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Enhancing effects of acute exposure to cannabis smoke on working memory performance

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Abstract

Numerous preclinical studies show that acute cannabinoid administration impairs cognitive performance. Almost all of this research has employed cannabinoid injections, however, whereas smoking is the preferred route of cannabis administration in humans. The goal of these experiments was to systematically determine how acute exposure to cannabis smoke affects working memory performance in a rat model. Adult male ($n = 15$) and female ($n = 16$) Long-Evans rats were trained in a food-motivated delayed response working memory task. Prior to test sessions, rats were exposed to smoke generated by burning different numbers of cannabis or placebo cigarettes, using a within-subjects design. Exposure to cannabis smoke had no effect on male rats' performance, but surprisingly, enhanced working memory accuracy in females, which tended to perform less accurately than males under baseline conditions. In addition, cannabis smoke enhanced working memory accuracy in a subgroup of male rats that performed comparably to the worst-performing females. Exposure to placebo smoke had no effect on performance, suggesting that the cannabinoid content of cannabis smoke was critical for its effects on working memory. Follow-up experiments showed that acute administration of either 9-tetrahydrocannabinol (0.0, 0.3, 1.0, 3.0 mg/kg) or the cannabinoid receptor type 1 antagonist rimonabant (0.0, 0.2, 0.6, 2.0 mg/kg) impaired working memory performance. These results indicate that differences in the route, timing, or dose of cannabinoid administration can yield distinct cognitive outcomes, and highlight the need for further investigation of this topic.

Keywords

cannabis; marijuana; smoke; working memory; executive function; cannabinoid

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1.0 Introduction:

Cannabis is the most frequently used illicit substance worldwide, and its use is expected to increase with recent changes in its legal status. Cannabis is used widely for recreational and medicinal purposes, but it also has actions on cognition. Most research in human subjects shows that acute cannabis administration induces cognitive impairments. For example, smoking a single cannabis cigarette or acute administration of Δ^9 -tetrahydrocannabinol (THC - the primary psychoactive component of cannabis) impairs performance on tests of divided attention, verbal memory, and working memory, among others (Broyd et al., 2016; Hindocha et al., 2017; Lundqvist et al., 2005; Block and Ghoneim, 1993; Solowij et al., 1995; Solowij et al., 2002; Pope and Yurgelun-Todd, 1996; Bolla et al., 2002; Ilan et al., 2004; Schwartz et al., 1989). Similar impairing effects are observed in animal studies, in which acute administration of THC or synthetic agonists at the cannabinoid receptor type 1 (CB1 receptor; at which THC is a partial agonist) causes deficits in numerous cognitive tasks (Arguello, 2004; Fadda et al., 2004; Jentsch et al., 1997; Varvel et al., 2001; Presburger and Robinson, 1990). These impairments are particularly notable in tests of executive function, including reversal learning, set shifting, and delayed match- and non-match-to-sample working memory tasks (Heyser et al., 1993; Hampson and Deadwyler, 1998, 1999; Cohen and Weintstein, 2018; Wright et al., 2013).

Despite the consistency in findings of cognitively-impairing effects of acute cannabinoid administration in animal models, almost all of these studies employed routes of administration that do not mimic human cannabis use. Whereas smoking is the most prevalent route of cannabis use in humans, most animal studies have employed intraperitoneal or subcutaneous administration. Cannabis smoke inhalation results in distinct patterns of THC pharmacokinetics compared to other routes of administration, and cannabis smoke can contain numerous cannabinoids in addition to THC, which can interact to yield unique neuropharmacological outcomes (Sharma et al., 2012; Agurell et al., 1986; Grotenhermen, 2003). Despite recent developments in cannabinoid vapor delivery (Nguyen et al., 2016; Lefever et al., 2017; McLaughlin, 2018; Manwell et al., 2014), very little preclinical cannabinoid research to date has employed a smoked route of administration, and there has been even less evaluation of the effects of cannabis smoke delivery on cognition (e.g., Niyuhire et al., 2007; Schulze et al., 1989).

There were two main goals of the experiments described herein. The first was to evaluate the effects of acute exposure to cannabis smoke on cognitive function in a rat model. A prefrontal cortex (PFC)-dependent delayed response working memory task (Sloan et al., 2006) was chosen for this evaluation, as a) similar tasks have been shown to be sensitive to acute cannabinoid administration in previous studies (Hampson and Deadwyler, 1998, 1999; Cohen and Weintstein, 2018), and b) the task is highly suitable for within-subjects experimental designs, which allow testing of multiple drugs and drug doses in a single subject (Banuelos et al., 2014; McQuail et al., 2016). The second goal was to evaluate sex differences in the effects of cannabis smoke. Males and females differ in CB1 receptor expression in several brain regions including PFC, and are differentially affected by both acute cannabinoid exposure and chronic cannabis use, with females often being more

sensitive than males (Castelli et al., 2014; Crane et al., 2013; Burston, 2010; Reich et al., 2009; Van Laere, 2008).

2.0 Methods:

2.1 Subjects:

Male (n=16) and female (n=16) Long Evans rats weighing 250-300 g upon arrival were obtained from Charles River Laboratories (Raleigh, North Carolina) and individually housed on a 12 h light/dark cycle (lights on at 0700). During behavioral testing, rats had free access to water but were food restricted to 85% of their free feeding weight (approximately 12 g food/day), with their target body weights increased by 5 g/week to account for growth. All animal procedures were performed in accordance with the University of Florida Institutional Animal Care and Use Committee as well as National Institutes of Health guidelines.

2.2 Smoke exposure apparatus and procedures:

Smoke exposure was conducted in a cigarette smoking machine (model TE-10, Teague Enterprises, Davis, CA). During the exposure sessions, rats were double housed in standard polycarbonate rat cages (38 × 28 × 20 cm) containing Sanichip bedding (P.J. Murphy Forest Products) and a wire top. Four cages at a time were placed into the exposure chamber of the smoking machine, and the chamber was sealed. Cannabis or placebo cigarettes were inserted into the ignition chamber, where they were lit and puffed (35 cm³ puff volume, 1 puff per min, 2 s per puff). Smoke from the ignition chamber was pumped into the exposure chamber and exhausted to the building exterior. Each cigarette required approximately 10 puffs to burn completely, after which it was replaced with a fresh cigarette and the process repeated. Toward the end of each exposure session, both carbon monoxide (CO) and total suspended particulate matter (TSP) were measured. CO measurements were conducted with a continuous CO monitor (Monoxor III, Bacharach, New Kensington, PA USA). TSP measurements were obtained by drawing smoke from the exposure chamber through a pre-weighed filter (Pallflex Emfab Filter, Pall Corporation, Port Washington, NY, USA) for 2 min. To calculate the TSP, the total weight gained by the filter was divided by the volume of airflow through the filter (Bruijnzeel et al., 2016; Bruijnzeel et al., 2011a; Bruijnzeel et al., 2011b; Small et al., 2010; Yamada et al., 2010). TSP and CO measurements were as follows: mean (SEM) cannabis TSP: 1 cigarette: 57.48 (2.42); 3 cigarettes: 121.04 (4.13); 5 cigarettes: 226.1 (21.14); cannabis CO: 1 cigarette: 250.759 (11.13); 3 cigarettes: 347.33 (7.42); 5 cigarettes: 464.75 (9.71); placebo TSP: 1 cigarette: 53.93 (1.34); 3 cigarettes: 102.77 (10.61); 5 cigarettes: 81.91 (6.94); placebo CO: 1 cigarette: 240.75 (14.40); 3 cigarettes: 359.00 (16.89); 5 cigarettes: 404.33 (7.88). At the completion of smoke exposure and TSP and CO measurements, there was a 15 min purge period to evacuate smoke from the exposure chamber, after which the rats were removed and brought to the operant chambers for testing in the delayed response task.

2.3 Working memory testing:

2.3.1 Apparatus: The apparatus and procedures for the delayed response task were identical to those reported previously by our lab (Beas et al., 2013). Testing was performed in 12 identical operant test chambers (Coulbourn Instruments) housed in sound attenuating

cabinets. The front wall of each chamber contained a food pellet delivery trough. The trough was located 2 cm above the floor and contained a photobeam to detect nosepoke entries into the trough as well as a 1.12 W lamp to illuminate the trough. A retractable lever (11 cm from the floor) was located on either side of the trough. The floor of the chamber was composed of stainless steel rods. A 1.12 W house light was mounted on the rear wall of each sound attenuating cabinet. Each chamber had an infrared activity monitor mounted on the ceiling, which allowed for locomotor activity assessment during the task. All chambers were interfaced with a computer running Graphic State 4.0 software (Coulbourn Instruments) to allow for experiment control and data collection.

2.3.2 Behavioral testing:

2.3.2.1 Shaping: Prior to testing in the delayed response working memory task, rats went through several stages of shaping in order to learn the various task components. Rats proceeded to each stage of shaping after meeting criterion on the previous stage. Shaping Stage 1 was magazine training, in which the 45 mg food pellets (5TUL soy free, TestDiet) were dispensed into the food-pellet delivery trough every 100 $\pm$ 40 seconds. Rats had to make a minimum of 100 nosepokes into the food trough in 64 min to reach criterion. Sessions in shaping Stage 2 involved learning to press the levers to earn food rewards. One of the levers (either left or right, counterbalanced across rats) was extended into the test chamber, and presses on this lever earned a single food pellet. Criterion was met when rats reached at least 50 presses in a 30 min session. Once criterion was met, rats were shaped in a subsequent session to press the other lever under the same criterion. In Shaping Stage 3, rats received multiple trials on which a single lever (left or right, randomized across pairs of trials) was extended into the chamber, and a press earned a single food pellet. Criterion in this stage was at least 30 presses on each lever in the 60 min session (Beas et al. 2013; Simon et al. 2009).

2.3.2.2 Delayed Response Working Memory Task: The delayed response working memory task was modified from Sloan et al. (2006), and consisted of multiple trials within each 40 min session (Figure 1). Each trial began with a single lever (the “sample” lever) being extended into the operant chamber. The left/right position of the sample lever was randomized within pairs of trials, and the lever was retracted once pressed, which initiated the “delay” phase. During the delay, rats were required to nosepoke into the food trough, in order to minimize the use of “mediating” strategies for performing the task such as remaining in front of the sample lever. The first nosepoke after the delay period expired initiated the “choice” phase, in which both levers were extended. Rats had to press the same lever extended in the sample phase (the “correct” lever) in order to receive a food pellet reward. An “incorrect” press on the opposite lever caused the levers to retract, the houselight to extinguish, and no food delivery. Both correct and incorrect responses were followed by a 5 s intertrial interval, after which the next trial began. The house light was illuminated throughout the sessions except during timeout periods following incorrect responses.

The durations of the delays between the sample and choice phases of the trials were increased over the course of training in the delayed response task. Initially, the delay duration was set at 0 s. Correction trials were used during this phase, such that if an incorrect

lever press was performed, rats were presented with the same sample lever on the next trial. Once rats achieved greater than 80% accuracy over 2 consecutive sessions on a set of delays, the delay durations were increased (delay set 1: 0, 1, 2, 3, 4, 5, 6 s; delay set 2: 0, 2, 4, 8, 12, 16 s; delay set 3: 0, 2, 4, 8, 12, 18, 24 s). Within each set of delays, the order of the delays was randomized within blocks of 7 trials. Delay set 3 was used for all behavioral testing reported in the manuscript (Sloan et al., 2006; Beas et al., 2013).

2.4 Drugs:

Cannabis cigarettes (approximately 700 mg each) were obtained from the NIDA Drug Supply Program, and contained approximately 5.6% THC, 0% cannabidiol (CBD), and 0.4% cannabitol (CBN) as per the accompanying analytical data. Placebo cigarettes, (consisting of cannabis plant material from which cannabinoids were extracted; 0.002% THC, 0.001% CBD, 0.004% CBN), were also obtained from the NIDA Drug Supply Program. Rats were exposed to smoke from 0, 1, 3, and 5 consecutively-burned cigarettes using a randomized, within-subjects design such that each rat was tested in each exposure condition. At least 48 h elapsed between successive exposure sessions with a single type of cigarette. THC in 100% ethanol was obtained from the NIDA Drug Supply Program. The ethanol was evaporated under nitrogen gas, and the remaining THC dissolved in a vehicle containing 5% kollophor, 5% ethanol, and 90% saline (0.9%) and administered i.p. at doses of 0, 0.3, 1.0, and 3.0 mg/kg, 30 min prior to testing. Rimonabant (SR141716A, Sigma) was dissolved in a vehicle containing 5% Tween 80, 20% DMSO, and 75% saline (0.9%) and administered i.p. at doses of 0, 0.2, 0.6 and 2.0 mg/kg, 10 min prior to testing. Rimonabant and THC doses were chosen based on prior studies that assessed their effects on working memory tasks (Panlilio et al., 2011; Deadwyler et al., 2007). Similar to smoke exposure, THC and rimonabant were administered using a randomized, within-subjects design, with at least 48 h between successive injections of a single drug.

2.5 Estrous Cycle Measurements:

Estrous cycle was assessed in female rats 1 h following test sessions in the delayed response task. During the procedure, rats were restrained and vaginal lavages performed using a plastic pipette and sterile saline. Samples were transferred directly to glass slides and assessed under a light microscope to determine estrous cycle phase as in our previous work (Orsini et al., 2016). Samples were collected daily until each phase was detected for each rat. To control for the effects of estrous cycle testing in female rats, male rats received sham lavage procedures, in which they were restrained and their tails lifted for approximately 2 s.

2.6 Experimental Design:

Rats were tested in two cohorts, each with n=8 males and n=8 females (one male rat in the first cohort died of unknown causes during shaping), using identical procedures. The rats were initially trained on the delayed response task until stable performance was achieved (see Statistical Analysis section for definition). At this point, estrous cycle measurements were obtained over the course of 8 days, until data from all 4 cycles were collected from each rat. Rats then underwent testing following smoke and drug administration in the following order: cannabis smoke, placebo smoke, rimonabant, and THC. Stable performance was re-acquired between testing with different smoke or drug conditions (at least 7 days of

testing). Although the order of drug administration was not randomized across cohorts, this design ensured that the data of greatest interest (performance following cannabis smoke) were obtained in naïve rats.

2.7 Statistical Analyses:

Stable choice performance on the delayed response working memory task was assessed separately in each sex using a two-factor repeated-measures ANOVA conducted on data from three consecutive sessions, with session and delay as within-subjects variables. Stability was defined as the absence of a main effect of session or interaction between session and delay. Male and female performance was compared using a two-factor repeated measures ANOVA, with sex as a between-subjects factor and delay as a within-subjects factor. Comparisons across phases of the estrous cycle were conducted using a two-factor repeated measures ANOVA, with both phase and delay as within-subjects variables. Analyses of the effects of smoke exposure and drug administration were conducted separately in males and females. These data were analyzed using two-factor repeated measures ANOVAs, with smoke or drug condition and delay as within-subjects variables. For analyses of choice accuracy, the main effect of delay was always statistically significant, and will therefore not be reported. Analyses of locomotor activity and number of trials completed/session were conducted using one-factor repeated measures ANOVAs, with smoke or drug condition as within-subjects variables. To determine whether the actual delay durations (the time between a press on the sample lever and the nosepoke that initiated the choice phase) were affected by the variables of interests, these data were analyzed using a repeated measures ANOVA, with programmed delay duration as a within-subjects variable, and sex, cigarette, or drug condition as between- or within-subjects variables as appropriate. In all cases, p values ≤ 0.05 were considered significant.

3.0 Results:

3.1 Baseline performance in the working memory task.

Female and male rats were first characterized on the delayed response working memory task (Sloan et al, 2006; Beas et al. 2013; Shimp et al. 2015) in which they had to remember the location of a sample lever over a brief delay period (0-24 s) in order to receive a food reward (Figure 1). Under baseline conditions (prior to any drug testing), female rats showed less accurate choice performance compared to males, particularly at longer delays [sex: $F(1,29)=3.31$ $p=0.08$; sex $\times$ delay: $F(6,174)=2.65$, $p=0.02$; Figure 2A]. In addition, females performed significantly fewer trials than males [sex: $t(14)=3.92$, $p=0.002$], displayed less locomotor activity than males during the test sessions [sex: $t(14)=2.89$, $p=0.01$] (see Table 1), and had longer actual delay durations [sex: $F(1,14)=5.00$, $p=0.04$; sex $\times$ delay: $F(6,84)=4.36$, $p<0.01$] (see Table 2). During this baseline period, estrous cycle was monitored daily in females to assess potential relationships between estrous phase and performance; there were no effects of estrous phase on choice accuracy [phase: $F(3,45)=0.77$ $p=0.52$; phase $\times$ delay: $F(18,270)=1.15$ $p=0.30$]; Figure 2B], nor were there effects on trials completed [$F(3,45)=2.46$, $p=0.08$] or locomotor activity [$F(3,45)=0.87$, $p=0.46$] (see Table 1).

3.2 Effects of acute exposure to cannabis smoke on working memory performance

Following baseline characterization, the effects of acute cannabis smoke exposure on working memory task performance were evaluated (note that data from two rats were excluded due to malfunctions in the operant chambers). In male rats, cannabis smoke exposure had no effect on choice accuracy [exposure: $F(3,42)=0.11$, $p=0.96$; exposure x delay: $F(18,252)=0.83$, $p=0.67$; Figure 3A]. In female rats, however, exposure to cannabis smoke significantly enhanced choice accuracy [exposure: $F(3,39)=3.80$, $p=0.02$; exposure x delay: $F(18,234)=1.67$, $p=0.67$; Figure 3B]. Follow-up two-factor ANOVAs comparing clean air (no cigarettes) and each exposure condition revealed that performance in females was significantly enhanced by smoke from 1 [exposure: $F(1,13)=5.66$, $p=0.03$; exposure x delay: $F(6,78)=2.33$, $p=0.04$], 3 [exposure: $F(1,13)=8.53$, $p=0.01$; exposure x delay: $F(6,78)=2.14$, $p=0.06$] and 5 [exposure: $F(1,13)=8.10$, $p=0.01$; exposure x delay: $F(6,78)=1.22$, $p=0.31$] cannabis cigarettes. In addition to its effects on working memory accuracy, cannabis smoke increased the number of trials completed in females [$F(3,39)=4.32$, $p=0.01$] but not in males [$F(3,42)=0.85$, $p=0.48$] and decreased locomotor activity in both sexes [males: $F(3,42)=3.07$, $p=0.04$; females: $F(3,45)=3.32$, $p=0.03$] (see Table 1) but did not influence actual delay durations in either sex (see Table 2).

Despite the fact that the enhancing effect of cannabis smoke exposure was evident only in female rats, baseline performance in females was significantly lower than in males (see section 3.1). This raises the possibility that the enhancing effects in females were due to their relatively worse baseline performance rather than to sex differences in the effects of cannabis *per se*. To address this issue, female rats were divided into two subgroups via a median split based on their mean choice accuracy under clean air conditions ($n=7$ /subgroup). Cannabis smoke significantly enhanced choice accuracy in the worse-performing subgroup [exposure: $F(3,18)=5.98$, $p<0.001$; exposure X delay: $F(18,108)=1.21$, $p=0.27$; Figure 3C] but had no significant effect on choice accuracy in the better-performing subgroup [exposure: $F(3,18)=1.34$, $p=.29$; exposure X delay: $F(18,108)=1.41$, $p=0.14$; Figure 3D]. For comparison, data were analyzed from the subgroup of male rats ($n=3$) whose performance under clean air conditions fell within the range of the worse-performing females. Within this subgroup of males, cannabis smoke significantly enhanced choice accuracy [exposure: $F(3,6)=3.24$, $p=0.10$; exposure X delay: $F(18,36)=2.24$, $p=0.02$; Figure 3E]. Although this analysis is *post hoc* in nature and would benefit from a larger sample size, it does suggest that enhancing effects of cannabis smoke exposure on working memory are not limited to females, but instead may be more robust under conditions of relatively poor baseline performance (Fishbein et al., 2012; Sarne et al., 2018).

3.3 Effects of acute exposure to placebo smoke on working memory performance

Passive exposure to smoke has the potential to increase arousal, which could account for the enhanced working memory performance observed following cannabis smoke exposure (Berridge & Arnsten, 2013). To address this possibility, rats were exposed to smoke from burning 1, 3, and 5 placebo cigarettes (consisting of cannabis plant material from which cannabinoids are removed) as well as a clean air control condition, following the same procedures used in the cannabis smoke experiment. There was no effect of placebo smoke exposure on choice accuracy in either male [exposure: $F(3,39)=0.80$, $p=0.50$; exposure x

delay: $F(18,234)=1.24$, $p=0.23$; Figure 4A] or female [exposure: $F(3,45)=1.04$, $p=0.38$; exposure x delay: $F(18,270)=0.89$, $p=0.59$; Figure 4B] rats, suggesting that the enhancing effects of cannabis smoke on working memory were due to the cannabinoid (likely THC) content of the cigarettes. Despite the absence of effects on working memory, placebo smoke did cause a reduction in the number of trials completed in females [$F(3,45)=5.1$, $p<0.01$] but not in males [$F(3,42)=0.6$, $p=0.59$], and a reduction in locomotor activity in both females [$F(3,42)=7.27$, $p<0.01$] and males [$F(3,45)=4.8$, $p<0.01$], but had no effects on actual delay durations in either sex (see Table 2). The fact that placebo smoke reduced locomotor activity indicates that it was behaviorally active, and that the enhancing effects of cannabis smoke on working memory accuracy in females were not secondary to its effects on locomotion (see Table 1).

Under clean air control conditions, the female rats performed slightly more accurately during the placebo smoke experiment than they did during the cannabis smoke experiment, raising the possibility that the failure to observe enhancing effects of placebo smoke resulted from a smaller parametric space in which performance could improve. To address this possibility, the effects of placebo smoke were evaluated in the subgroup of female rats ($n=8$) whose clean air control performance during the placebo smoke experiment fell within the same range as the worse-performing female rats during the cannabis smoke exposure experiment. In this subgroup, placebo smoke exposure had no effect on choice accuracy [exposure: $F(3,21)=1.57$, $p=0.23$; exposure x delay: $F(18,126)=0.79$, $p=0.71$]. For comparison, performance in the three worst-performing male rats was also analyzed (although note that these rats' performance under clean air conditions in the placebo smoke experiment fell outside the range of the worse-performing subgroup of female rats). Placebo smoke exposure also had no effect on choice accuracy in this male subgroup [exposure: $F(3,6)=0.54$, $p=0.67$; exposure x delay: $F(18,36)=0.74$, $p=0.75$]. Considered together, these data suggest that exposure to smoke conditions alone is not sufficient to enhance working memory performance.

3.4 Effects of acute rimonabant administration on working memory performance

The results of the smoke exposure experiments suggested that the cannabinoids in the cannabis smoke may cause enhancing effects on working memory performance. As THC exerts many of its actions through CB1 receptors, the role of these receptors in working memory was further investigated via acute administration of the CB1 receptor antagonist rimonabant. Rats received acute injections of rimonabant (0.2, 0.6 and 2.0 mg/kg; Deadwyler et al., 2007) or vehicle followed by testing in the working memory task, using a randomized, within-subjects design. In males, rimonabant had no effect on choice accuracy [dose: $F(3,42)=1.74$, $p=0.17$; dose x delay: $F(18,252)=0.82$, $p=0.67$; Figure 5A]. In females, however, rimonabant caused a significant reduction in choice accuracy [dose: $F(3,45)=6.83$, $p=0.001$; dose x delay: $F(18,270)=1.87$, $p=0.02$; Figure 5B]. Follow-up ANOVAs comparing vehicle and each dose in females revealed a significant difference between vehicle and 0.6 mg/kg [dose: $F(1,15)=2.02$, $p=0.18$; dose x delay: $F(6,90)=2.48$, $p=0.03$], as well as vehicle and 2.0 mg/kg [dose: $F(1,15)=11.13$, $p=0.005$; dose x delay: $F(6,90)=1.73$, $p=0.12$] but not between vehicle and 0.2 mg/kg [dose: $F(1,15)=0.25$, $p=0.63$; dose x delay: $F(6,90)=1.17$, $p=0.33$]. In addition to its effects on choice accuracy, rimonabant caused a significant

reduction in the number of trials completed in both females [$F(3, 45)= 21.68, p< 0.001$] and males [$F(3,42)=5.82, p=0.002$], a significant reduction in locomotor activity in females [$F(3,45)=4.39, p=0.009$] but not males [$F(3,42)=0.33, p=0.80$] (see Table 1), and significant increases in actual delay durations in both males [dose: $F(3, 42)=7.52, p<0.01$] and females [dose x delay: $F(18,270)=3.00, p<0.01$] (see Table 2).

3.5 Effects of acute THC administration on working memory performance

To further investigate the role of CB1 receptors in working memory, a final experiment assessed the effects of acute administration of THC on working memory performance. Rats received acute injections of THC (0.3, 1.0, 3.0 mg/kg; Panlilio et al., 2011) and vehicle followed by testing in the working memory task. In males, THC produced a significant impairment in choice accuracy [dose: $F(3,42)=5.96, p=0.002$; dose x delay: $F(18,252)=3.25, p=0.01$; Figure 6A]. Follow-up ANOVAs comparing vehicle and each dose revealed a significant reduction in choice accuracy at the 3 mg/kg [dose: $F(1,14)=11.77, p=0.004$; dose x delay: $F(6,84)=7.09, p<0.001$] and 0.3 mg/kg [dose: $F(1,14)=0.03, p=0.86$; dose x delay: $F(6,84)=2.22, p=0.049$] doses but not at the 1.0 mg/kg dose [dose: $F(1,14)=2.02, p=0.18$; dose x delay: $F(6,84)=1.85, p=0.10$]. In females, THC administration also produced a significant impairment in choice accuracy [dose: $F(3,45)=5.07, p=0.004$; dose X delay: $F(18,270)=3.03, p<0.001$; Figure 6B]. Follow-up ANOVAs comparing vehicle and each dose revealed a significant reduction in choice accuracy at the 3 mg/kg dose [dose: $F(1,15)=4.90, p=0.04$; dose x delay: $F(6,90)=4.07, p=0.001$] but not at the other two doses [vehicle vs. 0.3 mg/kg: dose: $F(1,15)=0.85, p=0.37$; dose x delay: $F(6,90)=0.38, p=0.89$; vehicle vs. 1 mg/kg: dose: $F(1,15)=0.57, p=0.46$; dose x delay: $F(6,90)=0.95, p=0.46$]. In addition to its effects on choice accuracy, THC caused significant changes in the number of trials completed in both females [$F(3, 45)=4.71, p=0.006$] and males [$F(3, 42)=5.12, p=0.004$], and a significant increase in locomotor activity in males [$F(3,42)=5.12, p=0.004$] but not females [$F(3,45)=1.54, p=0.22$] (see Table 1). In contrast, THC had no significant effects on actual delay durations in either sex (see Table 2).

4.0 Discussion:

The overwhelming majority of research in both animal models and human subjects shows that acute administration of cannabis and cannabinoids induces deficits in tests of cognitive function, including working memory. In contrast, the current experiments show that acute exposure to cannabis smoke enhanced working memory performance in a delayed response task in rats, particularly in females in which baseline levels of task performance were lower than those in males. These results highlight the need for further research on the effects of cannabinoids on cognition, particularly using models that mimic conditions of human intake.

Because the enhancing effects of cannabis smoke on working memory run counter to much of the existing preclinical literature (as well as conventional wisdom), it is important to consider factors that might account for these results. An obvious difference between the current and most prior studies is the route of administration. Most prior animal studies employed cannabinoid injections, whereas the current study used smoke inhalation, which is the most prevalent method of cannabis use in humans (Baggio et al., 2014). Hence, the

different pharmacokinetic profiles of injected vs. inhaled cannabinoids could produce distinct effects on cognitive performance. Arguing against this interpretation, however, are the few animal studies that have assessed effects of cannabis smoke inhalation and have found cognitively-impairing effects, albeit on different behavioral tasks than that used in the current study (Niyuhire et al., 2007; Lichtman et al., 2000; Fried and Nieman, 1973). More importantly, a number of human studies of cannabinoid effects on cognition have employed smoked cannabis and demonstrated impairing effects on working memory. Such data suggest that the smoked route of administration alone cannot account for the enhancing effects on working memory observed in the current study.

Several other factors unique to the context of smoke exposure can also likely be excluded as playing a causal role in the enhancing effects of cannabis smoke on working memory. Cannabinoids aside from THC (e.g., CBD) are thought to mitigate some of the deleterious effects of THC exposure in both rodents and humans (Klein et al., 2011; Izzo et al., 2009; Morgan et al., 2010), suggesting a means by which cannabis smoke (which often contains a rich mix of cannabinoids) might produce different effects from THC alone. The cannabis cigarettes used in the present study contained negligible levels of other cannabinoids, however, suggesting that THC likely accounted for the majority of the pharmacological effects of smoke exposure. Non-specific effects of smoke exposure are another potential contributor to the effects of cannabis smoke on working memory performance, as arousal (such as might be caused by smoke inhalation) can enhance working memory performance, albeit as part of an inverted U-shaped function (Berridge and Arnsten 2013; Arnsten, 2011). The fact that placebo smoke exposure did not enhance task performance, however, renders this explanation less likely.

Yet another possible account of the enhancing effects of cannabis smoke exposure concerns the THC doses to which the rats were exposed. Animal studies of the effects of acute cannabinoid administration on cognition have tended to employ doses that may not be rewarding/reinforcing. For example, Hampson and colleagues showed that in rats, i.p. administration of 1.0 - 2.0 mg/kg THC impaired performance on a delayed non-match to sample task similar in design to the delayed response task used here (Hampson and Deadwyler, 2000); the same doses impaired delayed response performance in the present study. Notably, however, THC doses of 1.0 mg/kg and higher can produce aversive effects in both taste aversion and place preference assays, suggesting that they may not model levels of recreational intake in most people (Elsmore and Fletcher, 1972; Braida et al., 2004; Maldonado and Rodriguez de Fonseca, 2002; Valjent and Maldonado, 2000). Indeed, cumulative daily doses of THC that are self-administered intravenously by rats are roughly 0.02 mg/kg/day (Spencer et al., 2018), which falls within the range that produces a conditioned place preference (e.g., Braida et al. 2004). Along similar lines, Kirschmann et al. (2017) showed that an experimenter-administered dose of the CB1 agonist WIN55,212-2 that impaired performance on an object recognition task in adolescent rats was 5 times higher than the dose of the same drug that was self-administered intravenously (which itself had no effect on object recognition).

Blood levels of THC were not assessed in the rats used in the present study. In a separate study however, we showed that exposing rats to smoke from the 5 cigarette condition in the

same apparatus produced plasma THC levels of approximately 10 ng/ml within minutes of removal from the smoke exposure chamber, as assessed by liquid chromatography-mass spectrometry (Ravula et al., 2018). Although this value is low compared to levels measured in humans following cannabis smoking [(and lower than values we reported previously with the same smoke exposure regimen as measured by ELISA; (Bruijnzeel et al. 2016)], they are comparable to levels in rats 30 min after acute i.p. injection of 3 mg/kg THC (Klein et al., 2011). Given the short half-life of THC in rat blood (30 min) (Klausner and Gingell, 1971), and the fact that the interval between completion of a smoke exposure session and the start of the delayed response task was 20 min, it is likely that THC levels were in the range of 5 ng/ml during the period of task performance (Ravula et al., 2018). Notably, several recent publications have shown that low doses of THC enhance performance in cognitive tasks in both mice and rats. Although the drug administration regimens in these studies [(surgically implanted minipump during testing (Bilkei-Gorzo et al., 2017) or a single THC injection 1-7 days prior to the start of testing; (Sarnecka et al., 2011)] differed substantially from the acute exposure used in the current work, they illustrate that THC may produce cognitively-enhancing effects at doses lower than those that impair cognition (see also Kirschmann et al., 2017; Hill et al. 2006). Interestingly, both Bilkei-Gorzo et al. and Sarnecka et al. found that cognitively-enhancing effects of THC were most evident in animals in which task performance was relatively poor under control conditions. These findings are comparable to those of the present study, in which enhancing effects of cannabis smoke were strongest in the subgroup of female rats whose working memory performance was worse under baseline conditions, as well as in a subset of males whose performance was comparable to these worse-performing females. These latter data further indicate that although cannabinoids are reported to produce sex-dependent effects on some aspects of cognition and behavior (Craft, 2005; Tseng et al., 2004; Castelli et al., 2014), the apparent sex difference in the effects of cannabis smoke on working memory is more likely attributable to a baseline performance difference rather than a true sex difference in THC's effects on the brain. Future work will determine whether enhancing effects of acute cannabis smoke exposure in the delayed response task would be more evident in male rats if baseline performance were made worse (e.g., by increasing the delay durations).

Of the four cigarette/drug conditions tested, only rimonabant caused a significant change (increase) in the actual delay durations (see Table 2). An increase in actual delay durations would be expected to render the task more challenging (as performance is highly delay-dependent), and hence this increase could account for rimonabant's impairing effects on accuracy. It is important to note, however, that such changes in actual delay durations do not account for the effects of cannabis smoke or THC on accuracy, as neither drug affected actual delay durations. This is particularly notable in the case of THC, which impaired accuracy to an even greater extent than rimonabant.

4.1 Potential mechanisms of cannabis smoke-induced enhancement of working memory

Working memory (the ability to hold information “in mind” for relatively brief periods of time) is thought to depend critically on sustained firing activity of PFC pyramidal neurons (Goldman-Rakic; 1995). These pyramidal neurons are modulated by several classes of co-distributed GABAergic interneurons, which have emerged as important regulators of

working memory (McQuail et al., 2015). For example, working memory impairments associated with aging are linked to enhanced PFC GABAergic transmission and reduced pyramidal neuron activity. Blockade of GABAergic signaling (particularly through the GABA(B) receptor) enhances working memory in aged rats, presumably by preventing GABA-induced suppression of pyramidal neuron activity (Carpenter et al., 2016; Banuelos et al. 2014). Notably, CB1 receptors in PFC are enriched on GABAergic interneurons, where they act to inhibit GABA release in an activity-dependent manner (Wedzony and Chocyk, 2009; McLaughlin et al., 2014). This anatomical arrangement suggests that by activating CB1 receptors on PFC GABAergic interneurons, THC could suppress GABA release, disinhibiting pyramidal neuron activity and facilitating working memory. Interestingly, some evidence suggests that PFC CB1 receptor density is lower in female compared to male rats (Castelli et al., 2014). Although speculative, this difference could account for the worse delayed response performance in females compared to males, as well as the greater improvement in females compared to males exposed to cannabis smoke. It will be important in future work to determine how pre-existing PFC CB1 receptor levels contribute to working memory performance and its modulation by cannabinoids. In addition, it will be important to assess the effects of cannabis smoke exposure on other aspects of executive function [e.g., on a set shifting task, in which performance has been shown to be inversely related to working memory (Beas et al., 2013)].

4.2 Sex differences in working memory performance

A secondary finding of these experiments was that under baseline conditions, female rats displayed less accurate choice behavior than males. To our knowledge, sex differences have not been evaluated previously in an operant delayed response task in rodents; however, a number of studies have compared male and female rats' performance in other tests of working memory, including T-maze delayed alternation and radial arm maze tasks (Koss et al., 2011; Gibbs and Johnson, 2008; Barha et al., 2007; Shansky et al., 2004; Jonasson, 2005). This prior work has revealed a range of outcomes (males better than females, females better than males, and no sex differences), as well as sex differences in the stress-sensitivity of task performance. The fact that both the T-maze and the radial arm maze can engage spatial cognition [for which there are documented sex differences (Gibbs and Johnson, 2008; Shansky et al., 2004)] further complicates attempts to understand sex differences in working memory. Notably, however, performance on the delayed response task employed in the present study depends upon an intact PFC but not the hippocampus (Sloan et al. 2006), suggesting that the differential choice accuracy in males and females is due to sex differences in executive rather than spatial functions. An additional factor to consider is that actual delay durations were significantly longer in females compared to males. The extent to which sex differences in working memory accuracy resulted from differences in actual delay durations is unclear; however, the fact that females were also significantly less active than males could be a contributing factor to their longer actual delays (in that less activity could translate into less frequent nose pokes into the food trough to initiate the choice phase of the trials).

The fact that performance in females did not differ across phases of the estrous cycle could be interpreted to mean that ovarian hormones do not strongly modulate task performance.

Although this is a reasonable conclusion in the context of normal variation within the estrous cycle, it does not mean that gonadal hormones are unimportant, even in an “activational” (as opposed to an “organizational”) sense. Indeed, in a previous study that showed better performance in males than females on the working memory component of a radial arm maze task, female performance was impaired by ovariectomy and restored by estradiol administration, demonstrating that gonadal hormones can modulate performance of at least some components of working memory (Shansky et al., 2004). Hence, further investigation is needed to determine how gonadal hormones contribute to the delayed response task employed here.

4.3 Conclusions

The results presented here show that acute exposure to cannabis smoke can enhance performance in a PFC-dependent delayed response working memory task. This effect was most robust in female (and a subset of poor-performing male) rats, but was not likely due to arousal or other non-specific effects of smoke exposure. Instead, the enhancing effects may be attributable to the relatively low dose of THC to which rats were exposed via smoke inhalation, and/or the unique pharmacokinetics of smoke delivery. Given that cannabis smoking is widespread, it will be important in future studies to determine how the conditions under which cannabis and cannabinoids enhance cognitive function are related to conditions of actual human use. Cannabinoid self-administration has historically been challenging to model in animal subjects, and models of inhaled and oral cannabinoid self-administration (the most prevalent routes of human use) are still early in development (e.g., McLaughlin, 2018; Barrus et al., 2018; Lefever et al., 2014). Hence, an important avenue of future work will be to assess cognitive outcomes in animal models that closely mimic the ways in which cannabinoids are used for medicinal and recreational purposes.

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Highlights:

Prior preclinical studies show that acute cannabinoid injections impair cognition

Here, effects of cannabis smoke on working memory were tested in rats

Cannabis smoke improved working memory accuracy

Placebo smoke did not affect working memory accuracy

Enhancing effects are likely due to THC dose and/or route of administration

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Figure 1. Delayed response working memory task.

The delayed response working memory task is comprised of 3 phases. During the first phase (sample phase) the rat is presented with one lever. Once pressed, the lever retracts and initiates the delay phase, which ranges from 0 to 24 seconds. Following the delay phase is the sample phase, in which the rat is presented with both levers. The rat must press the same lever presented in the sample phase to receive a food reward.

Figure 2. Sex differences in working memory performance.

A) Prior to drug testing, female rats were significantly less accurate in working memory performance compared to male rats. B) Estrous cycle phase did not modulate working memory performance in female rats.

Figure 3: Effects of cannabis smoke exposure on working memory performance.

A) Exposure to cannabis smoke had no effect on working memory accuracy in male rats. B) Exposure to cannabis smoke enhanced working memory accuracy in female rats compared to no-exposure control conditions C) Exposure to cannabis smoke enhanced working memory performance in the worse performing subgroup of female rats. D) Exposure to cannabis smoke had no effect on working memory accuracy in the better performing subgroup of female rats. E) Exposure to cannabis smoke enhanced working memory performance in the worst performing subgroup of male rats.

Figure 4. Effects of placebo smoke exposure on working memory performance.

Exposure to placebo smoke had no effect on working memory accuracy in either A) male or B) female rats.

Figure 5. Effects of systemic rimonabant administration on working memory performance.

A) Systemic administration of the CB1 receptor antagonist rimonabant had no effect on working memory accuracy in male rats. B) Systemic administration of rimonabant significantly impaired working memory accuracy in female rats compared to vehicle control conditions.

Figure 6. Effects of systemic THC administration on working memory performance.

A) Systemic administration of THC significantly impaired working memory accuracy in male rats compared to vehicle control conditions. B) Systemic administration of THC significantly impaired working memory accuracy in female rats compared to vehicle control conditions.

Table 1

Trials completed and locomotor activity in the delayed response working memory task in male and female rats

Session	Trials completed	Locomotor activity (units)
Working memory task baseline performance		
Male	133.88 (2.12)*	4198.69 (340.74)*
Female	128.54 (4.05)*	3093.79 (277.37)*
*significant difference between sexes (p<0.05)		
Estrous cycle		
Female		
Proestrus	115.50 (4.30)	3040.50 (311.87)
Estrus	118.50 (4.99)	3191.69 (290.33)
Metestrus	123.75 (2.56)	2982.56 (296.62)
Diestrus	125.63 (2.47)	3123.69 (309.94)
Cannabis smoke exposure		
Male		
0 cigarettes	142.00 (1.78)	4769.93 (379.61)
1 cigarette	142.07 (1.24)	4415.80 (374.31)
3 cigarettes	141.60 (1.50)	4286.20 (395.10)*
5 cigarettes	139.13 (2.97)	4219.40 (416.28)*
Female		
0 cigarettes	119.00 (5.45)	3894.28 (357.72)
1 cigarette	127.93 (4.06)*	3719.64 (380.22)
3 cigarettes	114.71 (6.33)	3140.07 (369.07)*
5 cigarettes	113.36 (6.15)	3216.71 (369.77)*
Placebo smoke exposure		
Male		
0 cigarettes	144.27 (1.11)	4637.67 (381.16)
1 cigarette	140.33 (4.28)	4509.47 (424.19)
3 cigarettes	141.47 (7.30)	4187.80 (441.53)*
5 cigarettes	141.00 (6.79)	4107.87 (427.9)*
Female		
0 cigarettes	128.38 (3.28)	3636.88 (360.10)
1 cigarette	117.63 (5.07)*	3328.56 (341.52)
3 cigarettes	115.81(5.56)*	3334.25 (340.49)*
5 cigarettes	109.06 (2.32)*	2927.75 (353.40)*
Rimonabant		
Male		
0 mg/kg	142.13 (1.79)	4570.33 (397.40)
0.2 mg/kg	141.40 (1.98)	4596.13 (405.97)
0.6 mg/kg	140.20 (2.37)	4469.60 (430.20)

Session	Trials completed	Locomotor activity (units)
2 mg/kg	125.80 (7.21) *	4429.93 (342.52)
Female		
0 mg/kg	112.81 (5.45)	2834.94 (309.46)
0.2 mg/kg	101.06 (5.55)	2889.50 (311.25)
0.6 mg/kg	77.69 (8.45) *	2405.81 (295.42) *
2.0 mg/kg	56.88 (8.79) *	2274.44 (152.15) *
THC		
Male		
0 mg/kg	137.79 (3.06)	4525.33 (501.46)
0.3 mg/kg	141.07 (2.43)	4848.60 (530.18) *
1.0 mg/kg	139.36 (1.69)	4944.80 (503.99) *
3.0 mg/kg	121.14 (7.82) *	4014.67 (396.19)
Female		
0 mg/kg	102.13 (4.52)	3180.38 (406.90)
0.3 mg/kg	113.13 (5.06)	3422.56 (431.34)
1.0 mg/kg	112.63 (4.57) *	3913.9 (949.90) *
3.0 mg/kg	82.38 (10.80)	3472.00 (522.67)

* significant difference from 0 cigarette or vehicle condition (p<0.05)

Table 2:

Mean (SEM) actual delay durations in seconds as a function of programmed delay durations.

Programmed Delay (s)	0	2	4	8	12	18
24						
Baseline						
Males						
24.93 (0.15)	0.55 (0.05)	2.26 (0.06)	4.40 (0.14)	8.35 (0.09)	12.41 (0.12)	18.80 (0.10)
Females						
28.17 (1.44)	0.63 (0.05)	2.42 (0.10)	4.73 (0.24)	9.14 (0.41)	13.50 (0.58)	21.85 (1.26)
Marijuana smoke						
Males						
0 cigarettes						
24.85 (0.15)	0.78 (0.22)	2.27 (0.07)	4.32 (0.10)	8.59 (0.19)	12.39 (0.12)	18.94 (0.17)
1 cigarette						
24.96 (0.20)	0.57 (0.06)	2.34 (0.14)	4.38 (0.11)	8.45 (0.11)	12.59 (0.17)	18.86 (0.17)
3 cigarettes						
24.86 (0.12)	0.53 (0.05)	2.35 (0.06)	4.31 (0.08)	8.38 (0.09)	12.69 (0.32)	18.88 (0.15)
5 cigarettes						
25.26 (0.36)	0.52 (0.03)	2.30 (0.07)	4.43 (0.12)	8.41 (0.11)	12.50 (0.14)	18.96 (0.20)
Females						
0 cigarettes						
26.63 (0.41)	0.63 (0.07)	3.10 (0.61)	4.62 (0.24)	11.03 (1.64)	13.03 (0.31)	21.75 (1.45)
1 cigarette						
26.22 (0.45)	0.62 (0.05)	2.49 (0.20)	4.66 (0.27)	9.17 (0.43)	13.05 (0.44)	20.06 (0.42)
3 cigarettes						
26.57 (0.68)	0.67 (0.10)	2.44 (0.12)	4.52 (0.17)	8.85 (0.27)	13.28 (0.77)	20.12 (0.46)
5 cigarettes						
29.24 (2.11)	0.77 (0.23)	2.47 (0.13)	6.91 (2.19)	8.73 (0.19)	12.74 (0.26)	20.58 (0.92)
Placebo smoke						
Males						
0 cigarettes						
24.70 (0.11)	0.48 (0.06)	2.25 (0.07)	4.23 (0.06)	8.34 (0.10)	12.33 (0.11)	18.61 (0.07)
1 cigarette						
25.92 (1.07)	0.56 (0.09)	2.66 (0.42)	4.57 (0.28)	8.58 (0.29)	14.01 (1.58)	19.45 (0.78)
3 cigarettes						
25.21 (0.22)	0.54 (0.10)	2.42 (0.10)	4.44 (0.19)	8.53 (0.18)	12.47 (0.16)	19.08 (0.27)
5 cigarettes						
24.69 (0.13)	0.56 (0.10)	2.28 (0.07)	4.30 (0.10)	8.43 (0.16)	12.78 (0.42)	18.75 (0.14)
Females						
0 cigarettes						
26.10 (0.31)	0.69 (0.07)	2.47 (0.12)	4.72 (0.18)	9.08 (0.37)	13.03 (0.33)	19.89 (0.34)
1 cigarette						
26.66 (0.40)	0.73 (0.09)	2.43 (0.13)	4.86 (0.30)	9.14 (0.35)	13.17 (0.34)	20.96 (0.63)
3 cigarettes						
27.11 (0.64)	1.35 (0.74)	2.80 (0.42)	8.08 (3.42)	9.06 (0.27)	15.43 (2.40)	20.91 (0.83)
5 cigarettes						
26.88 (0.49)	0.75 (0.09)	2.64 (0.22)	4.92 (0.30)	9.61 (0.54)	13.09 (0.31)	24.25 (2.64)
Rimonabant						
Males						

Programmed Delay (s) 24	0	2	4	8	12	18
0 mg/kg 24.86 (0.16)	0.47 (0.04)	2.26 (0.07)	4.27 (0.07)	8.40 (0.13)	12.56 (0.20)	18.82 (0.15)
0.2 mg/kg 24.88 (0.20)	0.46 (0.04)	2.27 (0.07)	4.36 (0.13)	8.57 (0.20)	12.68 (0.29)	19.01 (0.33)
0.6 mg/kg 24.81 (0.13)	0.48 (0.05)	2.35 (0.09)	4.36 (0.10)	8.48 (0.13)	12.55 (0.16)	18.93 (0.15)
2.0 mg/kg 26.21 (0.77)	0.53 (0.07)	2.38 (0.12)	4.72 (0.27)	9.00 (0.47)	13.76 (0.95)	20.26 (1.01)
Females						
0 mg/kg 28.36 (0.95)	0.56 (0.03)	2.52 (0.16)	4.72 (0.21)	9.08 (0.31)	14.23 (1.07)	21.20 (0.57)
0.2 mg/kg 29.96 (1.53)	0.60 (0.05)	3.11 (0.66)	5.91 (1.29)	10.50 (0.97)	13.63 (0.57)	22.42 (1.05)
0.6 mg/kg 28.32 (0.62)	0.94 (0.20)	3.53 (0.56)	6.17 (1.11)	10.16 (0.81)	13.90 (0.59)	24.66 (1.00)
2.0 mg/kg 52.18 (11.28)	1.59 (0.34)	11.72 (8.55)	13.45 (7.11)	15.52 (3.75)	15.25 (1.56)	32.43 (7.66)
THC						
Males						
0 mg/kg 24.85 (0.15)	0.78 (0.22)	2.28 (0.07)	4.32 (0.10)	8.59 (0.19)	12.39 (0.12)	19.94 (0.17)
0.3 mg/kg 24.96 (0.20)	0.57 (0.06)	2.34 (0.14)	4.38 (0.11)	8.45 (0.11)	12.59 (0.17)	18.86 (0.17)
1.0 mg/kg 24.86 (0.12)	0.53 (0.05)	2.35 (0.06)	4.31 (0.08)	8.38 (0.09)	12.69 (0.32)	18.88 (0.15)
3.0 mg/kg 25.26 (0.36)	0.52 (0.03)	2.30 (0.07)	4.43 (0.12)	8.41 (0.11)	12.50 (0.14)	18.96 (0.20)
Females						
0 mg/kg 26.44 (0.38)	0.62 (0.06)	3.06 (0.54)	4.70 (0.22)	10.80 (1.44)	13.10 (0.28)	21.64 (1.28)
0.3 mg/kg 26.11 (0.40)	0.62 (0.05)	2.45 (0.18)	4.81 (0.26)	9.12 (0.37)	13.01 (0.39)	19.95 (0.39)
1.0 mg/kg 26.40 (0.61)	0.74 (0.12)	2.43 (0.11)	4.53 (0.15)	8.91 (0.24)	13.21 (0.67)	20.07 (0.40)
3.0 mg/kg 28.69 (1.88)	0.72 (0.21)	2.46 (0.12)	6.63 (1.91)	8.77 (0.19)	12.70 (0.23)	20.36 (0.82)