JPET Fast Forward. Published on December 4, 2017 as DOI: 10.1124/jpet.117.244194
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Chronic Δ^9-THC in rhesus monkeys: effects on cognitive performance and dopamine D2/D3 receptor availability

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Running title: THC-related cognitive deficits in monkeys

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Text pages: 15

Tables: 4

Figures: 5

References: 69

Abstract: 250 words

Introduction: 713 words

Discussion: 1,537 words

List of non-standard abbreviations: D2/D3R, dopamine D2/D3 receptor; CANTAB, Cambridge Neuropsychological Test Automated Battery; CD, compound discrimination; CDR, reversal of compound discrimination; DMS, delayed match-to-sample; DVR, distribution volume ratio; ED, extradimensional shift; FR, fixed-ratio; ID, intradimensional shift; PET, positron emission tomography; ROI, region of interest; THC, Δ^9-tetrahydrocannabinol

Recommended section assignment: Behavioral Pharmacology

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ABSTRACT

Cannabis-related impairments to cognitive function may represent novel therapeutic targets for cannabis-use disorder, although the nature, persistence, and reversibility of those deficits remain unclear. Adult male rhesus monkeys (N=6) responded in the mornings on tasks designed to assess different cognitive domains using CANTAB touchscreens followed by responding maintained under a fixed-ratio (FR) 10 schedule of food presentation in different operant chambers. First, the acute effects of Δ^9-tetrahydrocannabinol (THC; 0.01-0.56 mg/kg, i.v.) on cognitive performance, FR responding and body temperature were determined. Next, THC (1.0-2.0 mg/kg, s.c.) was administered daily after FR10 sessions for 12 weeks during which the residual effects of THC (i.e., 22 hrs after administration) on cognition were examined and the acute effects of THC were redetermined. In a subgroup of monkeys, dopamine D2/D3 receptor availability was assessed after 4 weeks of chronic THC exposure and compared to drug-naïve controls using positron emission tomography and [^{11}C]-raclopride (N=4/group). Acute THC pretreatments dose-dependently decreased FR responding and body temperature, while impairment to cognitive performance was task specific. During chronic treatment, THC produced persistent residual impairment only to working memory; tolerance differentially developed to acute cognitive impairments. There was recovery from residual cognitive impairments to working memory within 2 weeks of abstinence. Compared to controls, D2/D3 receptor availability was not altered during chronic THC treatment. In conclusion, THC-induced disruptions in cognition were task-specific, as was tolerance development, and not related to changes in D2/D3 receptor availability. Intervention strategies for cannabis-use disorder that enhance working memory performance may facilitate positive treatment outcomes.

INTRODUCTION

Marijuana is the most widely abused illicit substance in the United States (CBHSQ, 2016); however, effective treatment options are limited (Copeland and Pokorski et al., 2016). One factor in particular associated with poorer treatment outcome and relapse is drug-induced disruption to cognitive function (Aharonovich et al., 2006, 2008; Carroll et al., 2011). Therefore, a better understanding of the mechanisms underlying THC-induced cognitive impairment may identify targets for cognitive enhancement as an adjunct treatment strategy for cannabis use disorder (Sofuoglu et al., 2013; Rezapour et al., 2016).

Studies in humans have shown that the acute effects of cannabis consistently impair executive cognitive functions such as attention (Hart et al., 2001; D'Souza et al., 2008; Anderson et al., 2010), working memory (Heishman et al., 1997; Hart et al., 2001, 2010) and inhibition/impulsivity (McDonald et al., 2003; Ramaekers et al., 2009). However, the residual effects of THC (i.e., after the intoxicating effects have subsided), which are relevant for treatments that target cognitive functioning, are equivocal for most cognitive domains (Crean et al., 2011; Crane et al., 2013). Some of these disparate findings may be the result of experimental factors that are difficult to control in studies with human subjects such as time since last cannabis use, amount and duration of cannabis use, previous/current drug histories, social variables, and innate cognitive differences prior to initiating cannabis use. Therefore, it is useful to employ longitudinal, within-subject studies in preclinical animal models that can control for these variables in a systematic manner.

The present study used a within-subjects study design in monkeys to examine the acute and residual effects of chronic THC administration on multiple cognitive domains including 1) attention and working memory via the delayed match-to-sample (DMS) performance, 2) associative learning via simple/compound discrimination task (SD, CD), 3) inhibitory/impulse control via compound discrimination reversal learning (CDR), and 4) cognitive flexibility via

attentional set-shifting tasks (intradimensional/extradimensional; ID/ED). Intact functioning within these particular cognitive domains is important for positive treatment response as they underlie the skills necessary for behavioral change and for reducing the likelihood of relapse during treatment (Rezapour et al., 2016). The effects of THC administration were also assessed on another behavioral baseline, fixed-ratio (FR) schedule of food reinforcement, and the effects of THC on body temperature were examined. We hypothesized that the effects of chronic THC administration would be different on cognition compared to simple operant responding under the FR schedule. Moreover, we hypothesized that working memory would be most vulnerable to THC-induced impairment based on previous research in nonhuman primates (Taffe et al., 2012; Wright Jr. et al., 2013; Kangas et al., 2016).

Prior work also suggests that the acute effects of THC on behavior may differ as a function of THC history (Tanda and Goldberg, 2003). As it relates to cognitive performance, studies in humans have demonstrated equivocal findings for the acute effects of cannabis in occasional vs. frequent cannabis users (Kirk et al., 1999; D'Souza et al., 2008; Ramaekers et al., 2009, 2016), which may have been due to differences in baseline performance and extent of cannabis use history. The use of within-subject experimental designs in animal models can address these confounds, which was the major goal of the present study..

Regarding neurobiological substrates mediating cognition and executive function, dopamine D2/D3 receptors (D2/D3R) are particularly important (Tomasi and Volkow, 2013). For instance, D2/D3R in the dorsal striatum have been shown to be associated with activation of prefrontal brain regions implicated in executive function (Volkow et al., 1993, 1998) and with frontostriatal circuitry implicated in inhibitory control (Ghahremani et al., 2012). Moreover, chronic drug abuse in humans has been associated with reductions in D2/D3R availability compared to healthy control subjects for a variety of substances (Volkow et al., 2012). Taken together, cognitive impairment resulting from chronic drug use may be related to low striatal D2/D3R availability. Indeed, cognitive impairment in long-term marijuana users has been

associated with reduced activity in frontal cortical regions (Block et al., 2002; Eldreth et al., 2004; Bolla et al., 2005; Gruber et al., 2005;). However, findings for D2/D3R availability in long-term marijuana users have been inconsistent (Albrecht et al., 2013; Stokes et al., 2012). Thus, a final aim of our study was to directly examine the association between chronic THC exposure, cognitive performance, and D2/D3R availability using PET and the D2/D3R selective radiotracer [^{11}C]-raclopride.

METHODS

Subjects. For Experiments 1 and 2, six individually housed adult male rhesus monkeys (*Macaca mulatta*), with extensive drug histories and indwelling intravenous catheters (John et al., 2015a, b, 2017) served as subjects. Each monkey was also implanted subcutaneously with a transponder (Model IPTT-300; Bio Medic Data Systems, Inc., Seaford, DE) to non-invasively measure body temperature. Two of these monkeys were used in PET imaging studies as part of Experiment 3, prior to any drug history and again after chronic THC administration; two additional drug-naïve monkeys were used in Experiment 3. Therefore, a total of 8 monkeys were used in these studies. Each monkey was fitted with an aluminum collar (Model B008, Primate Products, Redwood City, CA) and trained to sit in a primate restraint chair (Primate Products). Monkeys were housed in stainless-steel cages with visual and auditory contact with each other, *ad libitum* access to water in their home cage and were fed sufficient standard laboratory chow (Purina LabDiet 5045, St Louis, MO) to maintain healthy body weights as determined by the veterinary staff. Environmental enrichment was provided as outlined in the *Guide for the Care and Use of Laboratory Animals* (National Research Council, 2011) and was approved by the Animal Care and Use Committee of Wake Forest University.

Overview of behavioral procedures. Monkeys in Experiments 1 and 2 underwent two behavioral sessions each day (**Fig. 1**). At ~0900 cognitive performance was assessed using the CANTAB

apparatus; sessions typically lasted 1 hour. Immediately following, monkeys were transported to a different room and operant sessions were conducted in separate experimental chambers where monkeys responded under a fixed-ratio (FR) 10 schedule of food (1.0-g banana-flavored pellets) presentation with a 60-sec timeout (TO) following each reinforcer presentation. FR sessions were approximately 1 hr in duration.

Apparatus. The apparatus for food-maintained responding was a ventilated, sound-attenuating chamber (1.5 x 0.74 x 0.76 m; Med Associates, East Fairfield, VT) designed to accommodate a primate chair. Two photo-optic switches (5 cm wide) were located on one side of the chamber with a horizontal row of three stimulus lights 14 cm above each switch and a food receptacle between the switches. The food receptacle was connected with tygon tubing to a pellet dispenser (Gerbrands Corp., Arlington, MA) located on the top of the chamber for delivery of 1.0-g banana-flavored food pellets (Bio-Serv, Frenchtown, NJ). An infusion pump (Cole-Palmer, Inc., Chicago, IL) was located on the top of the chamber.

The apparatus for cognitive testing was a separate sound-attenuating, ventilated chamber (0.8 x 0.8 x 1.32 m) consisting of a CANTAB panel (0.38 x 0.56 x 0.31 m) that included a touch-sensitive screen (0.3 x 0.23 m), a stimulus light, a non-retractable response lever, and a pellet receptacle located on the right side. For each task, responding was maintained by presentation of 190-mg sucrose pellets.

Surgery. Monkeys included in behavioral experiments were surgically prepared with a chronic indwelling venous catheter (femoral, internal or external jugular) and subcutaneous vascular port (Access Technologies, Skokie, IL) using aseptic surgical procedures, as described in detail elsewhere (John et al., 2017). These monkeys were also implanted subcutaneously with a transponder (Model IPTT-300; Bio Medic Data Systems, Inc., Seaford, DE) to non-invasively measure body temperature.

General Procedures

Food-maintained responding. Sessions began with illumination of a white stimulus light above one of two photo-optic switches in the chamber, counterbalanced between monkeys (N=6). Ten consecutive responses (FR 10) emitted on the appropriate switch produced the delivery of a food pellet accompanied by extinction of the white light and illumination of a red stimulus light above the food receptacle for 3 sec. Responses emitted on the alternate photo-optic switch had no programmed consequence. Sessions lasted 60 min or until 30 reinforcers were obtained, whichever occurred first. Stable performance was defined as ± 20% of the mean rate of responding for 3 consecutive sessions, with no trends as used previously (John et al., 2017).

Delayed Match-to-Sample (DMS). The DMS task is a measure of working memory. Each trial began with the appearance of a "target" stimulus in the center of the screen (sample phase). A response on this stimulus was followed by a delay and then the presentation of a stimulus matching the previous image and at least two distractor stimuli that do not match (match phase). Responding on the match resulted in delivery of a sucrose pellet followed by a 10-s TO, whereas responding on the non-match resulted in trial termination and the 10-s TO. If a response was not emitted within 10-s during either phase, the trial was terminated. Three delays were randomly distributed throughout a total of 60 trials per session (20 trials/delay). Delays and number of distractor stimuli were individually determined (Table 1) in order to generate delay-effect curves that met the following criteria: short delay (80-100% accuracy); medium delay (60-79% accuracy); long delay (<60% accuracy). The stimuli used were unique abstract patterns preprogrammed in CANTAB software, which consisted of four rectangular quadrants of different colors and shapes. Each session lasted for a maximum of 100 min or after 60 trials were completed, whichever occurred first. Baseline performance was obtained prior to drug testing and was defined as 7 consecutive sessions with percent accuracy for each delay ±25% of the mean rate for those sessions with no trends.

Stimulus discrimination, reversal learning, and intradimensionl/extradimensional set-shifting (attentional set-shifting). This task consisted of 5 distinct stages that tested attention, rule learning and reversal, and executive function. Stages advanced within-session following acquisition of performance criteria, defined as 16 of 20 consecutive trials correct. Stage 1, simple discrimination (SD): Two stimuli (e.g. shapes) were presented and monkeys were required to discriminate between the two stimuli in which one resulted in reinforcement (S+) and the other resulted in trial termination (S-). Stage 2, compound discrimination (CD): The same stimuli as in the previous stage remained present, however, an additional dimension (e.g. lines) was superimposed to construct a compound stimuli that consisted of shape and line dimensions. The same stimulus dimension that was associated with reinforcement in the first stage (e.g. shapes) remained relevant for reinforcement in this stage, while the other dimension (e.g., lines) was irrelevant. Stage 3, compound reversal (CDR): Stage 2 rules were reversed such that the previously non-reinforced stimulus within the dimension was now reinforced. Stage 4, intra-dimensional shift (ID): A new pair of compound stimuli consisting of shape and line dimensions was presented; however, despite new stimuli, the same dimension of the stimuli as in stages 1 - 3 (e.g. shapes) remained relevant for reinforcement. Stage 5, extra-dimensional shift (ED): another novel set of compound stimuli was introduced; however, in this stage the previous irrelevant stimulus dimension (e.g. lines) became relevant for reinforcement. Each session lasted for a maximum of 100 min or after criterion was met for each stage, whichever occurred first. Baseline performance was obtained prior to drug testing and was defined as four consecutive sessions over two weeks with percent accuracy for each stage ±25% of the mean rate for those sessions with no trends.

Procedures

Experiment 1: Effects of acute and chronic THC administration on food-maintained responding. Once FR responding was stable, a non-contingent injection of vehicle or THC

(0.003-0.3 mg/kg, i.v.) was administered one minute prior to operant sessions, typically on Tuesdays and Fridays; all doses were tested 2-3 times in random order for each monkey. Body temperature was taken non-invasively using the implanted telemetry device immediately prior to THC administration and again 60-min after the start of the session. After completion of the THC dose-response curve and pre-chronic THC dose-response curve for Experiment 2 (see below), THC (1.0 mg/kg, s.c.) was administered daily approximately 5 minutes after the session for 10 weeks, followed by 2 weeks of 2.0 mg/kg (s.c.) THC for a total of 12 weeks of treatment. Hence, FR responding occurred ~23 hours after the daily THC administration. Doses were selected based on preliminary findings and other studies in rhesus monkeys showing these doses produced similar THC blood levels as humans smoking cannabis in controlled laboratory studies (Ginsburg et al., 2014). Following one week of chronic THC (1.0 mg/kg, s.c.) administration, the effects of acutely administered THC on FR responding were redetermined over the course of 2 weeks (Weeks 2-3) during which monkeys were still treated daily with 1.0 mg/kg THC post session. After 12 weeks of chronic treatment, responding was studied for 4-6 weeks after discontinuation of daily THC administration. The daily dose was not raised to 2.0 mg/kg in R-1710 due to marked behavioral disruption from 1.0 mg/kg THC, to which tolerance did not develop.

Experiment 2: Effects of THC on cognitive performance. Each week, DMS performance was assessed Monday-Wednesday and SD, CD, CDR, ID and ED set-shifting performance were assessed on Thursday and Friday. Following stable performance, the acute effects of vehicle and THC (0.003–0.3 mg/kg, i.v.) were determined on each task prior to chronic THC treatment. Each dose was administered 30-min prior to the start of the session, a pretreatment time based on the time course of cognitive impairment and subjective/euphoric effects following i.v. THC administration in humans and nonhuman primates (D'Souza et al., 2004; Lindgren et al., 1981;

Verrico et al., 2012). All doses were tested 2-3 times for each monkey and at least two intervening sessions were conducted where no drug or vehicle was administered.

After acute dose-response curves were determined, the residual effects (i.e., 22 hours post administration) of daily THC (1.0 mg/kg, s.c.) on cognition were assessed for 8 weeks. After 8 weeks of THC treatment, the acute effects of THC on cognitive performance were redetermined over the course of two weeks while monkeys still received 1.0 mg/kg THC at the end of FR sessions. During this period, acute effects were assessed approximately every other day, as long as performance was deemed stable in the preceding session. Following the redetermination of the acute effects, the dose of daily-administered THC was increased to 2.0 mg/kg (s.c.) for two weeks, in an effort to overcome tolerance, after which treatment was discontinued and performance during 4-6 weeks of abstinence was examined.

Experiment 3: Effects of chronic THC on D2/D3R availability. PET studies with [^{11}C]-raclopride were used to assess D2/D3R availability (Farde et al., 1985). Two groups of monkeys underwent [^{11}C]-raclopride PET scans; one group (N=4) was studied after 4 weeks of daily THC administration while another group (N=4) was drug-naïve and served as control subjects. Two subjects (R-1710, R-1711) were part of both groups. Approximately 30 min prior to the PET scan, the monkey was anesthetized with ketamine (10 mg/kg, i.m.) and transported to the PET Center. Anesthesia was maintained during the scan by inhaled isoflurane (1.5%). The monkey was placed in a GE Advance NXi PET scanner with 4 mm resolution and a catheter was inserted into an external vein for tracer injection and fluid replacement throughout the study. Body temperature was maintained at 40°C and vital signs (heart rate, blood pressure, respiration rate, and temperature) were monitored throughout the scanning procedure. A 5-min transmission scan was acquired in 3D mode. Next, the monkey received a bolus dose of [^{11}C]-raclopride (6.5–8.0 mCi) and a 90-min dynamic acquisition scan was acquired consisting of 18 frames over 90 min (18 × 5 min) in 3D mode (i.e., septa retracted). Image reconstruction of 3D

data was done using the 3D-reprojection method (Kinahan et al., 1989) with full quantitative corrections. Once scanning was complete, the transmission scan data were smoothed transaxially using a 4-mm Gaussian filter and segmented (Bettinardi et al., 1999). Emission data were corrected for attenuation and reconstructed into 128 × 128 matrices using a Hanning filter with a 4-mm cutoff transaxially and a ramp filter with an 8.5-mm cutoff axially.

Data Analysis. Experiment 1: The primary dependent variable for food-maintained responding was response rate (responses/second). Drug effects were expressed as a percentage of baseline responding, which was designated as 3 consecutive sessions of stable responding prior to the start of the study. Change in body temperature (°C) following administration of either vehicle or THC was determined by subtracting the temperature recorded prior to the start of the session from the temperature recorded at the end of the 60-min session. The potency of THC to decrease food-maintained responding was estimated by calculating the ED_{50} using the linear portion of the curve that crossed 50% reduction in baseline response rate. Potencies were considered statistically significant when 95% confidence intervals of ED_{50} values did not overlap. Subject R-1710 was excluded from analyses due to a significant reduction in response rates during chronic THC treatment that precluded a determination of ED_{50} values.

Experiment 2: The primary dependent variable for DMS and the SD, CD, CDR, ID and ED tasks was percent accuracy. Changes in percent accuracy from baseline were examined by two-way repeated measures ANOVA using delay (short, middle, long)/stage (SD, CD, CDR, ID, ED) and treatment condition (baseline, chronic, abstinence) as factors. Omitted trials were excluded from all analyses. Sessions during the chronic THC treatment phase where the total number of omissions exceeded 50% of the total trials were excluded from analyses. As a result, two subjects (R-1567, R-1710) were excluded from all DMS analyses; both subjects were also excluded from analyses of acute THC effects on ID/ED and R-1710 was excluded from analysis of chronic THC effects on ID/ED performance. One-way repeated measures ANOVAs were

conducted to compare number of omissions, response latency, and pellet retrieval latency between baseline and test conditions. Significant main effects were followed by post-hoc Fisher LSD tests. For all analyses, $p<0.05$ was considered statistically significant.

Experiment 3: PET data were co-registered to individual T1-weighted MRIs (acquired using a 3.0-T Siemens SKYRA scanner), which were used to anatomically define regions of interest (ROI) including the caudate nucleus, putamen, ventral striatum, and cerebellum. PMOD Biomedical Image Quantification Software (version 3.1; PMOD Technologies, Zurich, Switzerland) was used for image registration and was used to calculate distribution volume ratios (DVRs) for each ROI by implementing the "Logan method" of analysis (Logan et al., 1996) using the cerebellum as the reference region. DVRs for each region were not different between left and right sides therefore data from each monkey was expressed as a mean of both sides. To compare DVRs between control and THC-treated subjects, a two-way ANOVA was conducted using region and group as factors.

Drugs. Δ^9-tetrahydrocannabinol (THC) was provided by the National Institute on Drug Abuse (Rockville, MD) as 20-100 mg/ml ampules dissolved in 95% EtOH. THC was diluted in a vehicle containing one part Tween 80, one part ethanol, and 18 parts sterile water. THC was administered i.v., through the vascular access port, and followed by a 3 ml saline injection to ensure the entire dose was delivered.

RESULTS

Experiment 1: Effects of acute and chronic THC on food-maintained responding

Acute THC administration resulted in dose-dependent decreases in food-maintained responding (**Fig. 2A**, open circles) and body temperature (**Fig. 2A**, open triangles). The ED_{50} (±95% confidence limits) for THC to reduce response rates and body temperature was 0.035 (0.011-0.059) mg/kg and 0.07 (0.02-0.19) mg/kg, respectively. There were no residual effects of

chronic THC administration on food-maintained responding in any subject except for one (R-1710) in which tolerance to rate-decreasing effects did not develop (**Fig. 2B**). After one week of daily THC (1.0 mg/kg, s.c.) treatment, the ED_{50} (95% confidence limit) for THC to reduce food-maintained responding and body temperature was 0.34 (0.16-0.51) mg/kg and 0.46 (0.23-0.90) mg/kg, respectively (**Fig. 2A**, closed circles/triangles). The potency of THC to reduce food-maintained responding and body temperature was significantly reduced by approximately 9.7- and 6.6-fold, respectively, after one week of daily THC (1.0 mg/kg, s.c.) treatment.

Experiment 2: Effects of THC on cognitive performance

DMS performance. For each monkey, three delays (short, mid, long) and number of distractor stimuli were chosen (**Table 1**) that resulted in similar delay-effect curves between monkeys (**Table 2**). There was a main effect of dose [$F(4, 39)=11.66$, $p<0.0001$] for the acute effects of THC on DMS performance prior to chronic treatment; post-hoc analyses revealed that percent accuracy was significantly reduced by 0.03 mg/kg THC at the short delay and by 0.03 and 0.056 mg/kg THC at the middle and long delays compared to baseline (**Fig. 3A**, open symbols). When the THC dose-response curve was redetermined during chronic THC treatment, there was a main effect of delay [$F(2, 48)=31.63$, $p<0.0001$], dose [$F(5, 48)=4.07$, $p<0.01$], and a significant interaction [$F(10, 48)=2.59$; $p<0.01$). Post-hoc analyses indicated that tolerance developed to the performance-disrupting effects of THC such that 0.03 and 0.056 mg/kg THC did not alter percent accuracy from baseline at the short and middle delays (**Fig. 3A**, closed symbols). During chronic treatment, accuracy at the long delay was significantly lower than baseline following vehicle pretreatment indicative of sustained residual impairment. The number of omissions following acutely administered THC before or during chronic treatment was not significantly different from baseline or vehicle during the Sample or the Match phases (**Fig. 3B**).

The residual effects of THC (1.0-2.0 mg/kg, s.c., 22 hours after treatment) on DMS performance over the course of 12 weeks of treatment and 4 weeks of abstinence (**Fig. 4A**)

showed a significant main effect of study week (baseline, THC treatment or abstinence) on percent change in accuracy from baseline [F(14, 42)=2.29; p<0.05). Significant impairments were detected at the middle delay during week four of treatment and at the long delay during weeks 1-4, 6, and 7. After the dose of daily administered THC was increased to 2.0 mg/kg (s.c.), percent accuracy from baseline at the longest delay was significantly decreased again at Week 12, which persisted until Week 2 of abstinence (**Fig. 4A**). There were no residual effects of chronic THC treatment on omissions during DMS performance.

SD, CD, CDR, ID and ED performance. There was a main effect of stage [F(4,64)=13.96, p<0.0001] and dose [F(4, 98)=3.49, p<0.05] for the acute effects of THC on attentional set-shifting performance prior to chronic THC treatment. There was a significant increase in percent accuracy of the CD stage after 0.056 mg/kg THC compared to baseline (p<0.05). Post-hoc analysis also revealed a significant decrease in percent accuracy of ED at 0.056 (p<0.001and 0.1 mg/kg THC (p<0.05) compared to baseline (**Fig. 3C**). During chronic treatment, the acute effects of only 0.1 mg/kg THC were reexamined because it was the highest dose that produced impairments without producing a significant number of omissions in the initial assessment prior to treatment. Repeated measures two-way ANOVA demonstrated a main effect of stage [F(4, 36)=3.83, p<0.05]. During THC treatment, percent accuracy of ED after administration of 0.1 mg/kg THC was not significantly different compared to vehicle administration nor baseline prior to chronic treatment (**Fig. 3C**). Acute THC administration did not have a significant effect on omissions compared to baseline or vehicle for any stage.

The residual effects of THC on SD, CD, CDR, ID and ED set-shifting performance (**Fig. 4B**) showed a significant main effect of stage [F(4, 232)=14.46; p<0.0001] although post-hoc analysis revealed no significant decreases in percent accuracy from baseline during treatment or abstinence. There were no residual effects of THC on number of omissions for any stage. An

overall summary of the acute and residual effects of THC on all cognitive tasks is presented in **Table 3**.

Experiment 3: Effects of chronic THC on D2/D3R availability

In control monkeys, the distribution volume ratio (DVR) for [^{11}C]-raclopride was highest in the putamen, followed by the caudate nucleus and ventral striatum (**Table 4**). There were no significant differences in D2/D3R availability in the caudate nucleus, putamen, and ventral striatum between control and chronic THC-treated monkeys (**Table 2, Fig. 5**). Individual differences were noted between the two monkeys that participated in scans both before and during THC treatment such that DVRs in the putamen were decreased by 14.2 percent during THC treatment in one monkey (R-1710) and increased by 15.3 percent in the other (R-1711).

DISCUSSION

The main goal of the present study was to examine the effects of THC on multiple cognitive domains in nonhuman primates and to examine tolerance development following chronic THC administration on multiple behavioral baselines and to THC-induced changes in body temperature. Acutely administered THC dose-dependently decreased FR response rates and body temperature and tolerance developed to both effects during chronic THC administration. With regard to cognition, acute THC dose-dependently decreased working memory (i.e., DMS) and ED set-shifting performance. During chronic treatment, tolerance developed to acute impairments in ED set-shifting performance but not DMS working memory when the cognitive demand was high (i.e., at long delays using complex stimuli). The residual effects of chronically administered THC were specific to impairments in working memory at the long delay. Tolerance developed transiently to the residual deficits in working memory during chronic treatment; an increase in THC dose resulted in impairments to working memory at the long delay. Working memory deficits recovered within 2 weeks of abstinence. The present

findings show differential tolerance to cognitive performance relative to food-maintained operant responding and body temperature. In an effort to examine potential mechanisms mediating the effects of chronic THC, dopamine D2/D3R availability was examined and found not to be different compared to control monkeys.

Tolerance to the acute effects of cannabinoids following a period of chronic cannabinoid exposure can differ depending on the behavioral outcomes measured (Lichtman et al., 2005). As such, studies in humans comparing groups of frequent vs. infrequent cannabis users suggest that chronic THC exposure can produce differential tolerance to the acute effects of THC on cognition. For instance, compared to infrequent users, some studies report that frequent users were less impaired by acutely administered THC on measures of attention (divided attention task) and memory (DMS, spatial working memory) (D'Souza et al., 2008; Ramaekers et al., 2009) while other studies indicate a lack of tolerance (Ramaekers et al., 2016; Theunissen et al., 2012). However, these studies depended on the use of a group design, so the extent to which the drug history was actually a factor is unclear without knowing the effects of acute THC on cognitive performance prior to the initiation of frequent use. By using a within-subjects design, the present study showed THC history was a causal factor for the development of tolerance to THC-induced cognitive impairment within similar domains. These findings have implications for assessing THC-induced driving impairment, to the extent that the studied cognitive domains map onto driving performance, as well as other behavioral tasks and suggest that cannabis use history should be considered in addition to measuring THC plasma concentrations.

Prior to chronic treatment, acutely administered THC was more potent to decrease cognitive performance (i.e., ED set-shifting and working memory) than rates of FR responding and body temperature. However, dose was similar (i.e., 0.3 mg/kg) to decrease each behavioral measure compared to vehicle pretreatment during chronic THC treatment. Previous studies in rhesus monkeys demonstrated that the CB_1 receptor antagonist, SR 141716A, did not fully

attenuate THC-induced rate-decreasing and hypothermic effects, implicating a role for non-CB_1 mechanisms in mediating these behavioral effects (McMahon et al., 2005; McMahon, 2011). Therefore, the present results suggest a relatively greater role of CB_1 receptors in THC-induced cognitive impairment. However, a limitation of the present study was the different points during chronic treatment that acute effects were assessed on the various behavioral and physiological measures, which should be addressed in future studies.

With regard to the residual effects of chronic THC exposure on cognition, results from studies in humans have also shown task-specific deficits, although not all have been consistent with the present findings in monkeys. For instance, in contrast to the present results, several studies in recently abstinent (6 -36 hr) adult cannabis users showed no differences in working memory abilities compared to infrequent or non-users (Solowij et al., 2002; Pope Jr. and Yurgelun-Todd, 1996; Whitlow et al., 2004; Kanayama et al., 2004; Fisk and Montgomery, 2008). Human studies assessing the residual effects of cannabis use on cognitive flexibility are more mixed with some studies showing impairments (Pope Jr. and Yurgulen-Todd, 1996; Solowij et al., 2002) and others showing no deficits (Whitlow et al., 2004; Gruber et al., 2005; Hermann et al., 2007). Consistent with the present findings, associative learning in recently abstinent adolescent and adult users has been shown to be unaffected (Harvey et al., 2007; Fisk and Montgomery, 2008; Jager et al., 2010).

To our knowledge, only one preclinical study in nonhuman primates has examined the residual effects of chronic THC exposure (Verrico et al., 2014). In that study, adolescent rhesus monkeys were treated with THC (120 or 240 µg/kg, i.v.), 5 days per week for approximately 6 months during which working memory performance was assessed 23 hours after drug administration throughout the study. In contrast to our findings, there were no residual effects of chronic THC administration on object-working memory (i.e., DMS). However, spatial-working memory was impaired; tolerance or sensitization did not develop to these effects.

One possibility for discrepant findings among human studies may be due to the daily amount of THC consumed across studies. Although participants in the previously mentioned studies used marijuana on a nearly daily basis, the number of marijuana cigarettes smoked per day is often not reported, which makes it difficult to discern THC-induced effects across studies. It is also possible that cognitive deficits emerge after longer periods of chronic THC exposure than what was presently studied in monkeys. For instance, Solowij (2002) found that the severity of cognitive deficits after at least 12 hrs of abstinence in frequent cannabis users was correlated with years of use. Other factors such as cannabis strain, method of administration, and other smoking behaviors may have also contributed to inconsistencies in the literature. Furthermore, inconsistent results between studies could be a reflection of different cognitive demand across the tasks employed. For example, the previous study in nonhuman primates showing no effect of chronic THC treatment on DMS performance (Verrico et al., 2014) employed relatively short delay lengths and a less complex set of sample stimuli than our study. In line with these observations, previous studies of abstinent adolescent cannabis users demonstrated poorer working memory performance relative to control subjects but only during tasks that required greater memory load (Jacobsen et al., 2007; Hanson et al., 2010; Jager et al., 2010). Thus, the working memory task parameters employed in the present study may have had the highest cognitive demand of the other tasks making it most sensitive to impairment during chronic THC treatment.

Another explanation for the task-specific effects of chronic THC to impair cognitive performance in the present study may be the result of regional differences in brain CB_1 receptor function related to the domain of executive function. Electrophysiological and lesion studies demonstrate that working memory is preferentially activated by the ventrolateral prefrontal cortex (Mishkin and Manning, 1978; Wilson et al., 1993), while discrimination reversal learning and set-shifting are preferentially associated with activation of the orbitofrontal cortex and dorsolateral prefrontal cortex, respectively (Dias et al., 1996a,b). Thus, it is possible that

working memory impairments during chronic THC treatment were a reflection of greater dysregulation of CB_1 receptor dynamics (e.g., downregulation and/or densensitization) or signaling in regions that mediate working memory.

Previous studies in rhesus monkeys have observed physical signs of withdrawal (i.e., yawning, anorexia, piloerection, irritability, and headshaking) and the onset of cannabinoid receptor antagonist discriminative stimulus effects within 24-48 hours of discontinuation of chronic THC treatment (Deneau et al., 1971; Beardsley et al., 1986; Stewart and McMahon, 2010), thus raising the possibility that the cognitive deficits noted in the present study were related to withdrawal as opposed to sustained agonist effects of THC on brain function. Although cognitive assessments were made at similar time frames as withdrawal symptoms detected in previous studies, no overt physical signs of withdrawal were noted throughout the present study. This was mostly likely due to the once-a-day administration of a lower daily dose of THC compared to continuous infusion of higher daily doses used in previous studies documenting physical withdrawal symptoms (0.05-0.17 mg/kg/hr, Beardsley et al., 1986; 1.0 mg/kg/12hr, Stewart and McMahon, 2010). Nevertheless, a systematic procedure for monitoring of overt physical signs of withdrawal was not included in the present study design, which precludes more conclusive evidence. However, notwithstanding this limitation, rates of food-maintained responding and numbers of omissions were not altered during the course of chronic treatment or after discontinuation, which suggests that cognitive impairment was not the result of a non-selective disruption in operant responding.

The present study did not find differences in striatal D2/D3R availability measured by [^{11}C]-raclopride in monkeys chronically treated with THC compared to control subjects, even though deficits in working memory performance at the same time point were apparent. These findings are consistent with studies in humans also showing no differences in baseline D2/D3R availability between marijuana abusers and healthy controls (Stokes et al., 2012; Urban et al., 2012; Albrecht et al., 2013; Volkow et al., 2014). However, recent PET studies have shown

reduced striatal dopamine release (after amphetamine challenge) in cannabis abusers (Volkow et al., 2014; van de Giessen et al., 2017). Taken together, striatal dysfunction appears to be a component of cannabis abuse, albeit to a lesser extent perhaps than other drugs of abuse.

Acknowledgement. The authors thank Michael Coller, Jillian Odom, Phillip Epperly and Stephanie Danner for technical assistance.

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FOOTNOTE. This work was supported by the National Institutes of Health grants R37 DA10584 (MAN), P50 DA06634 (MAN), F31 DA041825 (WSJ) and T32 AA-007565 (WSJ).

FIGURE CAPTIONS

Figure 1. Experimental timeline. *Panel A*. Sequence of daily experimental sessions: Monkeys performed two operant behavioral sessions each day, which included cognitive testing immediately followed by responding under an FR 10 schedule of food presentation. *Panel B*. Sequence of treatments across weeks: Following stable performance, the acute effects of THC (0.003–0.3 mg/kg, i.v.) were first determined on FR responding (Exp. 1) and then on cognition (Exp. 2). Next, THC (1.0–2.0 mg/kg, s.c.) was administered chronically for 12 weeks (shaded segments) during which the residual effects were assessed on cognitive performance (i.e., ~22 hrs after daily administration) and FR responding (i.e., ~23 hrs after daily administration) (Exp. 2). During chronic treatment, dopamine D2/D3R availability was assessed using PET and [^{11}C]-raclopride at Week 4 (Exp. 3) and the acute effects of THC were redetermined on FR responding (Exp. 1) and cognition (Exp. 2) during Weeks 2-3 and 9-10, respectively (Exp. 1). Exp., Experiment; CANTAB, cognitive assessments including DMS, SD, CD, CDR, ID, and ED; + THC #2, redetermination of acute effects of THC.

Figure 2. *Panel A.* Mean ± SEM (N=5) effects of acute THC administration on FR food-maintained responding (circles) and body temperature (triangles) before (open symbols) and during (closed symbols) chronic THC (1.0 mg/kg, s.c.) treatment (Weeks 2-3). Abscissae: Dose of THC in mg/kg. Left ordinate: Mean response rate expressed as a percentage of baseline. Right ordinate: Mean change in body temperature expressed in °C. *Panel B.* Mean (±SD) FR food-reinforced response rates for sessions before (BL, baseline) during (i.e., 23 hrs after daily administration), and after chronic THC treatment for individual monkeys (N=6). Abscissae: Consecutive weeks before, during, and after chronic THC treatment. Ordinate: Response rate (responses/sec). Filled symbols indicate weeks during chronic THC treatment. Open symbols indicate weeks where THC was not administered. All monkeys were treated with 1.0 mg/kg THC

during weeks 1-10 and 2.0 mg/kg THC during Weeks 11-12 except for one monkey (R-1710) who was only treated with 1.0 mg/kg THC during weeks 1-8.

Figure 3. Mean ± SEM (N=4) effects of acutely administered THC on cognitive performance before and during chronic THC (1.0 mg/kg, s.c.) treatment (Weeks 9-10). THC (0.01–0.3 mg/kg, i.v.) was administered 30 min before experimental sessions. *Panel A*: DMS performance at short-, mid-, and long-delays before (left panel; open symbols) and during (right panel; closed symbols) chronic THC treatment. *Panel B*: Acute effects of THC on DMS phase 1 (circles) and phase 2 (triangles) omissions before (open symbols) and during (closed symbols) chronic THC treatment. Data point for 0.056 mg/kg THC before chronic treatment and for 0.3 mg/kg THC during chronic treatment represents N=2. *Panel C*: Acute effects of THC on SD, CD, CDR, ID, and ED set-shifting performance before (left panel) and during (right panel) chronic THC treatment. Filled symbols indicate significant difference from vehicle ($p < 0.05$). Data point for 0.056 mg/kg and 0.1 mg/kg THC before chronic treatment represents N=3. *$p < 0.05$, significant difference from baseline; #, significant difference from vehicle. BL, baseline; SD, stimulus discrimination; CD, compound discrimination; CDR, compound discrimination reversal; ID, intradimensional set-shifting; ED, extradimensional set-shifting.

Figure 4. Residual effects (i.e., 22 hrs after administration) of chronic THC treatment on delayed match-to-sample performance (*Panel A*; N=4) and discrimination, reversal learning and attentional set-shifting performance (*Panel B*; N=5). Data are expressed as percent of baseline performance. Filled symbols indicate a significant difference from baseline ($p < 0.05$). Break in x-axis indicates two-week period (Weeks 9-10) where acute effects of THC on cognitive performance were reassessed during THC treatment. Vertical dashed lines indicate discontinuation of chronic THC treatment after which effects during abstinence were assessed. Abbreviations as in Figure 3.

Figure 5. PET imaging data using [^{11}C]-raclopride to measure dopamine D2/D3R distribution volume ratios (DVRs) in control monkeys (open symbols) and in monkeys treated daily (after 4 weeks) with 1.0 mg/kg (s.c.) THC (closed symbols). Different shaped symbols represent individual subjects. Two monkeys (R-1710, R-1711) served as both control and THC-treated subjects.

Table 1. Individual parameters used throughout study that produced delay-dependent effects on DMS performance; dist., distractor stimuli.

	Delays (sec)			
Subject	Short	Mid	Long	# dist.
R-1567	1	30	60	2
R-1682	0	30	90	4
R-1710	0	10	30	2
R-1711	0	30	100	4
R-1713	0	30	60	3
R-1756	1	30	60	3

Table 2. Baseline percent accuracy (± SD) at each delay for individual monkeys responding under the DMS task.

	Delays		
Subject	Short	Mid	Long
R-1567	78.8 (7.2)	46.5 (17.2)	38.3 (9.9)
R-1682	81.7 (5.9)	70.9 (9.8)	58.7 (9.8)
R-1710	75.8 (4.0)	52.9 (11.4)	41.7 (13.6)
R-1711	82.2 (10.2)	72.0 (15.5)	58.1 (8.7)
R-1713	87.1 (5.7)	56.5 (10.2)	49.3 (14.0)
R-1756	72.7 (11.2)	52.0 (11.0)	48.5 (5.1)

Table 3. Summary of THC-induced effects on cognitive performance.

Task	Acute- 30 min PT (Pre-chronic Tx)	Residual- 23 hr PT (During chronic Tx)	Acute- 30 min PT (During chronic Tx)	Abstinence
DMS				
Short delay	↓	---	---	---
Middle delay	↓	---	---	---
Long delay	↓	↓	↓	↓
SD	---	---	---	---
CD	↓	---	---	---
CDR	---	---	---	---
IDS	--- ↓	---	↑	---
EDS		---	---	--- ---

Note: effects were dose-dependent; refer to figures for details.
DMS, delayed match-to-sample; SD, stimulus discrimination; CD, compound discrimination; CDR, compound discrimination reversal; IDS, intradimensional set-shifting; EDS, extradimensional set-shifting.
Upward arrow, significant decrease from baseline; downward arrow, significant increase from baseline; ---, no significant change from baseline.

JPET Fast Forward. Published on December 4, 2017 as DOI: 10.1124/jpet.117.244194
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Table 4. Individual and mean (±SEM) [^{11}C]-raclopride DVRs in control and THC-treated monkeys.

	Caudate	Putamen	Ventral Striatum
Control			
R-1710	2.36	2.55	1.51
R-1712	2.14	2.15	1.89
R-1711	2.39	2.15	1.82
R-1681	1.68	2.53	1.99
Mean	2.14	2.35	1.80
±SEM	0.16	0.11	0.10
Δ^9-THC			
R-1710	1.90	2.18	1.67
R-1711	2.35	2.48	1.89
R-1713	2.07	2.62	2.57
R-1682	2.04	2.32	1.54
Mean	2.09	2.40	1.92
±SEM	0.09	0.10	0.23
Difference	-2.3%	+2.1%	+6.7%

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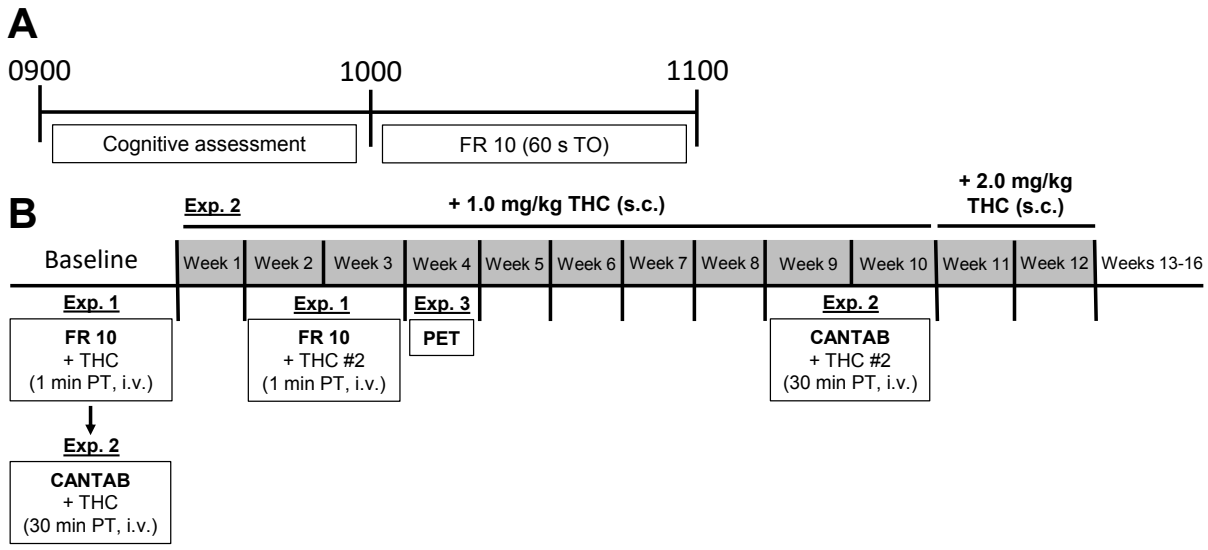

FIGURE 1

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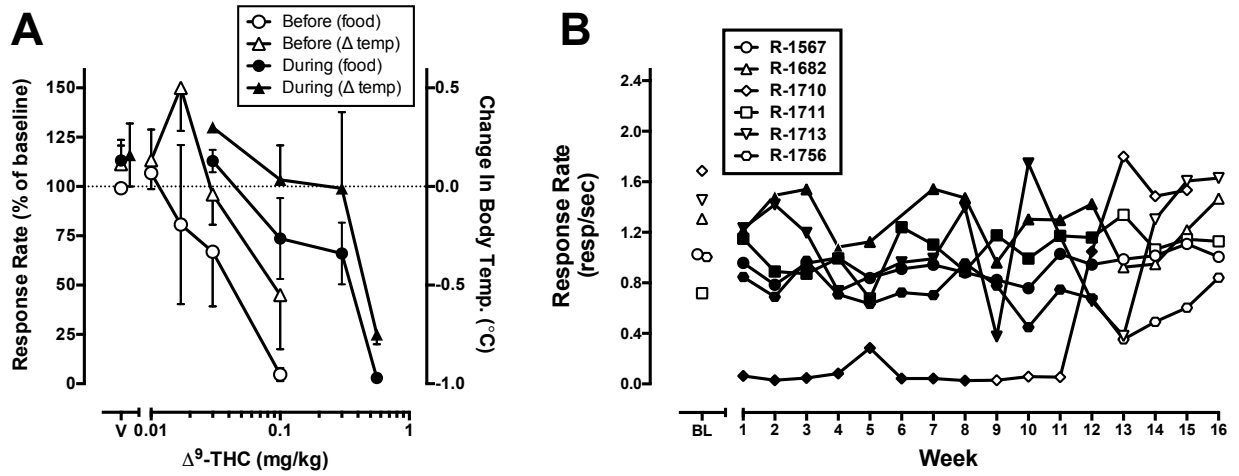

FIGURE 2

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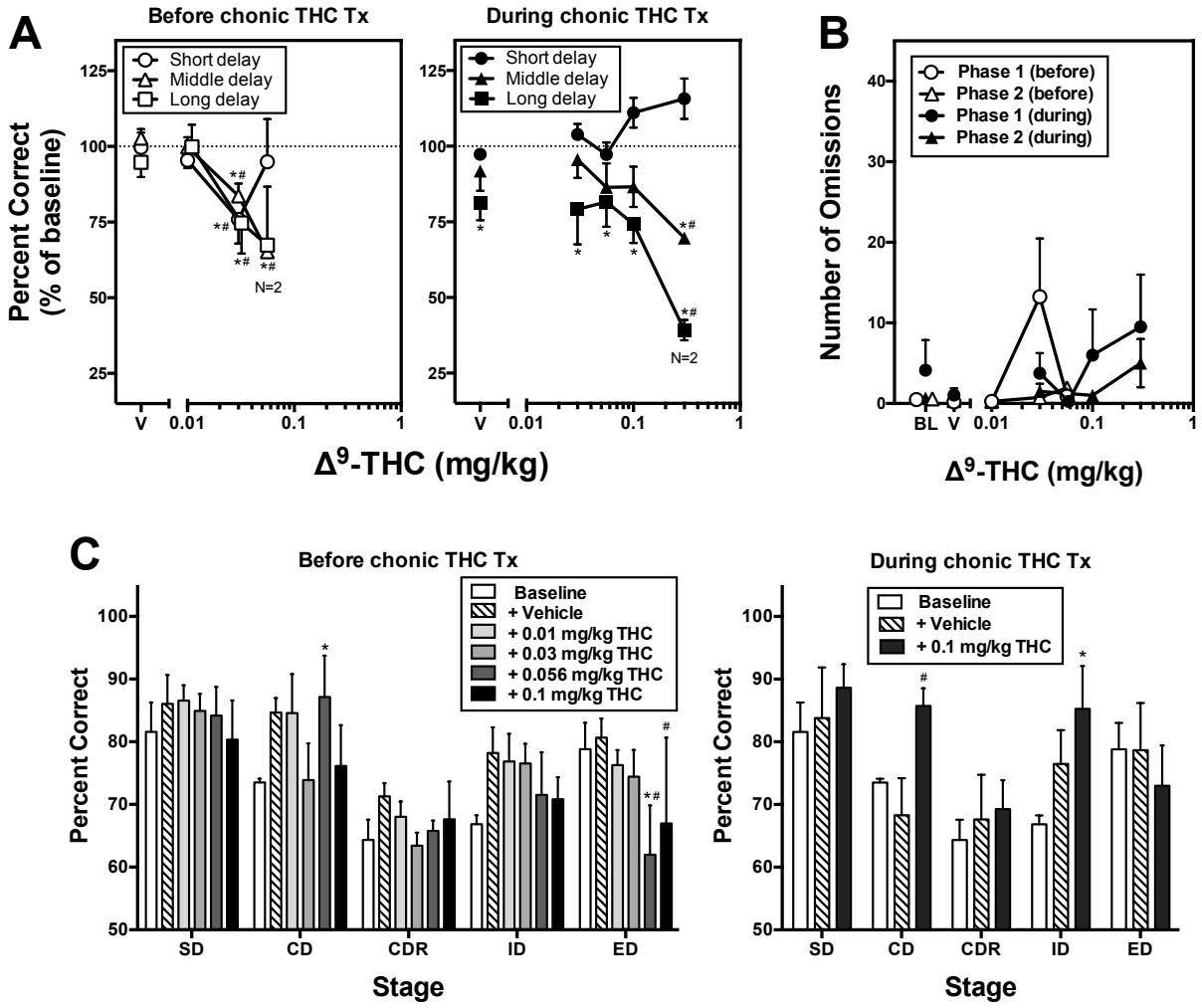

FIGURE 3

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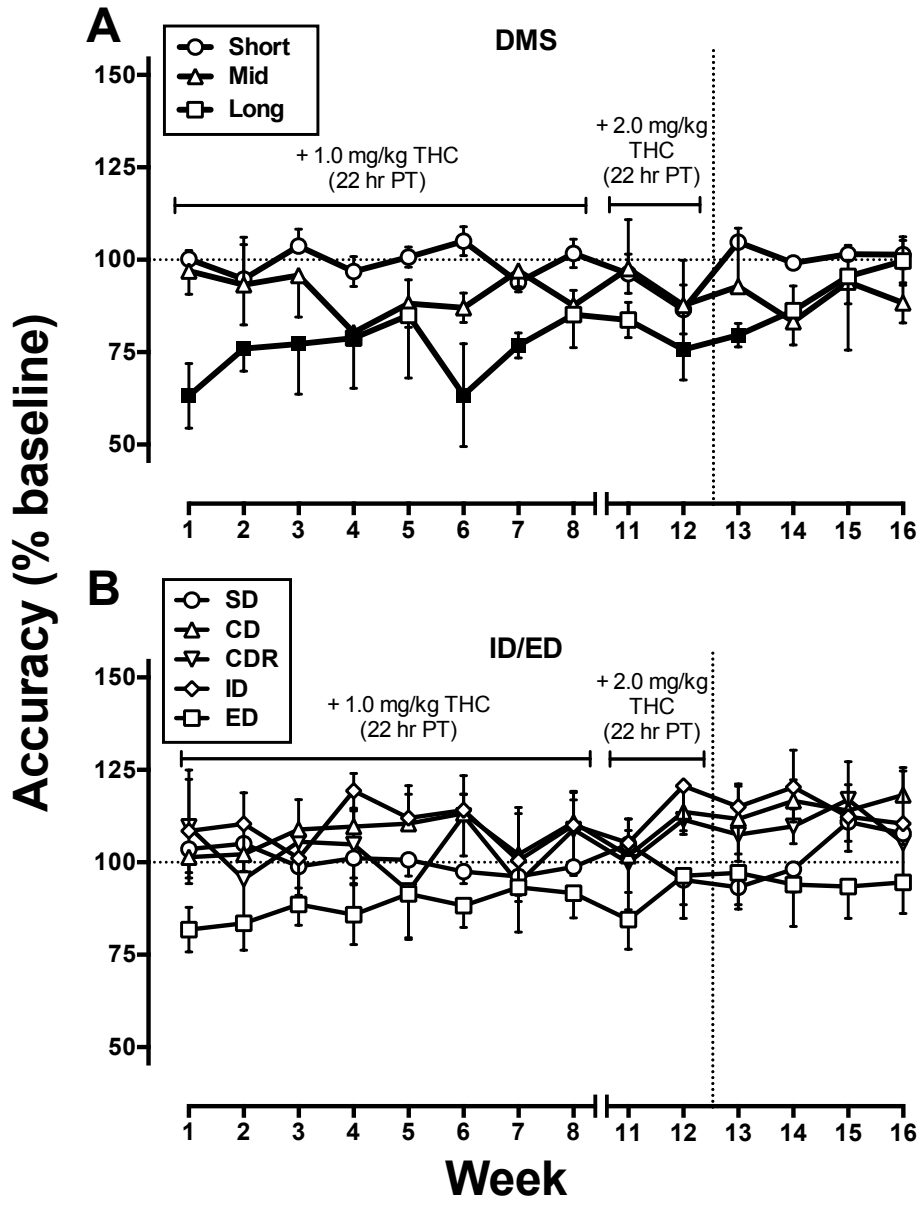

FIGURE 4

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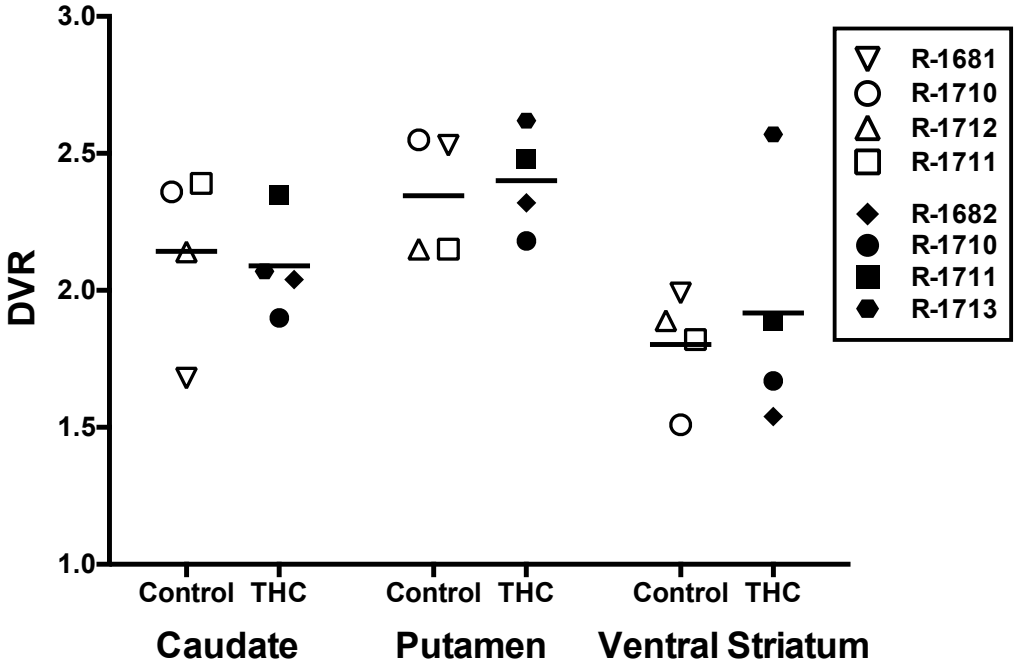

FIGURE 5