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Δ⁹tetrahydrocannabinol impairs visuo-spatial associative learning and spatial working memory in rhesus macaques

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Abstract

Cannabis remains the most commonly abused illicit drug and is rapidly expanding in quasi-licit use in some jurisdictions under medical marijuana laws. Effects of the psychoactive constituent Δ^9tetrahydrocannabinol (Δ^9THC) on cognitive function remain of pressing concern. Prior studies in monkeys have not shown consistent evidence of memory specific effects of Δ^9THC on recognition tasks and it remains unclear to what extent Δ^9THC causes sedative versus specific cognitive effects. In this study, adult male rhesus monkeys were trained on tasks which assess spatial working memory, visuo-spatial associative memory and learning as well as motivation for food reward. Subjects were subsequently challenged with 0.1-0.3 mg/kg Δ^9THC, i.m., in randomized order and evaluated on the behavioral measures. The performance of both vsPAL and SOSS tasks was impaired by Δ^9THC in a dose and task-difficulty manner. It is concluded that Δ^9THC disrupts cognition in a way that is consistent with a direct effect on memory. There was evidence for interference with spatial working memory, visuo-spatial associative memory and incremental learning in the latter task. These results and the lack of specific effect of Δ^9THC in prior visual recognition studies imply a sensitivity of spatial memory processing and/or working memory to endocannabinoid perturbation.

Keywords

cannabis; marijuana; Macaca mulatta; memory

1. Introduction

Data from the Monitoring the Future project (Johnston et al. 2011a; Johnston et al. 2011b) show that cannabis remains the most commonly abused illicit drug in the United States with rates of use exceeded only by the legal substances alcohol and nicotine. High rates of exposure to cannabis mean that *population* prevalence for dependence on cannabis is the highest for any illicit drug (Anthony et al. 1994; Schramm-Sapyta et al. 2009). The longitudinal trends show that cannabis use in high school seniors peaked in the late 1970s, declined to a nadir around 1991-1992, rebounded through the late nineties, declined gradually and has been on the rise 2007-2010. The data also suggest that cannabis use is initiated starting as early as middle school and by the senior year of high school 35% of the population have used cannabis in the past year, including 20% in the past month (Johnston et al. 2011a). Prevalence estimates of cannabis use during young adulthood show about 16%

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using cannabis monthly and about 4-6% using daily. Exposure to cannabis is therefore quite common in the US and begins in the adolescent period in a large fraction of users. The peak of daily use rates in the young adult ages identifies a critical window overlapping with the period of formal education, which raises concern about the cognitive effects of cannabis use.

It has been established that humans who smoke marijuana or are administered the presumed most-active component of marijuana smoke, Δ^9-tetrahydrocannabinol (Δ^9THC) exhibit alterations in cognitive performance within multiple domains including memory, attention and motor performance (Braff et al. 1981; Elwan et al. 1997; Fant et al. 1998; Heishman et al. 1997; Heishman et al. 1989; Kurzthaler et al. 1999; Wilson et al. 1994). Cannabis also alters sensitivity to reward in risky choice decision making (Rogers et al. 2007), impairs automobile driving skills (Gjerde and Kinn 1991; Liguori et al. 1998) and degrades flight simulator performance in experienced pilots (Janowsky et al. 1976; Leirer et al. 1989). Impairments are not always reported for all tasks and there is some initial evidence that increased levels of cannabidiol in marijuana may ameliorate detrimental cognitive effects presumably caused by Δ^9THC (Morgan et al. 2010b). Further determination of the precise roles played by Δ^9THC as well as other constituents of marijuana would be facilitated by animal models which are sensitive to learning and mnemonic effects of Δ^9THC. Nonhuman primate behavioral models offer significant advantages for the study of complex cognition.

The distribution of the CB1 receptor in macaque monkey brain is highly similar to that of human brain (Eggan and Lewis 2007). High levels of expression were noted in frontal and temporal lobe regions that have been extensively associated with higher level cognition including memory and executive function in monkeys (Castner et al. 2004; Murray and Wise 2004; Roberts 1996). Results of Eggan and Lewis, as well as others (see (Tanda et al. 1997) for review) also show a high level of CB_1 expression in regions of the brain that are critical for reward function and the development of drug abuse. Correspondingly, cannabis or THC exposure has also been demonstrated to disrupt behavior in a variety of nonhuman primate species including spontaneous locomotor activity in baboon (Hienz et al. 1992), schedule responding in macaque and squirrel monkeys (Brady and Balster 1980; Galbicka et al. 1980; Stark and Dews 1980), learning tasks in chimpanzee, orangutan, macaque and squirrel monkeys (Branch et al. 1980; Pieper 1976; Schulze et al. 1988; Schulze et al. 1989), recognition memory tasks in chimpanzee and macaque (Aigner 1988; Ferraro and Grilly 1974; Schulze et al. 1989), spatial delayed response in macaques (Rupniak et al. 1991) and time estimation in macaques, chimpanzee and orangutan (Pieper 1976; Schulze et al. 1988; Schulze et al. 1989; Snyder et al. 1975).

Although behaviors have been disrupted by THC in monkey models, evidence for specific effects on different domains of cognitive function are more ambiguous. The most consistent effect of Δ^9THC on behavioral performance of monkeys appears to be a reduction in response rate in various schedule-controlled operant paradigms, e.g., (Beardsley et al. 1987; Brady and Balster 1980; McMahon et al. 2005; Nakamura-Palacios et al. 2000). Even so, one study reports *increased* response rates in rhesus after 0.1 mg/kg Δ^9THC (Stark and Dews 1980). Evidence for a specific effect of Δ^9THC on memory is comparatively more equivocal. Minimal (Schulze et al. 1988) or delay-nonspecific (Zimmerberg et al. 1971) effects of THC have been reported on delayed (non)match-to-sample tasks (DNMS), although larger disruptions of DNMS have been reported in chimpanzees administered relatively high (1 mg/kg) doses of Δ^9THC (Ferraro and Grilly 1973). Studies also report increased errors on repeated-acquisition tasks in New World and Old World monkeys (Nakamura-Palacios et al. 2000; Winsauer et al. 1999) in which new learning was affected but recall of old sequences was not. Effects in that task, however, occur mostly at doses which produce substantial disruption of responding and appear in a subset of the subjects. Finally, rhesus monkeys were not impaired in learning a 20-pair object discrimination task

where DNMS performance was reduced in a delay-independent manner (Aigner 1988), although relatively high doses (2.0-4.0 mg/kg, p.o.) were necessary to see any effect. The existing data are strongest in showing that Δ^9THC is sedating and may decrease motivation for appetitive reinforcers, but much weaker in explicating the nature of any specific effects on memory in nonhuman primates.

The relatively modest, inconsistent and/or non-specific behavioral effects of Δ^9THC reported in monkeys to date may reflect the tasks chosen for study. Recent work with rodents and humans has focused on the effects of Δ^9THC on spatial and working memory and indeed one prior study which demonstrated a clear retention-interval dependent effect of THC in monkeys used a spatial delayed-response procedure thought to assess working memory (Rupniak et al. 1991). The present study was therefore conducted to contrast the effects of Δ^9THC on two memory tests which feature significant spatial and working-memory demands. The self-ordered spatial search (SOSS) task of the nonhuman primate version of the Cambridge Neuropsychological Test Automated Batter (CANTAB) was designed to assay spatial working memory. It has been shown that performance of this task depends on intact function in frontal regions in marmoset monkeys (Collins et al. 1998) (similar to humans (Owen et al. 1990; Owen et al. 1996)). Furthermore SOSS performance is impaired in a trial difficulty-dependent manner by amnestic drugs such as the muscarinic cholinergic receptor antagonist scopolamine (Taffe et al. 1999), the NMDA receptor, noncompetitive antagonist, ketamine (Taffe et al. 2002a) and the D2-like dopamine receptor antagonist raclopride (Von Huben et al. 2006). The CANTAB visuo-spatial paired-associate learning task (vsPAL) is highly sensitive to early stage Alzheimer's Disease (Blackwell et al. 2004; Fowler et al. 1997; 2002; Swainson et al. 2001) and has likewise been adapted for monkeys (Taffe et al. 2002b; 2004). Prior work has shown it is sensitive to challenge with the amnestic drugs scopolamine, ketamine, raclopride, the nicotinic cholinergic antagonist mecamylamine as well as to chronic alcohol drinking (Crean et al. 2011; Katner et al. 2004; Taffe et al. 2002b; Von Huben et al. 2006). These refined and pharmacologically validated behavioral tests may therefore provide improved sensitivity to mnemonic effects of acute Δ^9THC in rhesus monkeys.

The selected model also permits a higher degree of translational inference since parallel human CANTAB tasks are available. For example, one recent study in human adolescents found greater detrimental effects of chronic cannabis use on the human CANTAB SOSS task, but not on the vsPAL task (Harvey et al. 2007). Although users had been abstinent for at least 12 hrs in that study, this provides some indication that these tasks may be sensitive to disruption of endocannabinoid systems. The SOSS task challenges spatial memory and the vsPAL involves some degree of pattern recognition, similar to a DNMS task. Therefore it was predicted that acute effects of Δ^9THC will be greatest on the SOSS task in the present study.

2. Materials and Methods

2.1 Animals

Eight male rhesus monkeys (Macaca mulatta) were used in these experiments. Animals were 5-6 years of age, weighed 8.0-13.4 kg at the start of the study. Daily chow (LabDiet® 5038, PMI Nutrition International, Richmond, IN, USA; 3.22 kcal of metabolizable energy (ME) per gram) allocations were supplemented with fruit or vegetables seven days per week and water was available ad libitum in the home cage. Animals on this study had previously been immobilized with ketamine (5-20 mg/kg) no less than semiannually for purposes of routine care and some experimental procedures. Animals also had various acute exposures to challenge drugs in prior studies. The United States National Institutes of Health guidelines

for laboratory animal care (Clark et al. 1996) were followed and all protocols were approved by the Institutional Animal Care and Use Committee of The Scripps Research Institute.

2.2 Behavioral Testing

For behavioral testing, a touch-sensitive computer monitor was placed in front of the animal, unrestrained in a cage. All subjects had been trained to reach out of the cage to touch the location on the screen at which visual stimuli were presented to obtain a food pellet reward. The test battery consisted of four behavioral tasks, three of which (vsPAL, SOSS, PR) are part of the non-human primate CAmbridge Neuropsychological Test Automated Battery (CANTAB; Cambridge Cognition, Cambridge, UK). Comprehensive descriptions of the individual tasks and the procedural details have been previously reported (Taffe et al. 2004; Weed et al. 1999) and a task schematic is provided in Figure 1.

2.2.1 Self-Ordered Spatial Search (SOSS)—Two or more small colored rectangles (boxes) were displayed on the screen in positions randomly allocated from 16 possible locations, as previously depicted (Taffe and Taffe 2011). The animal was required to touch a box within 30 seconds of stimulus onset after which the color of the touched box was briefly (100 ms) changed, the screen blanked and a reinforcer delivered. After a 2 second delay, the boxes were re-displayed and the animal was required to touch a box which had not previously been touched within the trial for a successful trial completion. The trial was completed when the animal either touched all boxes without a repetition (correct), touched a box that had previously been selected in that trial (error) or failed to touch a box within 30 seconds of stimulus presentation (omission). Errors and omissions were followed by a tone and a 4 second timeout with a screen blank. After an inter-trial interval of 5 seconds, another trial was presented with stimuli in new (randomly allocated) positions. Each session consisted of 40 trials grouped into 8 blocks by trial type as follows: 5 (2 boxes), 5 (3 boxes), 5 (4 boxes), 5 (5 boxes), 5(3 boxes), 5(4 boxes), 5(5 boxes), 5 (2 boxes). Accuracy scores were calculated for each trial type by dividing the number of correctly completed trials by the number of trials in which there was at least one response. Six of the eight animals were trained to stable baseline performance on this task.

2.2.2 Visuo-Spatial Paired Associates Learning (vsPAL)—Colored abstract stimuli were displayed in one of four possible target locations (see (Taffe et al. 2002b)) and the subject was required to touch this sample stimulus, which then disappeared. The same pattern re-appeared during the choice phase in 2, 3 or 4 locations on the screen (the original location plus one or more novel locations) after a one second screen blank. The subject was required to touch the stimulus presented in the same location as the sample item to obtain a reinforce delivery. Subjects were allowed up to 10 additional attempts to successfully complete the set of stimulus-location associations in a given trial, thus measuring incremental learning. Each session consisted of 35 trials in sequential blocks including 5 × 1-stimulus (3 choice locations) trials, 10 × 2-stimuli (2 choice locations) trials, 10 × 3-stimuli (3 choice locations) trials and 10 × 4-stimuli (4 choice locations) trials. Performance was measured by percent correct trials on the initial-attempt to complete a trial and the percent correct of trials successfully completed within the allowed attempts (repeated-attempt completion). Four of the eight subjects had been trained to stable baseline levels of performance on this task.

2.2.3 Progressive-Ratio (PR) Schedule of Reinforcement—Subjects were required to respond to a single colored rectangle presented in the center of the screen for pellet reinforcement. The response requirement started at 1 touch and incremented by arithmetic progression within blocks of 8 reinforcers and by geometric progression between blocks of 8. (I.e., the first successive 8 ratios increase by 1, the second successive 8 increase by 2, the

third successive 8 increase by 4, etc.) The session was terminated after 10 minutes, or earlier if 3 minutes elapsed following a response. The primary dependent variable was the number of reinforcers acquired.

2.2.4 Bimanual Motor Skill Task (BMS)—A transparent polycarbonate board (10 cm wide × 25 cm high × 2.75 cm thick) drilled with 15 holes (spaced 13 mm apart in a 3 horizontal × 5 vertical array) was filled with raisins and mounted perpendicular to the door of the transport cage. Subjects acquire a technique wherein they push the raisin out of the hole with one finger before retrieving it with the opposite hand, thus entailing bimanual dexterity. The time elapsed to retrieve all 15 raisins was recorded.

2.2 Drug Challenges

Monkeys were administered acute doses (0.1-0.3 mg/kg, IM) of Δ^9THC 45 minutes prior to behavioral testing with active drug challenges being conducted no more than twice per week at 3-4 day intervals. Treatment order was pseudo-randomized across subjects and with respect to behavioral testing combinations (e.g., sessions of PR/SOSS/BMS versus vsPAL/BMS). For injection, Δ^9THC was suspended in a vehicle of absolute ethanol, Emulphor and saline in a 1:1:18 ratio. The Δ^9THC was provided by the U.S. National Institute on Drug Abuse.

2.3 Data Analysis

Analysis of the behavioral data employed randomized block analysis of variance (ANOVA) with a consistent within-subjects factor of drug treatment condition (baseline, vehicle, 0.1, 0.2, 0.3 mg/kg THC). Analysis of the SOSS data employed an additional repeated measures factor of trial difficulty (2-, 3-, 4- and 5-boxes). Two of the six animals failed to complete sufficient trials for analysis in the SOSS task in the 0.2 mg/kg condition, thus they are included only in the remaining conditions. Three factor repeated-measures analysis of the vsPAL performance was necessary to include a factor of initial versus repeated attempts (to demonstrate improvement with practice, or learning) as well as the trial difficulty (1, 2, 3 or 4 stimuli per trial). Post-hoc analyses of any significant main effects in the multi-factor ANOVAs was conducted using the Fisher LSD test including all pairwise comparisons. Post-hoc evaluation of the single factor PR and BMS tasks was conducted with the Dunnett procedure using the vehicle treatment condition for comparison with all other conditions. The criterion for significance in all tests was $p < 0.05$. Analyses were conducted with GB-STATv7.0; Dynamic Microsystems, Silver Spring MD

3. Results

3.1. Self-Ordered Spatial Search Task

Performance accuracy (percent correct trials) of the Self-Ordered Spatial Search (SOSS) task was ranked by trial difficulty and altered by drug challenge (Figure 2) as was confirmed by significant main effects of trial difficulty [$F_{3,15} = 130.5$; $p < 0.0001$] and drug treatment condition [$F_{4,20} = 6.4$; $p < 0.005$]. Furthermore, the post hoc test confirmed that under baseline, vehicle and 0.1 mg/kg Δ^9THC treatment conditions, trial completion success for each trial type was significantly different from every other trial type. Accuracy on the simplest, 2-box trials was significantly better than all other trial types, and the performance of 3-box trials remained better than the 4-box and 5-box trials, following the administration of the two highest doses of Δ^9THC as well. Trial completion success of the 4-box and 5-box trial types did not differ from each other after the two highest doses of Δ^9THC. The drug challenge also produced detrimental effects when considered within a trial difficulty type. The post-hoc test confirmed that relative to baseline, vehicle and the 0.1 mg/kg condition, 3-box trial success was significantly impaired when either 0.2 or 0.3 mg/kg Δ^9THC was

injected and 4-box trial success was impaired when 0.3 mg/kg was injected. The 5-box trial completion success was significantly lower after 0.3 mg/kg compared to the 0.1 mg/kg Δ^9THC condition, but no differences from either the baseline or vehicle condition were confirmed for 5-box trials after drug administration. Interestingly, the post-hoc test also confirmed that performance of the 4-box trials was improved after 0.1 mg/kg Δ^9THC relative to baseline, vehicle and the 0.3 mg/kg treatment conditions.

3.2. visuo-spatial Paired Associates Learning Task

The monkeys' trial completion success in the visuo-spatial Paired Associates Learning (vsPAL) task was determined by trial difficulty (number of stimuli) and improved with repeated attempts at the same trial (Figure 3). These differences were statistically reliable as was confirmed by main effects of trial difficulty [$F_{3,21} = 306.4$; $p < 0.0001$], of initial versus repeated attempts [$F_{1,7} = 292.6$; $p < 0.0001$] and the interaction between these two factors [$F_{3,21} = 78.8$; $p < 0.0001$]. The post-hoc test confirmed that initial-attempt performance of 3-stimuli and 4-stimuli trial types differed from all other trial types within treatment condition; 1-stimulus and 2-stimuli trial performances only differed after 0.3 mg/kg Δ^9THC. The repeated-attempt completion success for 4-stimuli trials differed significantly from all other trial types within each treatment condition as did 3-stimuli trial performance after 0.3 mg/kg Δ^9THC.

The performance of vsPAL was impaired by Δ^9THC in a dose by difficulty dependent manner. The ANOVA confirmed a significant main effect of drug condition [$F_{4,28} = 71.0$; $p < 0.0001$], of the interaction between drug condition and trial difficulty [$F_{12,84} = 5.8$; $p < 0.0001$] and of all three factors [$F_{12,84} = 14.0$; $p < 0.0001$]; the interaction between initial/repeated attempts and drug condition was not significant. The post-hoc test confirmed that relative to vehicle and baseline *initial-attempt* performance, 1-stimulus trials were impaired after 0.2 mg/kg and 2-stimuli trials after 0.3 mg/kg Δ^9THC was injected. Similarly, the 3-stimuli trial initial-attempt success was lower compared with baseline, vehicle and the 0.1 mg/kg treatment conditions when either 0.2 or 0.3 mg/kg Δ^9THC was administered. Finally, the 4-stimuli trial initial-attempt success was impaired relative to vehicle following 0.3 mg/kg Δ^9THC. The post-hoc test also confirmed that *repeated-attempt* trial completion success was impaired relative to baseline, vehicle and the 0.1 mg/kg conditions for 3-stimuli trials after 0.3 mg/kg and for 4-stimuli trials after 0.2 and 0.3 mg/kg Δ^9THC.

3.3. Progressive Ratio and Bimanual Motor Skill Tasks

The remaining tasks were also affected detrimentally by Δ^9THC as is shown in Figure 4. The ANOVAs confirmed that Δ^9THC significantly changed the number of reinforcers acquired on the progressive ratio task [$F_{4,24} = 6.11$; $p < 0.01$] and slowed raisin retrieval in the bimanual motor skill task [$F_{4,28} = 9.41$; $p < 0.0001$]. The post-hoc analysis further confirmed that compared with the vehicle condition, PR performance was lower after 0.3 mg/kg Δ^9THC and raisin retrieval was slower after either 0.2 or 0.3 mg/kg Δ^9THC.

3. Discussion

This study shows that the administration of Δ^9-tetrahydrocannabinol (Δ^9THC) interferes with the performance of two spatial memory tasks in rhesus monkeys. The detrimental effects of Δ^9THC were task specific within both the Self-Ordered Spatial Search (SOSS) and visuo-spatial Paired Associates Learning (vsPAL) tasks of the nonhuman primate CANTAB battery (Taffe et al. 2004; Weed et al. 1999) since the monkeys' performance was degraded in a dose by trial difficulty manner. Although the simpler tasks that are intended to index motivated responding (PR task) and psychomotor function (bimanual motor skills task) were also affected these effects were incremental rather than a complete disruption of

behavior at the doses administered (0.1-0.3 mg/kg i.m.). Similarly, performance of the easiest trial types within both SOSS and vsPAL tasks was minimally affected, or unaffected, by Δ^9THC. Additional evidence that motivation does not explain the pattern of effects on the memory tasks is provided by a prior demonstration that degrading the motivation for the food reinforcer disrupts SOSS and vsPAL performance in a trial-difficulty independent manner (Taffe 2004). Therefore the present data are most consistent with an interpretation of a specific disruptive effect of Δ^9THC on spatial working memory, visuo-spatial associative memory and incremental learning.

Prior cognitive studies in Old World monkeys have not generally found effects of Δ^9THC on memory procedures that are clear demonstrations of a highly specific impact. That is, drug effects were not task-difficulty dependent and in some cases were observed at doses which produced significant general behavioral disruption. For example Aigner found that Δ^9THC (2-4 mg/kg p.o.) impaired overall performance on a sequential-list version of Delayed Non-Match to Sample (DNMS) but no retention-interval or list-length dependent effects were reported (Aigner 1988). Similarly, Schulze and colleagues found that Δ^9THC did not impair a traditional sample/response DNMS (with a 7-stimulus set) task in a retention-interval dependent manner (Schulze et al. 1988). Interestingly this same task *was* impaired in a retention-interval dependent manner by the inhalation of marijuana smoke by monkeys (Schulze et al. 1989). Winsauer and colleagues showed that Δ^9THC impaired the acquisition of a novel stimulus-response chain but not the expression of an already-learned chain of responses (Winsauer et al. 1999; Winsauer et al. 2011). In this procedure, however, there was no report of within-task difficulty levels for either the acquisition or performance components, the drug effects on accuracy were observed in about half of the subject population and following doses that significantly suppressed responding.

As is obvious from the present findings on the PR and BMS tasks, Δ^9THC is very likely to have behavioral effects that may not be specific to learning or memory. It was also the case that choice latency was altered in the complex tasks. For example in the vsPAL task choice latency was slowed from about 800 ms in the vehicle condition (for all trial types) to 1500 ms for 2-stimuli trials and 2630 ms for 4-stimuli trials after 0.3 mg/kg Δ^9THC. Interestingly, mean correct response latency in the SOSS task was slowed only slightly (1864-2169 ms vs 1410 under vehicle conditions) in the easy, 2-box trials after 0.2-0.3 mg/kg Δ^9THC, however response latencies were faster for the more difficult trial types under baseline and vehicle conditions (~900-1100 ms) and were not slowed any more than the 2-box trials under Δ^9THC challenge. We have shown previously that experimentally extending the retention interval in these tasks (Crean et al. 2011; Weed et al. 1999) by a duration similar to the slowing induced by Δ^9THC does not introduce an accuracy cost similar to the one produced by Δ^9THC in this study. This again supports the interpretation of a selective effect of Δ^9THC on the mnemonic aspects of the SOSS and vsPAL tasks.

One prior report that did identify detrimental effects of Δ^9THC on monkey performance (Rupniak et al. 1991) using a spatial delayed-response task, raising the possibility of a cognitive domain selective, and potentially regionally-selective, effect. That is, spatial working memory tasks are often characterized as depending primarily on frontal mechanisms whereas recognition memory procedures such as DNMS are thought to depend primarily on the medial temporal lobe memory systems. The present SOSS task has been shown to depend on intact function of prefrontal cortex in marmoset monkeys (Collins et al. 1998) and therefore this commonality may suggest a specific or enhanced sensitivity of frontal cognitive mechanisms to Δ^9THC. The visuo-spatial associations that are intrinsic to the vsPAL procedure likely involve frontal-temporal circuitry (Browning and Gaffan 2008), perhaps with a dominance of orbital regions and excluding dorsolateral prefrontal areas (Baxter et al. 2008). Nevertheless dominant contribution to pattern/spatial associative

memory appears to be in the temporal lobe systems (Browning and Gaffan 2008; Malkova and Mishkin 2003). Furthermore, a recent study of humans with Mild Cognitive Impairment found functional Magnetic Resonance Imaging differences in hippocampal regions, but not frontal regions, during the performance of a human analog of vsPAL (de Rover et al. 2011). This is consistent with reports of relatively high density of the CB_1 endocannabinoid receptor in the dentate gyrus and CA fields of the hippocampus, entorhinal cortex, Areas 9 and 46 (dorsolateral prefrontal cortex) and Areas 10 and 11 (anterior prefrontal, orbitofrontal) in the macaque brain (Eggan and Lewis 2007).

The current data also provide further insight into potential species differences in responses to Δ^9THC when considered in combination with a recent report from this laboratory showing that the same dose range reduces the body temperature of freely moving monkeys (Harvey et al. 2007). Prior studies have shown that while doses of about 0.5-1.0 mg/kg, i.p. disrupt cognition in rats (Egerton et al. 2005; Han and Robinson 2001), the considerably higher doses (~10-30 mg/kg) required to decrease body temperature in the rat also produce hypolocomotion and/or catalepsy (Smirnov and Kiyatkin 2008; Whitlow et al. 2002). In macaque monkeys, doses that are higher than the 0.1-0.3 mg/kg used in the current study produce behavioral disruption, sedation, task refusal and other non-cognitive effects (McMahon et al. 2005; Winsauer et al. 1999), consistent with pilot studies conducted to establish the appropriate dose range for the current investigation. With the dose range of 0.1-0.3 mg/kg, monkeys were visibly intoxicated (most frequently evidenced by drooping eyelids, slowed reaction to conspecifics and investigative staff) within about 15-20 minutes of injection. Nevertheless they still responsive in the behavioral tasks, completing sufficient numbers of trials for analysis across the difficulty conditions and generating response latencies that were quantitatively slower but did not reflect total task disruption, as noted above.

The present results can also be compared in magnitude with prior pharmacological challenge of these same memory tasks with drugs more generally established to have amnestic effects. For example, the SOSS task is impaired in a difficulty- and dose-dependent manner by the muscarinic antagonist scopolamine (Taffe et al. 1999) and the NMDA noncompetitive antagonist ketamine (Taffe et al. 2002b) but not by the nicotinic antagonist mecamylamine (Katner et al. 2004) or the D1-like dopamine receptor antagonist SCH23390 (Von Huben et al. 2006). SOSS performance may be improved by a rapid tryptophan depletion approach which decrements central serotonin function (Taffe et al. 2003). It has also been shown that ketamine, mecamylamine and the D2-like dopamine receptor antagonist raclopride impair the initial memory component of vsPAL while leaving the learning component intact (Katner et al. 2004; Taffe et al. 2002b; Von Huben et al. 2006). In contrast scopolamine impairs both initial memory and incremental learning (Taffe et al. 2002b). The present results therefore put Δ^9THC on a similar scope and scale of effect as more canonical amnestic drugs. Within this CANTAB model of NHP cognition, Δ^9THC had an impact comparable to that of scopolamine, which is one of the few drugs that has been shown to disrupt incremental learning as well as initial memory in the NHP vsPAL task.

In summary, this study used refined and validated memory procedures to assess the cognitive effects of the primary psychoactive constituent of marijuana, Δ^9-tetrahydrocannabinol, in a nonhuman primate model. The results show that while there is a general behavioral impact (as with many prior studies in which operant responding was disrupted) there do appear to be selective effects on spatial working memory, visuo-spatial associative memory and incremental learning. This selective effect was evidenced in both vsPAL and SOSS tasks by preservation of the easiest trial conditions and dose-dependent impact on the harder trial conditions. These studies confirm that cognitive/behavioral models in the macaque monkey are likely translatable to the human condition and identify

cannabinoid sensitive assays that will be of further utility in additional studies of exo- and/or endocannabinoid regulation of cognitive function and complex behavior. For example a recent study of human adolescent regular cannabis users, tested at least 12 hrs after last use, on human CANTAB which were impaired on SOSS (but not on vsPAL) compared with the occasional-use controls (Taylor and Fennessy 1978). The present nonhuman primate model can now be used to explore effects of chronic exposure to Δ^9THC and/or other marijuana constituents (Morgan et al. 2010a; Morgan et al. 2010b), withdrawal and degree of recovery from chronic Δ^9THC dosing.

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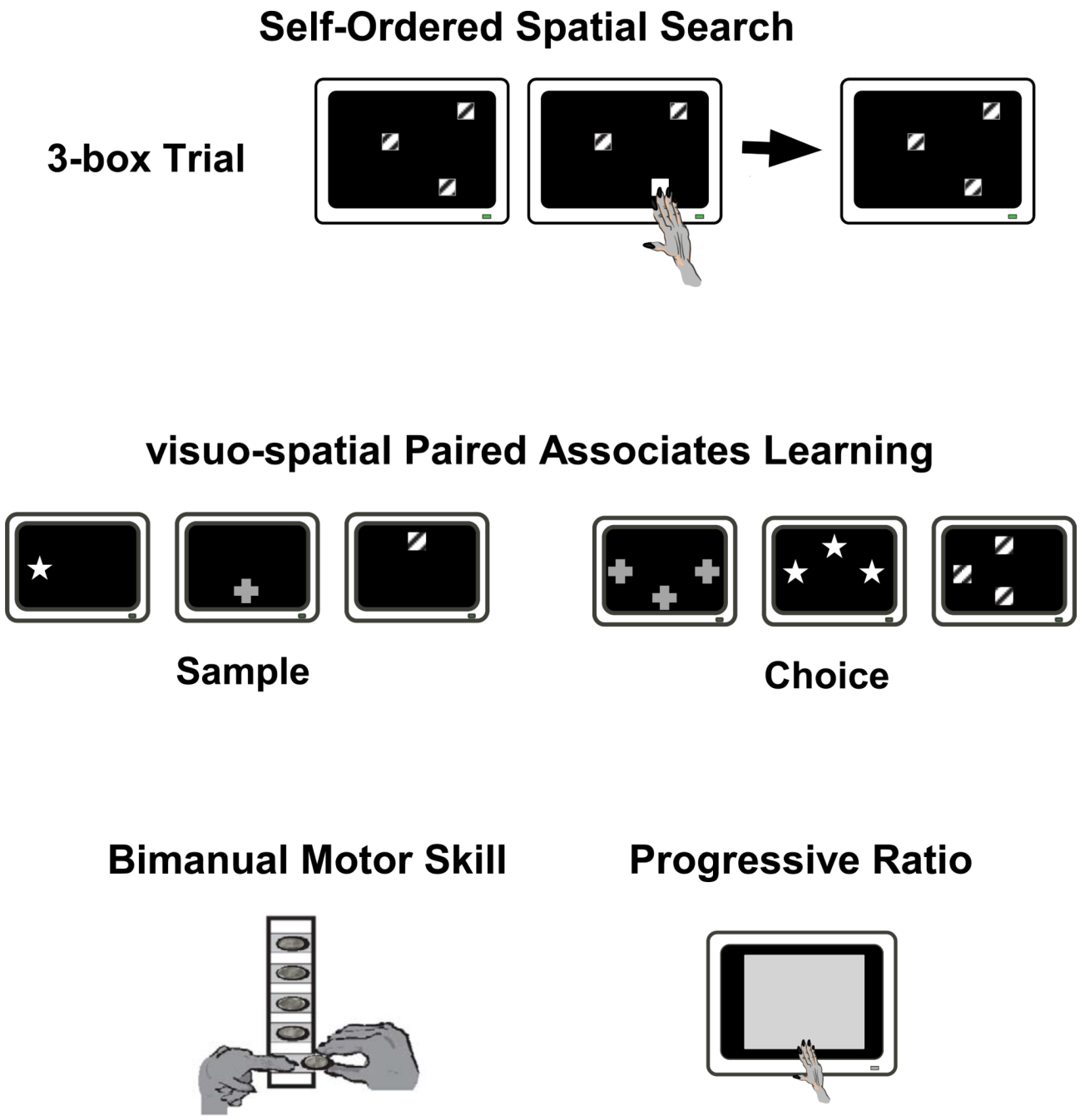

Figure 1.
A depiction of the behavioral tasks included in this study including a sample stimulus display for the 3-box difficulty condition in the Self-Ordered Spatial Search task (top) and the 3-stimuli by three choice locations trials in the visuo-spatial Paired Associates Learning task. See Materials and Methods and (Taffe et al. 2002b; 2004; Weed et al. 1999) for further specification of the tasks.

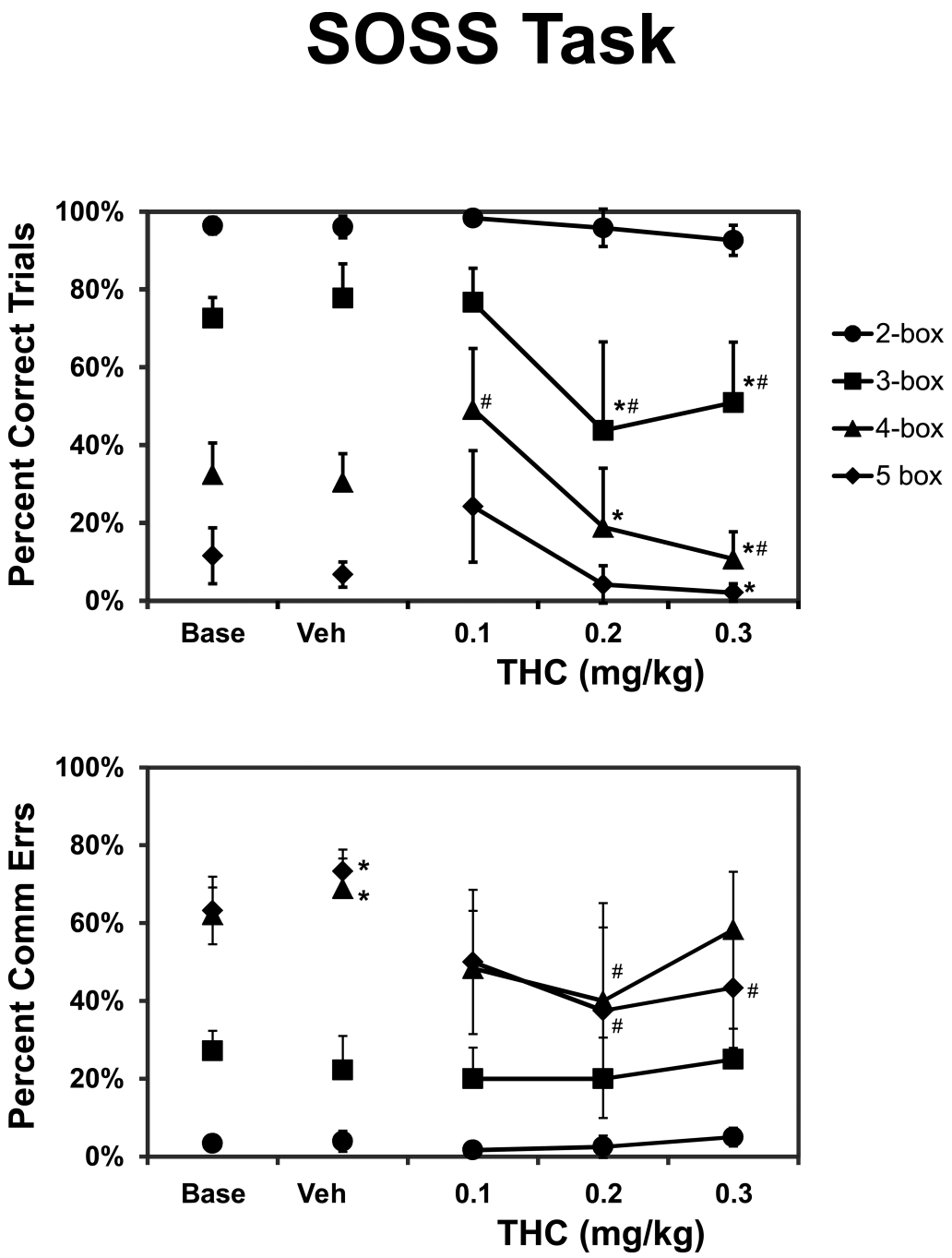

Figure 2.

The mean (N=6; ±SEM) trial completion accuracy on the SOSS task is presented by trial-difficulty and drug treatment condition. A significant difference from the vehicle and baseline conditions is indicated by #, and a difference from the 0.1 mg/kg condition by * within a given trial-difficulty level. The percent correct on all trial types differed significantly from all of the other trial types within a treatment condition, save that 4-box and 5-box completion success did not differ after 0.2 and 0.3 mg/kg THC.

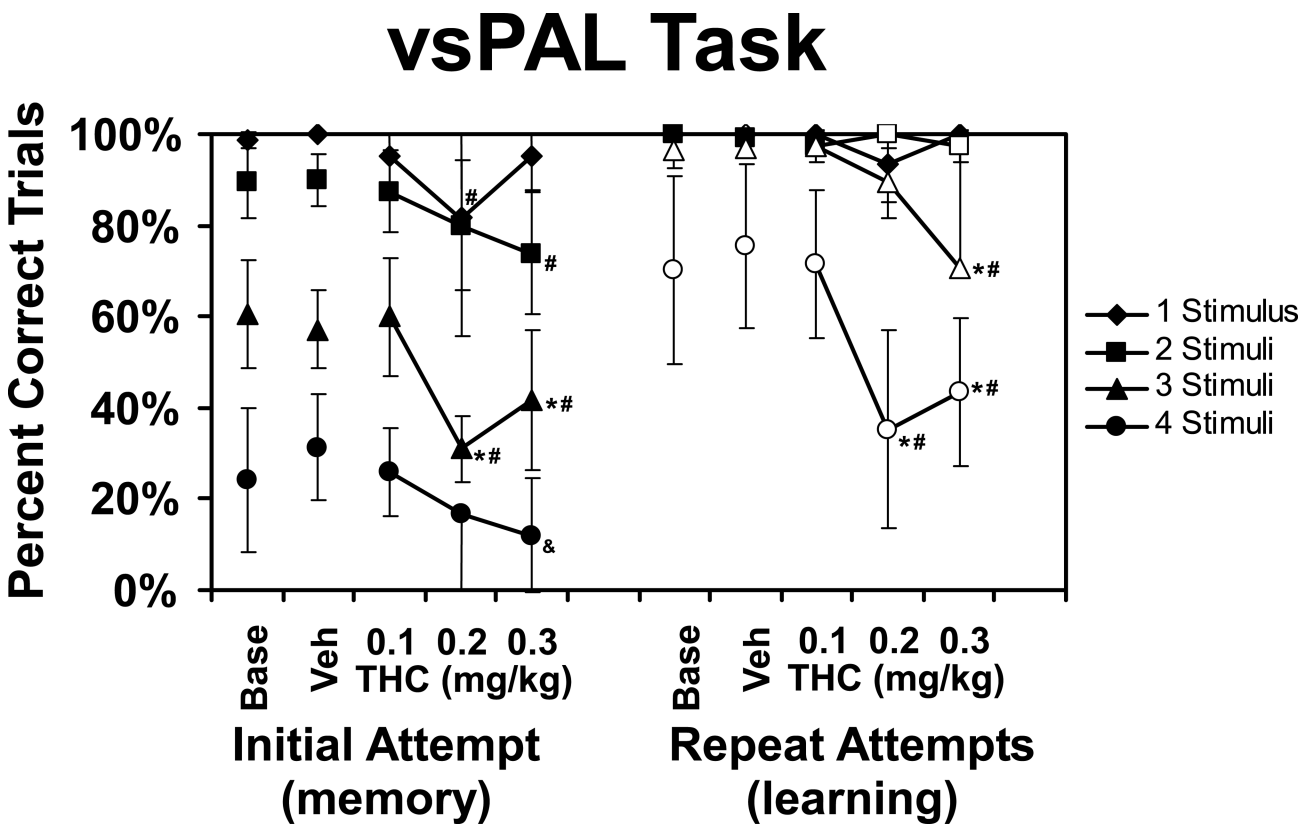

Figure 3.

The mean (N=4; ±SEM) percentage of trials correctly performed in the vsPAL task on the first attempt, and after a maximum of 6 attempts, are presented for baseline, vehicle and THC treatment conditions. The open symbols indicate significantly improved trial completion after repetition when compared with the initial attempt for a given treatment condition and trial type. Within a given trial-difficulty level, a significant difference from the vehicle and baseline conditions is indicated by #, from the vehicle condition (only) by &, and a difference from the 0.1 mg/kg condition by *.

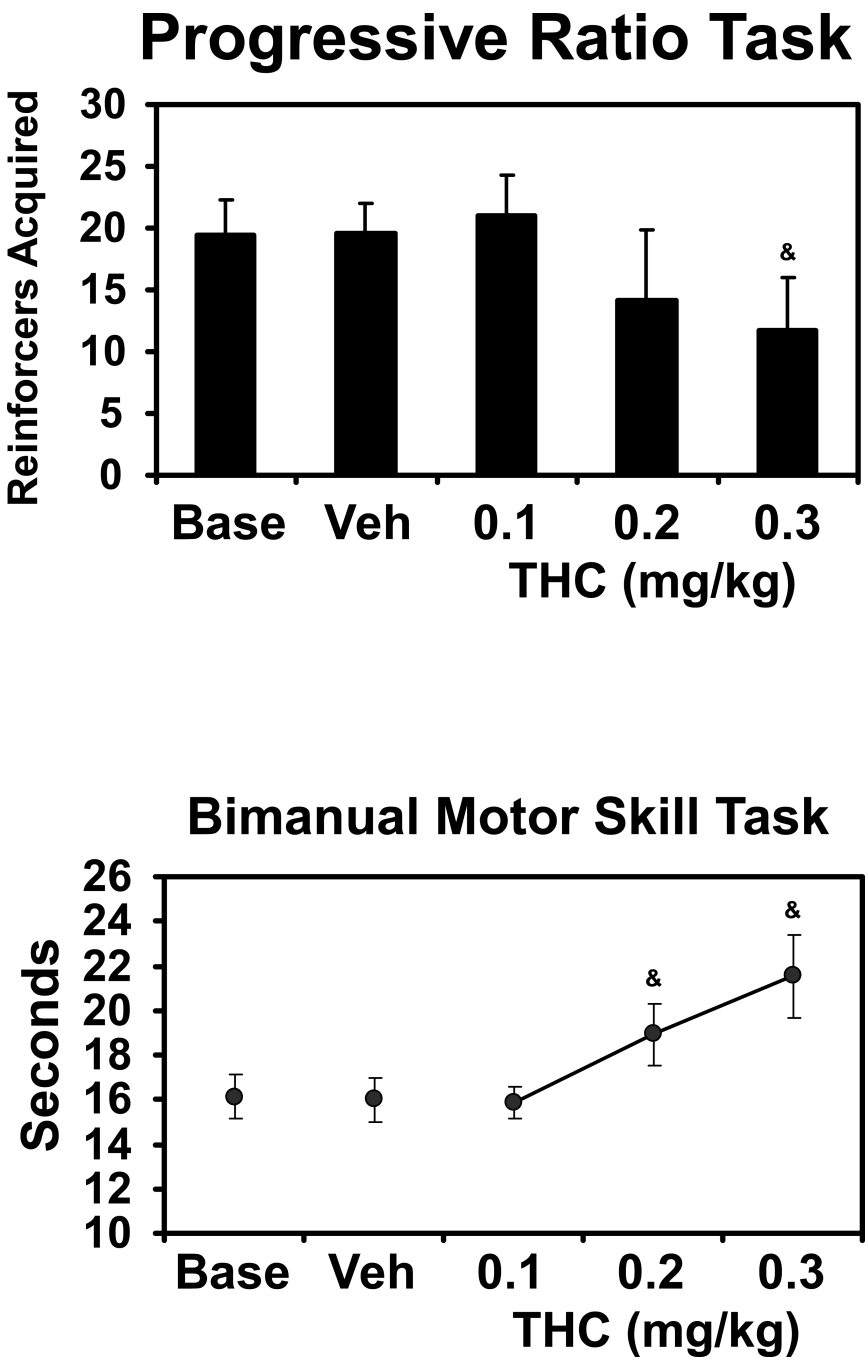

Figure 4.
Mean (Bars indicate SEM) performance on the Progressive Ratio (N=7) and Bimanual Motor Skill (N=8) tasks. A significant difference from the vehicle condition is indicated by &.