychopharmacology (see Spear, 2000). An early study noted THC-induced differences in the performance of young and old animals on the rotating rod apparatus (Barnes and Fried, 1974). Previous studies have shown that THC impairs learning in both operant (Heyser et al., 1993; Mallet and Beninger, 1998) and maze (Da and Takahashi, 2002; Lichtman et al., 1995; Nakamura et al., 1991;) tasks in adult rats, and that
the spatial learning deficit can be prevented with a CB1 cannabinoid receptor antagonist ([Da and Takahashi, 2002]). The present finding, that THC impairs both spatial and non-spatial learning in the water maze more in adolescent rats than adults, indicates developmental sensitivity to the effects of THC on learning. While the effects of THC on spatial learning may reflect changes in hippocampal function (see Lichtman et al., 1995), the fact that we also observed developmentally-mediated effects on non-spatial learning suggests that other brain regions may also be differentially affected by THC in adolescents compared to adults. The developmental sensitivity to the effects of THC on spatial learning is analogous to the effects of ethanol, which attenuates spatial memory acquisition more in adolescent rats than in adults (Markwiese et al., 1998). However, ethanol did not inhibit the acquisition of non-spatial learning in an age-dependent way (Markwiese et al., 1998). This suggests that while the developmental sensitivity to the effects of ethanol on learning may be mediated by greater potency against memory-related hippocampal functions (Swartzwelder et al., 1995a,b; Pyapali et al., 1999), the developmental sensitivity to the effects of THC on learning may be mediated by more diffuse mechanisms.

Although the precise mediating mechanisms for these THC effects on learning are not certain, cannabinoid inhibition of glutamate release represents one possibility. Cannabinoids act presynaptically to inhibit the release of several transmitters in the hippocampus including GABA, norepinephrine, acetylcholine and glutamate (Katona et al., 1999; Shen et al., 1996; Sullivan, 2000). Through this action, cannabinoids can decrease postsynaptic depolarization sufficiently to prevent the relief of $\text{Mg}^{2+}$ blockade of NMDA receptors that is necessary for LTP and learning to occur (Misner and Sullivan, 1999). Long-term depression of GABA release from inhibitory interneurons represents an alternative glutamate-dependent cannabinoid-mediated form of plasticity that could mediate enhanced cannabinoid effects on memory in adolescents (Chevaleyre and Castillo, 2003). Further study of activation of critical glutamate pathways may provide some insight into the developmentally-mediated effect of THC on learning.

An alternative hypothesis is that tolerance to THC is less robust in adolescents than in adult rats. In the present experiments, THC produced comparable performance deficits on Day 1 of training in both adolescent and adult animals. However, at least in the spatial task, the adult animals learned the task to the extent that their performance was comparable to controls by the end of testing, whereas the performance of the adolescents remained consistently worse than their controls. It could be that the adult rats simply became tolerant to the amnesic effects of THC, thus allowing them to learn the task by the end of the training sequence. Adolescent rats have been shown to become “tolerant” to the disruptive effects of THC in the rotating rod apparatus faster than adults, which suggests a possible age difference in THC tolerance development (Barnes and Fried, 1974). However, if the adolescent animals had become more rapidly tolerant in our studies, one would expect them to have been less, rather than more, affected than the adults in the final days of the water maze sessions. Nonetheless, though we favor the hypothesis that memory-related neural structures are more physiologically compromised in adolescents than in adults, it is possible that differential tolerance may contribute to the observed developmental differences.

Similarly, we cannot completely rule out the possibility that THC interfered with abilities unrelated to learning. For example, a developmentally different effect on visual function or on motivation in negative reinforcement tasks, like the water maze task, could account for the observed deficits, as could a metabolic difference between adolescent and adult rats. The possibility of a motivational difference seems unlikely since the adolescent and adult rats did not differ with respect to swim speed in the maze. Although developmentally different effects of visual function are plausible, we are not aware of any studies demonstrating such an effect. Neither are we aware of any studies to indicate a difference in THC metabolism between adolescent and adult rats. Given the accumulating literature regarding the effects of THC on learning and memory, we are more inclined to interpret the performance deficits observed in this study as related to deficits in learning.

Another issue that is not addressed in the present experiments is the possible interaction of sex with development in determining the effects of THC on learning. We used male rats only in this study specifically to avoid the potential confound of the onset of the estrus cycle during the THC exposure periods. However, future studies will address such possible sex differences, and we have preliminary data which suggest that both adolescent and adult female rats may be more sensitive to the acute effects of THC on spatial learning in the water maze.

If the adolescent brain is more sensitive to the acute effects of THC, it could be that adolescence represents a period during which an individual is more vulnerable to neural changes induced by THC, which could result in long-lasting or even permanent deficits in cognitive functioning. This remains an open question. However, our results indicate that there is not a long-lasting impairment of water maze performance, regardless of whether the THC exposure had occurred during adolescence or adulthood. The lack of a long-lasting impairment in adults is consistent with early reports that used even higher doses of THC (Stiglick and Kalant, 1983, 1985).

The present findings contrast with a recent report showing that chronic THC exposure in rats during adolescence, but not adulthood, results in impaired subsequent object recognition learning and changes in progressive ratio operant performance (Schneider and Koch, 2003). However, the absence of an effect of chronic THC exposure on subsequent water maze learning does not rule out the possibility that it induced more subtle deficits that could be “unmasked” under certain conditions. In a previous study of the effects of chronic ethanol exposure on subsequent spatial learning (White et al., 2000), we challenged the animals with a single dose of ethanol and found that those that had been exposed to ethanol as adolescents were more sensitive to acute ethanol-induced learning impairment. We did not conduct such an experiment in the present series, so it is possible that prior THC treatment could result in a greater subsequent sensitivity to THC, or other drug, effects on learning. In this regard, it is also...
important to recognize that no behavioral technique can assess all aspects of learning. Therefore, THC could be expected to affect performance on some indices and not others. One possibility would be that while there was no effect of THC pre-treatment on acquisition in the water maze, an index of retention, such as a probe trial, may have revealed more subtle deficits. The literature on human THC exposure does suggest that adolescence may be a vulnerable period for producing enduring cognitive deficits. Some studies have identified cognitive deficits in marijuana users who began use during adolescence but not in those whose use began in adulthood (Ehrenreich et al., 1999; Jacobsen et al., 2004; Pope et al., 2001). However, these studies lack the level of experimental control that would be required to draw causal conclusions. Finally, it is possible that increased THC exposure or a different, possibly longer, treatment paradigm may be necessary to cause measurable, long-lasting learning deficits.

Others have found learning impairments at the same dose used here (although for a longer period of time) through use of the 8-arm radial maze task (Nakamura et al., 1991), and by treating animals for six months at 20 mg/kg and testing them in the Hebb–Williams closed-field maze (Fehr et al., 1976). The increase in cannabinoid receptors that occurs between birth and postnatal day 60 in rats (Belue et al., 1995) could indicate that the long-term effects of chronic exposures during development may depend upon critical periods within this interval.

The present results address a question of pressing relevance to adolescent drug treatment, public policy, education, and law: is adolescence a period of heightened sensitivity to the neurobehavioral effects of THC? The data suggest that the answer is “yes.” However, this study addresses only two learning tasks, and indicates that though acute impairment on these tasks is greater in adolescents, persistent effects are not observed after a period of abstinence. More research will be needed to determine which cognitive and behavioral functions, and which developmental periods are most vulnerable to the effects of THC. Future studies must also address possible developmental differences in the sensitivity of learning-related neurophysiological measures to THC and other cannabinoid agents as well as the effects of chronic cannabinoid treatment on other components of cognition, such as executive functioning.

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