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Delay- and Dose-Dependent Effects of Δ^9-Tetrahydrocannabinol Administration on Spatial and Object Working Memory Tasks in Adolescent Rhesus Monkeys

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Among adolescents, the perception that cannabis can cause harm has decreased and use has increased. However, in rodents, cannabinoid administration during adolescence induces working memory (WM) deficits that are more severe than if the same exposure occurs during adulthood. As both object and spatial WM mature in a protracted manner, although apparently along different trajectories, adolescent cannabis users may be more susceptible to impairments in one type of WM. Here, we evaluate the acute effects of a range of doses (30–240 μg/kg) of intravenous Δ^9-tetrahydrocannabinol (THC) administration on the performance of spatial and object WM tasks in adolescent rhesus monkeys. Accuracy on the object WM task was not significantly affected by any dose of THC. In contrast, THC administration impaired accuracy on the spatial WM task in a delay- and dose-dependent manner. Importantly, the THC-induced spatial WM deficits were not because of motor or motivational impairments. These data support the idea that immature cognitive functions are more sensitive to the acute effects of THC.

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INTRODUCTION

Administration of the cannabinoid 1 receptor partial agonist Δ^9-tetrahydrocannabinol (THC), the primary psychoactive molecule in cannabis (*cannabis sativa*), transiently impairs working memory (WM) performance in adults (Ranganathan and D'Souza, 2006). The ability to perform WM tasks, and the patterns of prefrontal cortical (PFC) activity that support spatial WM, progressively matures across adolescence (Luna *et al*, 2010; Crone *et al*, 2006). These findings suggest that adolescents, who constitute an increasing proportion of cannabis users (SAMHSA, 2010), may be particularly susceptible to cannabis-induced WM deficits. Indeed, cannabis use before age 17 has been associated with more severe cognitive deficits (Ehrenreich *et al*, 1999; Pope *et al*, 2003) and smaller cortical gray matter volumes (Wilson *et al*, 2000) than cannabis use during adulthood. Consistent with these observations, cannabinoid administration causes more profound effects on WM in peripubertal than in adult rats (Cha *et al*, 2006; Quinn *et al*, 2008), and the effects of THC on WM are evident at doses that do not impair performance on other cognitive or motor tasks (Varvel *et al*, 2001; Jentsch *et al*, 1997). Collectively, these results suggest that immature cognitive abilities, and especially WM, are particularly sensitive to the effects of exogenous cannabinoids.

Tasks that involve WM require the circuitry of the PFC, with spatial WM tasks preferentially activating the dorsal PFC and object WM tasks preferentially activating the ventral PFC (Courtney *et al*, 1996; Wilson *et al*, 1993). Interestingly, adult levels of performance are achieved later in development for dorsal PFC-dependent than ventral PFC-dependent tasks (Distler *et al*, 1996; Conklin *et al*, 2007), consistent with findings that developmental refinements in white matter structure (Reiss *et al*, 1996) and cortical gray matter volume (Gogtay *et al*, 2004) are more prominent in dorsal than ventral PFC regions during adolescence. In concert, these findings suggest that cannabis use during adolescence may be more likely to impair spatial than object WM.

However, directly testing this hypothesis in humans is limited by potential confounding factors such as premorbid

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differences in cognitive abilities; the type, dose, pattern, and duration of cannabis use; and comorbid use of other drugs. Although rodent studies are informative, monkey models of cognitive functions are more robust reflections of human performance (Murray and Wise, 2010). In addition, like humans, macaques have an extended period of cognitive development (Watts and Gavan, 1982) characterized by protracted refinements in PFC circuitry (Lewis, 1997) and an increasing engagement of dorsal PFC circuitry in the mediation of spatial WM task performance (Goldman and Alexander, 1977). Thus, rhesus monkeys are well suited for experimental assessments of the effects of cannabis exposure during adolescence on WM function.

Studies of the effects of THC administration on WM performance in adolescent monkeys would ideally employ doses of THC that are most likely to mimic the amount of THC ingested by cannabis-using adolescents. However, idiosyncratic smoking techniques and variation in THC content across sources of cannabis (Chiang and Rapaka, 1987; Adams and Martin, 1996) make it difficult to estimate the actual dose of THC consumed by adolescents. On the other hand, intravenous administration reduces the inter- and intra-individual variability in plasma THC levels associated with smoking (Azorlosa *et al*, 1992), and both the peak and time course of plasma THC levels are comparable following smoking of a cannabis cigarette or intravenous injection of THC (Agurell *et al*, 1986). In fact, acute intravenous doses of THC that disrupt performance of a WM task in adult rhesus monkeys (Schulze *et al*, 1988) are comparable to the total doses of THC self-administered by squirrel monkeys (Tanda *et al*, 2000; Justinova *et al*, 2003) and within the range of doses that impair WM function of adult humans in a controlled setting (Volkow *et al*, 1996; D'Souza *et al*, 2004).

Consequently, we conducted a dose-response study of the acute effects of intravenously administered THC on spatial and object WM performance in adolescent rhesus monkeys. The range of THC doses included amounts known to impair WM function in adult humans and monkeys. We sought to determine if acute THC administration (1) differentially affects performance accuracy on spatial relative to object WM tasks during a developmental stage when only spatial WM performance accuracy is still maturing, and (2) similarly affects non-mnemonic measures of task performance that are shared by spatial and object WM tasks. Specifically, we predicted that low doses of THC would not affect performance on either task, intermediate doses would impair spatial but not object WM performance, and higher doses would impair non-mnemonic performance measures on both tasks.

SUBJECTS AND METHODS

Animals

Experimentally naïve, Chinese origin male rhesus monkeys (*Macaca mulatta*), 14–18 months of age, were obtained as a single cohort ($n = 14$). Before shipment to the University of Pittsburgh, animals were bred and housed at Covance Research Products (Alice, TX). All monkeys had different dams and, with the exception of two animals, different sires. Animals were weaned at 6–8 months of age and subsequently 5 were housed in corral or field cage production units, containing 3–5 males, 30–40 females and their offspring, and the other 9 were housed in cribs containing 1 male, 7–10 females and their offspring. For at least 3 months before shipment, all 14 monkeys were in crib housing units. Upon arrival at the University of Pittsburgh, animals were singly housed in the same room with environmental enrichment. Lights were on in the housing room from 0600 to 1800 hours and the animals were fed at ~0800 hours. Housing and experimental procedures were conducted in accordance with United States Department of Agriculture and National Institutes of Health guidelines and with approval of the University of Pittsburgh's Institutional Animal Care and Use Committee. At 23.9 ± 0.6 months of age, animals were trained to respond to computerized touch-screens as previously described (Verrico *et al*, 2011). See Supplementary Materials and Methods for details.

Spatial Delayed Response (SDR) Task

The SDR task, which had trials with one reinforcement condition (SDR-1x), was a location-recall WM test. To respond accurately to the WM trials, monkeys had to form, maintain, and recall a spatial location-matching rule. To control for non-mnemonic effects, we included control trials that had the same stimulus sequence, cue locations, delay durations, and motor requirements as the WM trials; however, only a single choice probe appeared at the location occupied by the sample stimulus earlier in the trial. Thus, for the SDR control trials, monkeys were required to touch the single choice stimulus to earn a reward.

There were four delay periods (1, 4, 8, or 16 s) for the SDR task, which was designed to have 32 WM trials (8 per delay) and 8 control trials (2 per delay) per run. The resulting 40 trials were divided into two 20-trial blocks and within each block, all delays and both WM and control trials were randomly interwoven. Monkeys were allowed 20 s to respond to the sample stimulus and 20 s to respond to a choice stimulus. If a monkey failed to respond during the allotted times, the trial was recorded as an omission. Figure 1a provides a schematic for the SDR task, which is described in detail in the Supplementary Materials.

Delayed Match to Sample (DMTS) Task

The DMTS task was an object-recall WM test. To respond accurately to the WM trials, monkeys had to form, maintain, and recall color- and shape-matching rules. To ensure that the SDR measures were not confounded by floor effects that might be induced by the partial satiation associated with water reinforcement, the SDR task was always performed before the DMTS task. In an effort to offset a potential order effect, the DMTS task had two stimulus sets, which were used trial-to-trial and day-to-day; one stimulus set indicated trials that had the same reinforcement condition as the SDR trials (DMTS-1x) while the other stimulus set indicated trials that had twice the water reward as the SDR trials (DMTS-2x). As shown in Table 1, this manipulation resulted in baseline performance on the DMTS-2x trials that was greater than on DMTS-1x trials and comparable to SDR-1x trials. To control for non-mnemonic effects, we included control trials that had the

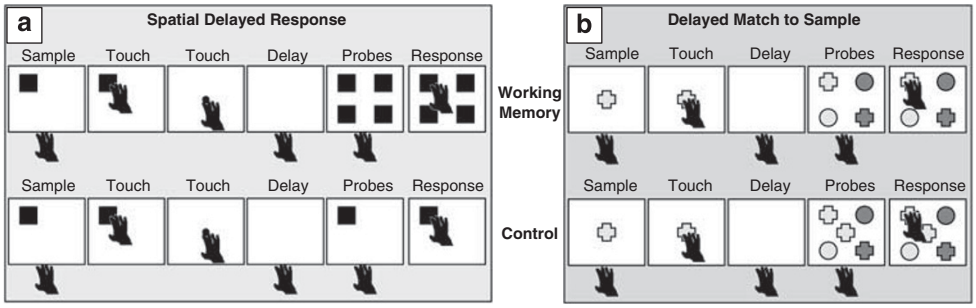

Figure 1 The spatial and object working memory (WM) tasks. (a) Schematic illustration of a SDR WM and control trial. WM (top left): a sample appeared at one of the four corners of the touch screen. The monkey had to touch it. Immediately following this response, a fixation cue stimulus appeared at the center of the screen. The monkey had to touch the fixation cue stimulus and thus could not remember the target location by continuing to touch it. A randomly selected delay ensued. At the end of the delay period, choice probes appeared at each corner of the screen. The monkey had to touch the probe at the location occupied by the sample stimulus earlier in the trial. Control trials (bottom left) were distinguished by the appearance of a single choice probe at the same location that the sample stimulus occupied earlier in the trial. (b) Schematic illustration of a DMTS WM and control trial. WM (top right): a sample appeared at the center of the touch screen. The monkey had to touch it. A randomly selected delay followed. At the end of the delay period, choice probes appeared at the corners of the screen, distinct in color and shape. The monkey had to touch the probe that matched the sample stimulus earlier in the trial. Control trials (bottom right) were distinguished by the reappearance of the sample stimulus at the center of the screen.

Table 1 Baseline Performance on the Working Memory Trials

Group	Pair	SDR-1x	DMTS-2x	DMTS-1x
THC	1	38%	46%	38%
	2	52%	48%	30%
	3	64%	43%	34%
	4	65%	36%	45%
	5	52%	45%	36%
	6	33%	41%	37%
	7	40%	35%	28%
Vehicle	1	51%	47%	33%
	2	52%	42%	37%
	3	40%	42%	35%
	4	44%	49%	48%
	5	63%	40%	38%
	6	33%	48%	48%
	7	41%	41%	33%
Means ± SD		48% ± 11%	43% ± 4%	37% ± 6%[a]
Repeated-measures ANOVA		$F_{(2, 26)} = 6.00$, $p = 0.0072$		

[a]Different from SDR-1x at $p < 0.05$.

Performance accuracy (percent correct across all delays) on each task and both reinforcement conditions for each monkey during the final week of baseline training. Performance accuracy here was defined as the number of correct trials divided by the total number of trials (ie, correct, incorrect and omitted WM trials). A *post hoc* repeated-measures ANOVA on the WM accuracy rates revealed significant differences between SDR-1x and DMTS-1x trials.

sample stimulus reappear at the center of the screen during the choice phase. Thus, for the DMTS control trials, monkeys were required to touch the choice probe that matched the simultaneously presented sample stimulus to earn a reward.

There were four delay periods (1, 4, 8, or 16 s) for the DMTS task, which was designed to have 6 trials per delay for the DMTS-1x condition and 6 trials per delay for the DMTS-2x condition, for a total of 48 WM trials, and 16 control trials (2 per delay per stimulus set) per run. The resulting 64 trials were divided into two 32-trial blocks and within each block, all delays, both reinforcement conditions and both WM and control trials were randomly interwoven. Monkeys were allowed 20 s to respond to the sample stimulus and 20 s to respond to a choice stimulus. If a monkey failed to respond during the allotted times, the trial was recorded as an omission. Figure 1b provides a schematic for the DMTS task, which is described in detail in the Supplementary Materials.

Pairings and Group (THC or Vehicle) Assignments

At an average age of 27.6 ± 1.1 months (range 26.4–30.0 months) and before the THC/vehicle administration, monkeys completed 4–5 weeks of baseline training on the SDR and DMTS tasks as previously described (Verrico *et al*, 2011). Importantly, monkeys performed both tasks in two sessions per day, spaced 30 min apart, ~5 days per week. See Supplementary Materials and Methods for additional details. In order to insure that baseline performance was comparable between the groups, a paired design was used where assignments to a group were counterbalanced such that THC was administered to the animal with the poorer performance in the first pair and the better performance in the second pair, and so on. As expected from this design, mean baseline performance accuracy on the SDR-1x ($t_{(12)} = 0.47$, $p = 0.64$), DMTS-2x ($t_{(12)} = -0.94$, $p = 0.36$), and DMTS-1x ($t_{(12)} = -1.04$, $p = 0.32$) trials did not differ between the vehicle and THC groups (Table 1).

Design of the Behavioral Sessions and THC/Vehicle Administrations

At least 2 months before beginning THC/vehicle administrations, a polyethylene catheter was inserted into the jugular vein, tunneled subcutaneously, and attached to a vascular access port (Access Technologies; Skokie, IL) at the center of the animal's back (Wojnicki *et al*, 1994). Monkeys resumed training following a 10-day recovery period.

THC/vehicle exposure was initiated at an average age of 28.6 ± 1.2 months (range 27.4–31.1 months), an age when dependence of spatial WM ability on dorsal PFC circuitry is still developing (Alexander and Goldman, 1978). THC, provided by the NIDA Drug Supply Program, and vehicle (~50 µl Tween 80 dissolved in saline) were prepared as previously described (Verrico *et al*, 2004). Each day (Monday–Friday), both monkeys in a pair simultaneously completed both the SDR and DMTS tasks during the first (run-1) of the two daily behavioral test sessions. Immediately following run-1, THC or vehicle was administered via the vascular access port before the pair of monkeys was returned to their home cages. 30 min later, the pair of monkeys simultaneously completed the second test session (run-2). The 30-min interval between THC/vehicle administration and testing on the WM tasks was selected based on the pharmacokinetics of THC in plasma after similar exposures in male rhesus monkeys (Slikker *et al*, 1991; Paule *et al*, 1987; Schulze *et al*, 1989), the pharmacodynamics of THC associated with the clinical 'high' (Agurell *et al*, 1986; Lindgren *et al*, 1981; Ohlsson *et al*, 1980), and the time course of THC-induced cognitive impairments in humans following intravenous administration (D'Souza *et al*, 2004).

In general, monkeys received escalating doses of THC (or a comparable volume of vehicle); an ascending sequence was chosen to minimize the possibility of adverse side effects (eg, anxiety in the testing chambers). However, two monkeys received THC doses with a few differences from the ascending order. Preliminary analyses revealed that the results from all seven pairs of monkeys were indistinguishable from the five pairs with ascending doses. Thus, the combined data from all seven pairs are presented.

All seven monkeys in the THC group received doses of 30, 60, 120, and 180 µg/kg; five animals also received 240 µg/kg. Each dose of THC/vehicle was administered for 3 to 5 days during a single week, with at least 23 h between drug administrations and ~72 h (the approximate elimination half-life of THC; Johansson and Halldin, 1989) between each change in dose. See Supplementary Materials and Methods for additional details.

Data Analysis

For both run-1 and run-2, six measures were determined for each task: (1) WM accuracy rates, (2) control accuracy rates, (3) completion rates on all trials, (4) initiation latencies on all trials, (5) WM reaction latencies, and (6) control reaction latencies. Accuracy is the proportion of correct trials among the completed trials for a given delay; completion rate is the proportion of completed trials among presented trials; and latency is the time difference between the appearance of the sample (initiation) or choice (reaction) stimuli and the responses to those stimuli. Preliminary analyses indicated no significant day-to-day differences within a week for any measure, so a suitably obtained single weekly value for each measure was used.

To ensure that any effects of THC were not confounded by floor effects, primary analyses were conducted on a corrected data set (see Supplementary Materials and Methods) whose appropriateness was validated by comparisons with analyses of the full data set. Except where indicated, reported analyses are based on the corrected data set.

Methodology to analyze performance of vehicle-exposed monkeys is described in the Supplementary Materials and Methods.

To assess the effects of THC administration relative to vehicle administration, statistical modeling and analyses were done separately within delay for each task; this approach provides more readily interpretable results than an all-encompassing single model including dose, group, delay, task, and the pertinent interactions. To account for the repeated doses within a monkey for each delay and task, monkey was treated as a random normal effect while all other effects were treated as fixed effects. Exploratory analyses and graphics indicated the assumption of a linear dose-response effect for each group (THC and vehicle) was appropriate.

For each task, the percent change from run-1 to run-2 was determined for each animal on all six performance measures, and the difference between THC and vehicle groups was estimated based on the appropriate linear model. As the effects of THC were expected to be most evident at longer delay intervals and higher doses, the analyses for each measure began with the longest delay and a step-down multiple comparison procedure was used to control the family-wise error rate for this delay across doses, starting with the highest dose (see Supplementary Materials and Methods).

RESULTS

General Observations

In the vehicle group, there were notable differences between run-1 and run-2 performance measures for each task. Specifically, the percentage change in WM accuracy rates between run-1 and run-2 significantly ($F_{3,297} = 3.36$, $p = 0.019$) declined as the delay interval increased even in the absence of THC exposure. Across all tasks, the percentage change in between-run performance significantly differed between the 16 s and each of the 1 s (13.7%, $p = 0.003$), 4 s (12.3%, $p = 0.007$), and 8 s (9.7%, $p = 0.037$) delay trials. This effect of run on accuracy also differed ($F_{2,298} = 3.19$, $p = 0.042$) as a function of task. For example, DMTS-1x trials significantly differed between runs relative to SDR-1x trials (9.4%, $p = 0.012$). This difference might reflect a lower motivation to perform DMTS-1x trials during run-2 because the DMTS task was always performed after the SDR task. On the other hand, the percentage change in WM accuracy rates from run-1 to run-2 did not differ ($p = 0.200$) between SDR-1x and DMTS-2x trials, presumably because the higher level of reinforcement, and thus greater motivation, associated with DMTS-2x trials counteracted the partial satiation of thirst obtained from performing the SDR task.

Spatial Delayed Response (SDR-1x)

In order to assess the effects of THC dose and delay interval on WM performance, we estimated the percent change from

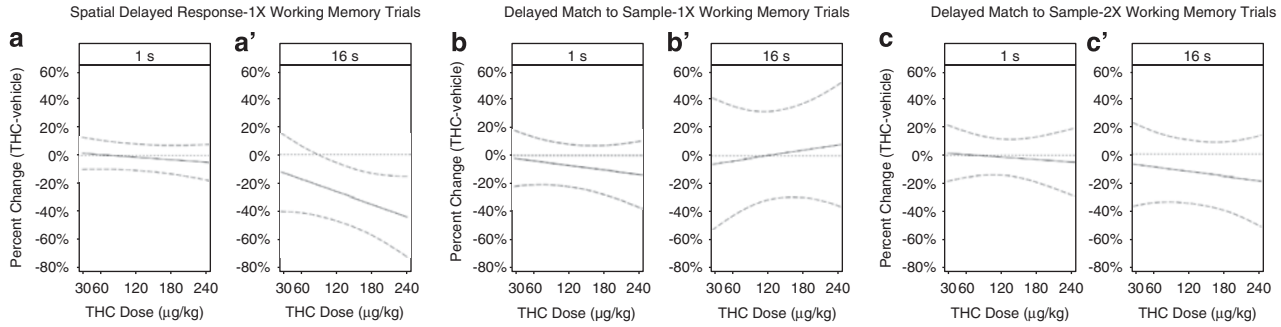

Figure 2 Spatial and object working memory (WM) accuracy. Linear model estimated dose-response curves (and 95% confidence intervals for the true differences) of the between-group (THC-vehicle) percentage change in WM accuracy for the SDR-1x (a, a′), DMTS-1x (b, b′) and DMTS-2x (c, c′) 1- and 16-s delays. Examination of the dose-response curves for SDR-1x trial accuracy rates indicates that the curve for the 16-s (a′) delay falls below the curve for the 1 s (a) delay. The graph for the 16-s delay also reveals a pronounced dose-response effect and the upper confidence limits are below 0 for the 120, 180, and 240 μg/kg doses. For both the DMTS-1x and DMTS-2x trials, the percentage change in WM accuracy rates for the 1- and 16-s delays appear similar, indicating little effect of THC on object WM with increasing delay or dose at either reward level. DMTS-1x indicates trials that had the same reinforcement as the SDR trials and DMTS-2x indicates trials that had twice the water reward as the SDR trials.

run-1 to run-2 for each group based on the linear model, and then compared the estimates between groups (ie, THC minus vehicle). The resulting value is subsequently referred to as the between-group percentage change for each measure.

Step-down testing of the percentage change in accuracy for 16-s delay trials revealed a progressive decline in spatial WM performance with increasing dose of THC (Figure 2a′); the between-group effects were significantly different following administration of the 240 (−43.9%, $p=0.004$), 180 (−34.9%, $p=0.004$), and 120 (−25.9%, $p=0.019$) μg/kg doses, but not for the 60 (−16.9%, $p=0.155$) or 30 (−12.3%, $p=0.365$) μg/kg doses of THC. Analysis of the full data set revealed the exact same pattern of THC-induced effects; the between-group percentage change in accuracy grew larger as the dose of THC increased (240 μg/kg (−34.2%; $p=0.044$), 180 μg/kg (−27.8%; $p=0.038$), and 120 μg/kg (−21.5%; $p=0.070$)). The greater level of impairment at each of these doses in the corrected relative to the full data set is consistent with the expectation that the full data set would be confounded by floor effects associated with chance performance levels before THC/vehicle administration (ie, run-1) for some weeks. In contrast to 16-s delay trials (Figure 2a′), analyses of the corrected (Figure 2a) and full data sets revealed that THC did not affect the performance of 1-s delay trials, at any dose. The between-group percentage change in accuracy on the SDR-1x control trials was not affected by any dose of THC, suggesting that THC did not affect the non-mnemonic components of task performance.

An effect of delay on WM performance was also evident from both data sets for each of the three highest THC doses. Graphical depiction of the model estimates for the difference in percentage change across delay intervals for the three highest doses of THC (Figure 3) illustrates that the between-group percentage change in WM accuracy progressively grew larger with increasing delays. This pattern indicates the deleterious impact of increasing THC doses across delays, especially the longer delays.

The between-group percentage change in completion rates and initiation latencies were collapsed across delay intervals, and step-down testing was conducted across THC

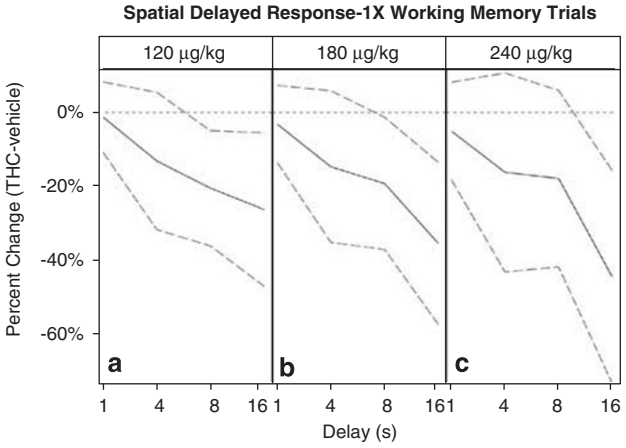

Figure 3 Delay- and dose-dependent effects of THC on spatial working memory (WM) accuracy. Linear model estimates (and 95% confidence intervals for the true differences) of the between-group (THC-vehicle) percentage change in SDR-1x WM trial accuracies plotted against all delays for the 120 (a), 180 (b), and 240 (c) μg/kg doses. Notably, the curve for the 240 μg/kg dose falls below the curve for the 180 μg/kg dose, which falls below the 120 μg/kg dose, showing the increasing negative effect of acute THC administration on spatial WM. Moreover, examination of the individual curves demonstrates that the acute effect of THC on spatial WM increased as the delay increased.

doses. The between-group percentage change in completion rates (Figure 4a) and initiation latencies (Figure 4a′) grew larger with increasing doses of THC; THC significantly decreased completion rates at the 240 (−19.4%, $p<0.001$) and 180 (−12.7%, $p=0.006$) μg/kg doses and significantly increased initiation latencies at the 240 (74.4%, $p=0.009$) and 180 (49.2%, $p=0.032$) μg/kg doses. Analysis of the full data set revealed exactly the same pattern, with completion rates most affected at the highest dose (−19.6%; $p<0.001$) and least affected at the lowest dose, and with initiation latencies most affected at the highest dose (71.5%; $p=0.011$) and least affected at the lowest dose. The between-group percentage change in response latencies on control and WM trials were not significantly affected by any dose of THC in either data set.

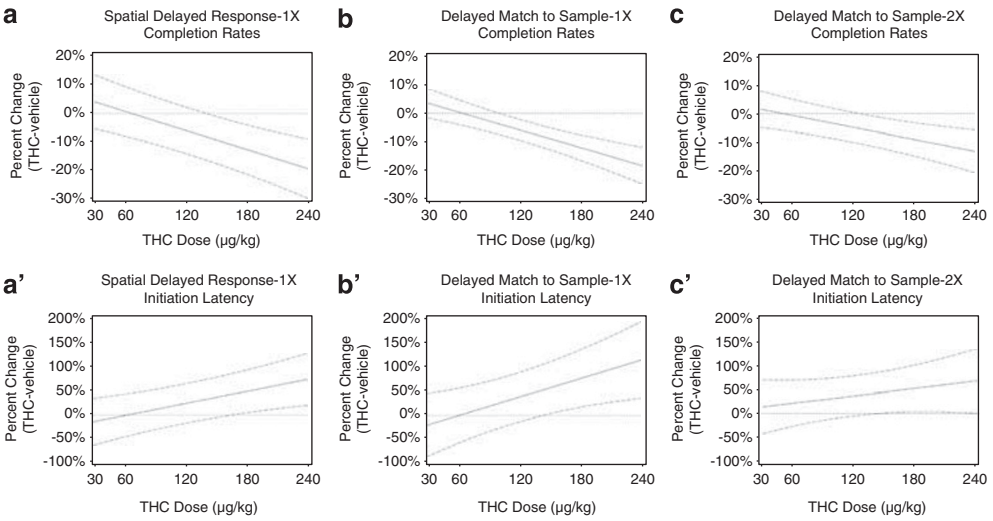

Figure 4 Effects of THC on non-mnemonic measures of both tasks. Linear model estimated dose-response curves (and 95% confidence intervals for the true differences) of the between-group (THC-vehicle) percentage change in completion rates for the SDR-1× (a), DMTS-1× (b), and DMTS-2× (c) trials and initiation latencies for the SDR-1× (a′), DMTS-1× (b′), and DMTS-2× (c′) trials along with the 95% point-wise confidence intervals for the true dose-response curves. The graphs for completion rates (collapsed across all delays) indicates that as the dose increased, completion rates decreased for the SDR-1× (a), DMTS-1× (b), and DMTS-2× (c) trials. Comparison of these dose-response curves indicates that the upper confidence limits are below 0 for DMTS-1× (b) and DMTS-2× (c) trials at the 120, 180, and 240 µg/kg doses. In contrast, the upper confidence limits are below 0 for SDR-1× (a) trials at the 180 and 240 µg/kg doses, but not the 120 µg/kg dose. The graphs for initiation latencies indicate that as the dose increased, initiation latencies increased for the SDR-1× (a′), DMTS-1× (b′), and DMTS-2× (c′) trials. Comparison of these dose-response curves indicates that the lower confidence limits are above 0 for SDR-1× (a′), DMTS-1× (b′), and DMTS-2× (c′) trials at the 180 and 240 µg/kg doses. DMTS-1× indicates trials that had the same reinforcement as the SDR trials and DMTS-2× indicates trials that had twice the water reward as the SDR trials.

Delayed Match to Sample

Delayed match to sample-1x. Step-down testing of the percentage change in DMTS-1x accuracy did not reveal significant between-group performance differences at any dose or delay (Figures 2b and b′), a marked divergence from the substantial dose- and delay-dependent effects observed on the SDR task. In contrast, the between-group percentage change in completion rates (Figure 4b) and initiation latencies (Figure 4b′) grew larger with increasing doses of THC; THC significantly decreased completion rates at the 240 (−18.1%, $p<0.001$), 180 (−11.9%, $p<0.001$), and 120 (−5.74%, $p=0.005$) µg/kg doses and significantly increased initiation latencies at the 240 (115.3%, $p=0.0006$), and 180 (76.5%, $p=0.014$) µg/kg doses. The between-group percentage change in response latencies on the control and WM trials and accuracy on the DMTS-1x control trials were not significantly affected by any dose of THC.

Delayed match to sample-2x. Similar to the percentage change in DMTS-1x accuracy, step-down testing of the percentage change in DMTS-2x accuracy did not reveal significant performance differences between groups at any dose or delay (Figures 2c and c′); at the 16-s delay, the percentage change of performance between groups was modestly affected at the highest dose (18.9%, $p=0.240$) and least affected at the lowest dose of THC. The between-group percentage change in completion rates (Figure 4c) and initiation latencies (Figure 4c′) grew larger with increasing doses of THC; THC significantly decreased completion rates at the 240 (−13.1%, $p<0.001$), 180 (−8.93%, $p<0.004$) µg/kg doses and significantly increased initiation latencies at the 240 (67.3%, $p=0.05$) and 180 (51.9%, $p=0.037$) µg/kg doses. The between-group percentage change in response latencies on the control and WM trials and accuracy on the DMTS-2x control trials were not significantly affected by any dose of THC.

DISCUSSION

The present dose-response study was designed to assess the impact of acute THC administration on WM performance in male rhesus monkeys during adolescence, when spatial but not object WM is reportedly still maturing. We found that intravenous administration of THC to adolescent rhesus monkeys (1) impairs spatial WM accuracy in a delay- and dose-dependent manner, (2) marginally affects object WM accuracy only at the highest dose (240 µg/kg) and longest delay interval (16 s) at the 2x, but not the 1x, reward level, and (3) shows similar dose-response effects on completion rates and initiation latencies on both tasks. These data support the hypothesis that immature cognitive abilities are particularly sensitive to the acute effects of THC.

THC Impairs Accuracy on the Spatial WM Task

We found that THC impaired spatial WM accuracy at doses that did not significantly impair object WM accuracy. However, because 240 µg/kg of THC reduced DMTS-2x accuracy by −18.9% at the 16-s delay, which trended toward significance ($p=0.240$), a larger sample size may well have revealed a significant effect. Nonetheless, the different effects of THC on spatial and object WM are particularly striking because the greater memory load associated with the DMTS task would likely have greater sensitivity to even

mild WM impairments. That is, in contrast to the single memorandum of the SDR task, the DMTS task required the monkeys to maintain both the shape and color features of an object, and doing so successfully would have required proactive inhibition of shape- or color-reward associations on some trials.

The acute effects of THC on the SDR task were observed during adolescence when the ability to perform spatial WM tasks is progressively improving and becoming increasingly dependent on dorsal PFC in rhesus monkeys (Alexander and Goldman, 1978). For example, temporarily disrupting dorsal PFC activity (by reversible cooling) while a monkey performs a spatial WM task does not significantly impair performance until monkeys are ~30 months of age (Goldman and Alexander, 1977) and the percentage of dorsal PFC neurons that are active during the delay period of a spatial WM task doubles between 12 and 36 months of age (Alexander, 1982). Although spatial and object WM have some overlapping representations in the lateral PFC (Rainer *et al*, 1998), electrophysiological and lesion studies have revealed that spatial WM preferentially activates dorsal PFC (Wilson *et al*, 1993; Goldman and Rosvold, 1970; Goldman *et al*, 1971), whereas object WM preferentially activates ventral PFC (Funahashi *et al*, 1989; Wilson *et al*, 1993; Mishkin and Manning, 1978; Jones and Mishkin, 1972). This neuroanatomical dissociation suggests that spatial and object WM may have different developmental trajectories in rhesus monkeys, as they do in humans (Distler *et al*, 1996; Conklin *et al*, 2007), with object WM reaching adult levels of performance earlier in development. Thus, our finding of a more potent effect of THC on spatial WM in adolescent monkeys supports the hypothesis that immature cognitive abilities are particularly vulnerable to the acute effects of THC.

THC Similarly Affects Non-Mnemonic Measures on Both WM Tasks

Control trial accuracy rates, reaction latencies on WM trials, and reaction latencies on control trials for both the SDR and DMTS tasks did not differ between the THC- and vehicle-exposed monkeys. The similarities between the groups on these measures suggest that THC, across all doses, did not impair the motor skills necessary to perform the tasks.

The two highest doses of THC (180 and 240 μg/kg) significantly reduced completion rates and increased initiation latencies on both tasks, suggesting that at these doses THC may have increased fatigue (sedation) and/or decreased motivation, relative to the vehicle group. However, these non-mnemonic effects appear to be independent of WM processes because performance accuracy on the DMTS-1x and DMTS-2x trials was unaffected (in both data sets) by the doses (120, 180, and 240 μg/kg) that impaired spatial WM. In fact, although the DMTS task should have had greater discriminating power than the SDR task because it was theoretically more difficult, object WM accuracy was not significantly affected at any dose. In addition, the non-mnemonic processes required to perform both of the WM tasks employed here were highly similar. For example, the spatial and object WM tasks were identical with respect to motivational demand (ie, thirst), reinforcer (ie, water), and motor requirements (ie, responding to stimuli on a touch screen). Thus, the differential deficit reported here is consistent with the selective spatial WM impairments observed in rodents (Jentsch *et al*, 1997; Varvel *et al*, 2001) and potentially meaningful because (1) the non-mnemonic requirements of both tasks were similarly affected; (2) accuracy rates of the THC and vehicle groups on the more difficult DMTS task were similar; and (3) the differences between the THC and vehicle groups were greater on the task with poorer discriminating power.

Performance accuracy on one task can reflect multiple sources of variance because accurate recall/retrieval of information is dependent on other, potentially independent, cognitive processes (ie, encoding and maintenance). However, the delay-dependent effects of THC on spatial WM in these adolescent monkeys suggest that the impairments resulted from changes in rates of forgetting (maintenance and/or retrieval processes) but not from changes in the encoding process. In fact, the design of the WM tasks employed here required the animals to touch the sample stimulus, which ensured that the animals attended to the sample stimulus. This fact, coupled with the finding that performance of the 1- and 4-s delay trials was not impaired at any dose, strongly suggests that the sample stimuli were perceived and converted (encoded) into a stored (maintained) construct, but that as the delay lengthened to 8 or 16 s, THC increasingly impaired the animal's ability to recall the sample stimulus that was most recently encoded. Indeed, this is consistent with the delay-dependent WM deficits observed in rodents (Heyser *et al*, 1993; Hampson and Deadwyler, 1999) and humans (D'Souza *et al*, 2004), which supports the idea that THC affects maintenance and/or retrieval, but not encoding, processes.

Thus, the hypothesis that the effect of THC on spatial WM in these adolescent monkeys was not because of motor, motivational, or encoding/attentional processes is supported by both the differential WM deficit observed across two tasks that rely on highly similar non-mnemonic processes, and the delay dependence of the spatial WM deficits, which is consistent with the specific mnemonic deficits observed in humans following cannabis exposure (Lane *et al*, 2005).

Potential Confounds Associated with the Study Design

The animals used in this study will also be included in a longitudinal study exploring the effects of long-term THC administration on age-related improvement in WM ability, thus the animals had only 4–5 weeks of baseline training before THC/vehicle administration (Verrico *et al*, 2011). This approach ensured that (1) the monkeys understood how to perform the tasks, (2) performance accuracy increased in a delay-dependent manner from the first to last week of baseline training, and (3) performance accuracy was well below ceiling levels. Thus, a stable level of performance was not established before THC/vehicle administration and, as expected, the performance of all animals was not significantly above chance at the longer delays before beginning THC/vehicle administration, which could have limited our ability to detect THC-induced impairments. Nonetheless, to mitigate possible floor effects on the primary measure of interest, the SDR task was always performed before the DMTS task. In addition, statistical analyses were performed on the full data set and a corrected data set, which included only data in which performance

was significantly above chance levels before THC/vehicle administration. Importantly, comparison between the two data sets revealed highly consistent findings and similar overall patterns of results with the primary measures on performance accuracies similarly affected (SDR) or not (DMTS).

The sequential order of task presentation (ie, the SDR task was always presented before the DMTS task) could have confounded the comparisons of performance across tasks. In an effort to offset a potential order effect, the DMTS-2x trials had twice the reinforcement as the SDR and DMTS-1x trials, and were randomly interwoven with the DMTS-1x trials within a session. The success of this manipulation to increase motivation in partially satiated animals is evident from the fact that baseline performance on the DMTS-2x trials was not significantly different than performance on the SDR-1x trials. Nonetheless, because the 240 μg/kg dose of THC reduced DMTS-2x accuracy (−18.9%) at the 16-s delay, it is possible that presentation of the DMTS task first following THC administration would have revealed performance impairments at a lower dose and/or at shorter delays. However, in a previous study of rhesus monkeys (3 to 6 years of age), the effects of intravenous administration of THC (3–300 μg/kg) on DMTS task performance were similar to our findings, even though the DMTS task was always the first task presented; that is, accuracy on the DMTS task was not significantly affected by THC at any dose or delay (Schulze *et al*, 1988). In addition, these investigators found that 100 and 300 μg/kg of THC significantly decreased completion rates and significantly increased response latencies (Schulze *et al*, 1988), similar to the effects we observed at the 120 and 240 μg/kg doses of THC. Together, these findings suggest that the DMTS task is not as sensitive as the SDR task to the effects of intravenous THC administration in rhesus monkeys.

The escalating schedule of THC dose administration was chosen to minimize the possibility of adverse side effects (eg, anxiety in the testing chambers) in the adolescent monkeys. Although the dose escalation design of our study might have caused tolerance to the effects of THC, this would have resulted in a rightward shift of the dose-response curve. As such, a counterbalanced design may have revealed WM impairments at a lower dose. However, it seems highly unlikely that the differential effect on spatial, relative to object, WM would differ between an escalating *vs* a counterbalanced dose design.

Finally, the short washout periods within a dose (~23 h) and between doses (~72 h) could have led to an accumulation of THC in tissue, especially because THC is highly lipophilic. However, the acute effects of THC, which refer to effects that occur while a subject is directly intoxicated from THC administration, typically last no more than 2 to 4 h (Vadhan *et al*, 2009) and our analyses indicated no significant effects of the day-to-day variation of responses within a week. Thus, it seems unlikely that a prolonged washout period would have altered the differential effect of THC on spatial and object WM performance.

CONCLUSIONS

Comparable to monkeys, humans undergo a protracted period of cognitive maturation during adolescence that is associated with progressively greater efficiency of executive control capacities, as well as more focal and specialized PFC activity while irrelevant and diffuse activity is reduced (Durston *et al*, 2006; Tamm *et al*, 2002; Yurgelun-Todd, 2007). Importantly, age-related changes in spatial WM have been reported to extend into adulthood (Kwon *et al*, 2002), whereas maturation of object WM performance has been reported to be completed by adolescence (Crone *et al*, 2006). This difference in the trajectory of functional maturation is consistent with regional differences in prefrontal white (Reiss *et al*, 1996) and gray matter volume changes during adolescence, which are more prominent in dorsal than ventral PFC regions (Gogtay *et al*, 2004). In concert with findings in monkeys, the results of these human studies suggest that dorsal PFC-dependent spatial WM does not achieve adult levels of performance until after ventral PFC-dependent object WM is mature.

These differences suggest the hypothesis that THC exposure during adolescence is more likely to impair the still immature dorsal PFC circuits subserving spatial WM than the mature ventral PFC circuits subserving object WM. Indeed, studies of chronic users suggest that cannabis may impact adolescents differently than adults. For example, chronic users who began cannabis use during adolescence may be more vulnerable to neural dysfunction, as well as WM impairments, relative to those who began use in adulthood (Schweinsburg *et al*. 2008). Additionally, cannabis use during adolescence is associated with an increased risk of developing schizophrenia (Arseneault *et al*, 2002; Moore *et al*, 2007), a disorder characterized by persistent WM impairments. Although the current findings indicate that the effect of acute THC administration on spatial WM did not persist into the following test session ~23 h later, the protracted maturation of dorsal PFC circuits and functions during adolescence may render this circuitry especially vulnerable to repeated THC exposure.

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