Effects of adolescent THC exposure on the behavioral effects of cocaine in adult Sprague-Dawley rats

Amy L. Friedman, and Dr. Emily M. Jutkiewicz, Ph.D.

Department of Pharmacology, University of Michigan Medical School, Ann Arbor, MI

ABSTRACT

Marijuana is the most popular illegal drug used in America among adolescents. Exposure to marijuana's main psychoactive ingredient Δ9-tetrahydrocannabinol (THC) during adolescence may have enduring effects on behavior during adulthood. This study investigated the effects of chronic adolescent exposure to THC on the reinforcing effects of cocaine and sensitization to the psychomotor stimulating effects of cocaine in male Sprague-Dawley rats. During adolescence (P28-45), rats were given once daily i.p. injections of either vehicle or 1 mg/kg THC. On P90 when considered full adults, we analyzed cocaine self-administration behavior by evaluating (1) within-session cocaine dose-effect curves, (2) acquisition of a small dose of cocaine (0.1 mg/kg/inj), (3) breakpoints on a progressive ratio schedule of reinforcement, and (4) locomotor activity sensitization to cocaine. Rats treated with THC during adolescence showed potentiation of the reinforcing effects of small doses of cocaine and were more likely to acquire self-administration with access to small cocaine doses. However, the breakpoint or motivation for cocaine was unchanged between the two treatment groups under the progressive ratio testing. There was no difference in locomotor sensitization to cocaine, but rats treated with THC during adolescence showed an overall increased response to the psychomotor stimulating effects of cocaine. Together, these results demonstrate that exposure to THC during adolescence alters the reinforcing and psychomotor stimulating effects of cocaine, suggesting that adolescent THC exposure produces long-lasting changes in the brain, specifically in reward systems.

INTRODUCTION

According to the 2012 results from the National Survey on Drug Use and Health, marijuana was the most commonly used illicit drug. Each day, an average of about 6,600 people 12 years and older are introduced to marijuana for the first time. The percentage of past month marijuana users among youths aged 12 to 17 increased from 6.7% in 2008 to 7.2% in 2012, which is now slightly higher than past month cigarette users (6.6%) (SAMHSA, 2013). Among 10th and 12th graders, 4% and 6.5% respectively used marijuana daily (NIDA, 2013). This large prevalence of marijuana use among teens has prompted the National Institute on Drug Abuse to work towards understanding the effects of marijuana and its active ingredients on brain and behavioral development in adolescents.

Adolescence is defined as a gradual developmental phase between childhood to adulthood consisting of rapid physical, emotional, and behavioral changes (Spear, 2000). During this time period, many mammalian species develop skills necessary for independent survival, increasing social affiliations and exploration of novel areas to provide new sources of food, water, and mates (Spear, 2000). As they mature, adolescent rats, like humans, demonstrate an increase in risk-taking and novelty-seeking behaviors while the motivational and reward-related brain regions are undergoing changes in development (Doremus-Fitzwater *et al*, 2010). These tendencies may drive adolescents to experiment with drugs of abuse. In fact, some previous studies have demonstrated that drugs may be more reinforcing to adolescents. Studies in rats with short daily access to morphine show that adolescents may have heightened sensitivity to the reinforcing effects of opioids (Doherty *et al*, 2009). There is some evidence that adolescents are particularly sensitive to the novel stimuli produced by

drugs, which could make them particularly vulnerable to drug abuse. For example, adolescent rats were more sensitive to the reinforcing effects of morphine in a cue-induced reinstatement procedure (Doherty *et al*, 2009) and nicotine in the conditioned place preference test (Shram and Lê, 2010).

Additionally, many plastic changes occur in the brain during adolescence, including synaptogenesis and synaptic pruning, myelination, and changes in neurotransmitter concentrations and receptor number, making the adolescent brain especially vulnerable to disruption of normal neurobiological development (Rice and Barone Jr., 2000). The high prevalence of marijuana usage among adolescents draws interest to the role of the endocannabinoid system in neurodevelopment. In rats, the number of cannabinoid receptors in the brain (CB1) increase during puberty, reaching maximum levels during adolescence and decreasing afterwards during adulthood (Rodríguez De Fonseca *et al*, 1993). Higher concentrations of CB1 receptors during adolescence may represent a period of vulnerability to the effects of exogenous cannabinoids, potentially resulting in deleterious effects on normal development upon over-activation of this system. A recent study examined the relationship between age of onset of marijuana use and white matter microstructure, finding that earlier marijuana use was associated with lower white matter fiber tract integrity possibly due to interruption of normal myelination (Gruber *et al*, 2014).

Further research has shown that adolescent THC exposure in rats produces elevated levels of the endogenous cannabinoid anandamide in the nucleus accumbens (NAc) in adulthood, which is implicated in reward-related behavior (Ellgren *et al*, 2008). Evidence suggests that the endogenous cannabinoid system is an activity-dependent

modulator of dopamine transmission, as shown by sensitization to the motor effects of dopaminergic agonists upon CB1 stimulation (Rodríguez De Fonseca *et al*, 2001). This suggests that elevations in endocannabinoid levels may produce enduring changes in normal dopaminergic signaling, an important neurotransmitter involved in the reward pathway. In addition to the mesolimbic dopamine system, the endocannabinoid system is also a major regulator of glutamate signaling (Bossong and Niesink, 2010). Glutamatergic projections to the NAc core have also been shown to be involved in drug seeking and reward (LaLumiere and Kalivas, 2008; McFarland *et al*, 2004), and exogenous cannabinoids can disrupt the normal regulatory role of endocannabinoids on this glutamate system (Hoffman *et al*, 2007). Therefore, THC exposure may disrupt normal endocannabinoid system functioning, causing persistent changes in reward processing.

These neural changes as a result of drug use also have long-term consequences on behavior. Studies have revealed that nicotine exposure in adolescence increases nicotine reward in adulthood as measured in a conditioned place preference test (Kota *et al*, 2009), and chronic exposure to THC in adolescence increases heroin self-administration in adulthood (Ellgren *et al*, 2007). These data suggest that adolescent drug exposure can have enduring effects on adult behavior and potential drug abuse later in life.

Consistent with these behavioral findings, epidemiological studies have shown that early marijuana use is a predictor for later drug abuse, with the highest prevalence of heroine, cocaine, and psychotherapeutics users being among those who initiated marijuana use before 15 years old (Gfroerer *et al,* 2002). To further understand this

increased propensity for drug abuse, the present study investigated the effects of adolescent exposure to the active ingredient in marijuana (Δ9-tetrahydrocannabinol (THC)) in male Sprague-Dawley rats on the reinforcing and behavioral effects of cocaine in adulthood.

METHODS

Subjects. Male Sprague-Dawley rats were obtained from Harlan (Indianapolis, IN) on P21, and they were maintained in a temperature- and humidity-controlled environment, with a 12-hour light/dark cycle with lights on at 7:00 AM. All rats were group-housed 2 or 3 per cage from post-natal (P) day 21-P85 with free access to food and tap water before the start of behavioral experiments. Starting on P90, rats self-administering drug or sugar pellets were restricted to 20 g of food per day and singly-housed for the duration of the experiments. Rats used in locomotor activity studies were free feeding and group-housed 2 or 3 per cage. All studies were performed in accordance with the Guide for the Care and Use of Laboratory Animals, as adopted and promulgated by the National Institutes of Health, and all experimental procedures were approved by the University of Michigan Committee on the Use and Care of Animals.

Drugs. THC and cocaine were obtained from the National Institute on Drug Abuse (Bethesda, MD). Ketamine was purchased from Henry Schein (Denver, PA) and xylazine was purchased from Lloyd Laboratories (Shenandoah, IA). THC was diluted in 20% ethanol, 20% alkamul, and 60% sterile water. All other drugs were dissolved or diluted in sterile saline.

Surgery. For self-administration experiments, rats (P84-85) were implanted with chronic indwelling catheters in the left femoral vein under ketamine:xylazine (90 : 10 mg/kg intraperitoneal (IP) anesthesia as previously described (Collins and Woods, 2007). Catheters were tunneled under the skin and attached to stainless steel tubing, which exited the body through a mesh button sutured between the scapulae. Immediately and 24 h after surgery, rats were given 5 mg/kg carprofen s.c. Following the surgical procedure, rats were singly housed and given 5-7 days to recover post-surgery before self-administration. Catheters were flushed daily with 0.5 ml of heparinized saline (50U/ml) during recovery, and before the start and after completion of self-administration sessions.

Apparatus. All drug and food self-administration sessions were conducted in operant conditioning chambers (30.5 cm W x 24.1 cm D x 21 or 29.2 cm H; Med Associates, St Albans, VT) placed inside sound-attenuating cubicles. Each chamber was equipped with two nose-poke devices (ENV-114BM; Med Associates) positioned 3 cm above the stainless steel grid floor and a white house light on the top of the wall on the same side as the nose-poke devices. Each chamber was equipped with a syringe driver (PMH-107; Med Associates) that delivered solutions through Tygon tubing connected to a fluid swivel (Instech Laboratories, Plymouth Meeting, PA) and spring tether, which was held in place by a counterbalanced arm. For food self-administration sessions, chambers were also equipped with a pellet dispenser (ENV-203M-45) and trough-type pellet receptacle (ENV-200R2M). Locomotor activity was measured in Plexiglas chambers (44.5 cm W x 44.5 cm D x 20.5 cm H) with grids of infrared light beams to detect

horizontal and vertical motion detected as beam breaks (Columbus Instruments, Columbus, OH).

Experimental Design

Adolescent treatments. On P28-45 (Spear, 2000), adolescent rats were treated once daily with either vehicle solution or 1 mg/kg THC via intraperitoneal (i.p.) injection. After P45 and before the start of behavioral experiments, rats were left undisturbed in their cages with the exception of weekly weight determinations and twice weekly cage changes. Separate groups of rats (N=6-9 rats per treatment group) were used in each of the experiments 1-5 listed below (see Figure 1).

Cocaine or sugar pellet self-administration. Each operant chamber was equipped with two nose-pokes, one of which was illuminated with a yellow stimulus light (active nose-poke) and one that was not illuminated (inactive nose-poke). The active and inactive nose-pokes were counterbalanced throughout each experiment. Completing the response requirement on the active nose-poke produced reinforcer delivery with simultaneous turning off of the nose-poke lights and illumination of the house light. Responses on the inactive nose-poke were recorded but had no programmed consequence. Following reinforcer delivery, there was a 10 sec blackout (no stimuli, no reinforcer availability) during which responses were recorded but had no scheduled consequence.

Experiment 1: Cocaine self-administration: multiple dose procedure. On P90, rats were placed in operant conditioning chambers for daily self-administration sessions. Rats began with 60-min training sessions during which cocaine was available on a fixed ratio (FR) 1 schedule of reinforcement, such that rats received one intravenous infusion of

0.32 mg/kg/injection cocaine for each active nose-poke response. The rats were trained for 2 weeks with increasing FR requirements (FR1, FR2, FR3, and finally FR5). After these two weeks, the rats progressed to a multiple dose procedure (total duration of 133 minutes) for 2 weeks. Rats responded for increasing doses of cocaine in ascending order in 25-min components (0, 0.032, 0.1, 0.32, 1.0 mg/kg/injection) with 2 min blackout between components. The dose was manipulated by holding constant the drug solution concentration and changing the infusion duration (~0, 0.1, 0.3, 1, and 3 sec, respectively).

Experiment 2: Acquisition of a small dose of cocaine. On P90, rats were placed in operant conditioning chambers for daily self-administration sessions for 60 min during which 0.1 mg/kg/injection cocaine was available on a FR1 schedule of reinforcement for 25 sessions.

Experiment 3: Sugar self-administration. Starting on P90, rats began with 20-min training sessions during which a 45 mg sugar Dustless Precision Pellet (BioServ, Frenchtown, NJ) was available on a FR 1 schedule of reinforcement. The rats were trained for 15 sessions with increasing FR requirements (FR1, FR2, FR3, and finally FR5), and continued to self-administer for an additional 15 sessions on FR5. Acquisition and maintenance of food self-administration behavior was observed.

Experiment 4: Progressive ratio testing. On P90, rats were placed in operant conditioning chambers for daily self-administration sessions for 60 min during which 0.32 mg/kg/injection cocaine was available on a FR1 schedule of reinforcement for 7 days. After these 7 days of training, rats were evaluated in a progressive ratio (PR) schedule of reinforcement for 7 days. Rats were divided into two dose groups to self-

administer either 0.1 or 0.32 mg/kg/injection cocaine on the PR schedule. The progression of increasing response requirements for reinforcement were calculated by the equation: response ratio=(5 x $e^{(0.2 \times \text{infusion number})}$)-5, rounded to the nearest integer (Roberts and Bennett, 1993). For example, the response requirement begins at 1 and progresses through PR steps to 1, 2, 4, 6, 9, 12, 15, 20, 25, 32, 40, 50, 62, 77, 95…The final ratio (breakpoint), or last ratio successfully completed to earn an infusion of cocaine, was recorded daily for each animal.

Experiment 5: Locomotor activity and sensitization. Beginning on P90, rats were removed from their home cages and habituated to the locomotor chamber for 30 min. The rats were injected with saline (i.p.), and 30 min later with escalating cumulative doses of cocaine (1, 3.2, 10, 18 mg/kg i.p.) with 20 min between doses. Data was collected for 60 min after the final dose of cocaine. On P91-94, rats were injected with 15 mg/kg cocaine i.p. and placed into the locomotor chamber for 60 min. After a 10 d drug-free period (P104), rats were removed from their home cages and habituated to the locomotor chamber for 30 min. The rats were injected with saline (i.p.) and 30 min after the saline injection, rats were injected with escalating cumulative doses of cocaine (1, 3.2, 10, 18 mg/kg i.p. with 20 min between doses). Data was collected for 60 min after the final dose of cocaine.

Data Analysis. Adolescent weights were analyzed by two-way ANOVA with repeated measures (2 adolescent treatments X postnatal day), and then averaged per treatment group and analyzed by Student's t-test on the final day of adolescent injections, P45. For self-administration studies, data are presented as the mean number of active/inactive nose-poke responses and reinforcers $\pm$ the standard error of the mean

(S.E.M.) and analyzed by two-way ANOVA with repeated measures (2 adolescent treatments X sessions). Cocaine dose-effect curves in the multiple dose schedule of reinforcement were analyzed 1) by repeated measure ANOVA (5 doses of cocaine X 3 session groups X 2 adolescent treatments) and 2) by calculating the area under curve (AUC) for the daily dose-effect curve for each rat, AUCs were averaged within each treatment group, and analyzed by two-way ANOVA with repeated measures. Locomotor activity was recorded as XY ambulatory beam breaks, defined as the consecutive interruption of two infrared beams in the horizontal plane, and Z total number of beam breaks. Locomotor activity was summed across treatment group and is presented as sum XY ambulatory + Z total number of beam breaks ± S.E.M. Locomotor activity was analyzed by repeated measure ANOVA (4 doses of cocaine X 2 days X 2 adolescent treatments) using PASW Statistics 18 (SPSS, IBM, Chicago, IL). Locomotor activity on days 1 and 16 were compared for 30 min following the 18 mg/kg dose of cocaine for rats treated with vehicle and THC using a two-way ANOVA with repeated measures. Bonferroni tests were used in all post hoc analyses (GraphPad Prism; GraphPad Software, San Diego, CA). Statistical significance was set at $p<0.05$ and trends considered for $p<0.10$.

RESULTS

Effect of THC treatment on body weight. All rats gained approximately 5-8 g per day from P28-45 independent of adolescent treatment condition. Rats treated with THC showed a less rapid increase in body weight during adolescent injections, resulting in lower body weight compared to vehicle-treated rats during the adolescent treatment

period (Fig. 2). Two-way ANOVA with repeated measures revealed a significant main effect of day [$F(22,594)=3480$, $p<0.0001$] and adolescent treatment [$F(1,27)=11.60$, $p=0.0021$]. There was no difference in body weight between rats treated with vehicle and THC at the start of injections on P28 ($p>0.05$). Rats treated with 1 mg/kg THC during adolescence showed significantly decreased body weight compared to vehicle-treated rats during the period of adolescent injections on P31 and 41-45, as well as post-injections on P52, 59, and 80 ($p<0.05$). On the final day of injections, P45, the mean body weight of vehicle-treated rats (197.95 g $\pm$ 2.55) was significantly greater than the mean body weight of THC-treated rats (179.5 g $\pm$ 4.15) [$t(621)=3.910$, $p<0.01$].

Acquisition of responding for 0.32 mg/kg/inj cocaine. Figure 3 shows that adolescent exposure to THC did not affect the acquisition of 0.32 mg/kg/inj cocaine. There was a main effect of session on active NP responses [$F(15,270)=47.70$, $p<0.0001$]. Rats treated with vehicle and 1 mg/kg THC during adolescence increased number of active nose-poke (NP) responses across consecutive sessions as the FR requirement increased (Fig. 3a). There was no main effect of adolescent treatment on active NP responses [$F(1,18)=0.2293$, $p=0.6378$]. Inactive responses remained low throughout, with no significant main effect of session [$F(15,270)=0.8248$, $p=0.6496$] or adolescent treatment [$F(1,18)=1.240$, $p=0.2801$, Fig. 3b]. There was a main effect of session [$F(15,270)=5.968$, $p<0.0001$] on number of infusions earned, as rats earned more infusions across consecutive sessions and as FR requirement increased (Fig. 3c). There was no main effect of adolescent treatment [$F(1,18)=22.63$, $p=0.2439$] on number of infusions earned, demonstrating that THC exposure during adolescence did not affect the acquisition of 0.32 mg/kg/inj cocaine.

Effect of adolescent THC treatment on cocaine dose-effect curves. Figure 4 shows the within-session determinations of the cocaine dose-effect curves, displayed as the number of active NP responses recorded from rats treated with vehicle or THC averaged across days 1-5 (Fig. 4a), 6-10 (Fig. 4b), and 11-15 (Fig. 4c). In the first 25-min component, rats did not receive any infusions of cocaine, and active NP responses were low across all sessions during this component. Both treatment groups showed upward shifts in the cocaine dose-effect curves across days. In sessions 1-5 and 6-10, rats responded the greatest amount for 0.1 mg/kg/inj cocaine in both treatment groups. In sessions 11-15, rats treated with vehicle responded the greatest amount for 0.1 mg/kg/inj cocaine, consistent with earlier sessions, but rats treated with THC responded the greatest amount for a lower dose of cocaine (0.032 mg/kg/inj). Repeated measures ANOVA revealed a significant main effect of day [$F(1,14)=18.673$, $p<0.001$] and cocaine dose [$F(1,14)=61.739$, $p<0.001$]. Analysis of between-subjects effects showed a trend of adolescent treatment [$F(1,14)=3.740$, $p=0.074$]. There was a significant interaction of day by cocaine dose [$F(1,14)=13.11$, $p=0.001$] and cocaine dose by adolescent treatment [$F(1,14)=5.229$, $p=0.013$], with a significant difference in the cocaine dose-effect determinations between vehicle and THC-treated rats. Post hoc analyses reveal that for days 1-5, there was a significant interaction of adolescent treatment by cocaine dose [$F(4,64)=5.457$, $p=0.0008$], with a significant increase in active NP responses in rats treated with THC compared to vehicle at 0.1 mg/kg/inj cocaine ($p<0.001$). For days 6-10, there was a significant interaction of adolescent treatment by cocaine dose [$F(4,64)=4.353$, $p=0.0035$], with a significant increase in active NP responses for rats treated with THC compared to vehicle at 0.032 mg/kg/inj cocaine ($p<0.01$). For days 11-

15 of the multiple dose procedure, there was a significant interaction of adolescent treatment by cocaine dose [$F(4,56)=7.145$, $p=0.0001$], with a significant increase in active NP responses for rats treated with THC compared to vehicle at 0.032 mg/kg/inj cocaine ($p<0.0001$). These data overall suggest a significant upward or leftward shift of the ascending limb of the cocaine dose-effect curve in rats treated with THC compared to rats treated with vehicle during adolescence.

The AUCs of the averaged daily cocaine dose-effect determinations did not display a significant interaction of adolescent treatment by session [$F(24,196)=0.9$, $p=0.59$], but showed a main effect of session [$F(14,196)=9.5$, $p<0.0001$] (Table 1), with an increase over consecutive sessions. The dose-effect functions were significantly elevated in rats exposed to THC during adolescence as compared to vehicle-treated rats, shown by a main effect of adolescent treatment [$F(1,14)=7$, $p=0.02$]. Post hoc analyses revealed a significant difference in the average AUC on day 9 ($p<0.01$).

Acquisition of responding for 0.1 mg/kg/inj cocaine. Figure 5 shows the number of active and inactive NP responses during self-administration sessions for 0.1 mg/kg/inj cocaine on an FR1 schedule of reinforcement. Animals that did not complete 25 sessions (due to failed catheters) were removed from the data set. One rat was also removed after being determined a statistical outlier because its responding differed from the mean by more than two standard deviations. There was a significant interaction of adolescent treatment by session [$F(24,480)=3.975$, $p<0.0001$], a significant main effect of adolescent treatment [$F(1,20)=7.181$, $p=0.0144$] and a significant main effect of session [$F(24, 456)=12.89$, $p<0.0001$] on the number of active NP responses and corresponding infusions of 0.1 mg/kg/inj cocaine (Fig. 5a). Post hoc analyses revealed a significant

increase in number of active NP responses in THC-treated rats compared to vehicle-treated rats during sessions 17-24 ($p<0.05$). There was no main effect of session on the number of inactive NP responses [$F(24, 432)=0.4482$, $p=0.4482$], as the number of inactive NP responses remained low across all sessions for both treatment groups (Fig. 5b).

We also considered the number of sessions it took rats to reach acquisition of responding for cocaine. In order to meet criteria for acquisition, rats were required to consistently make at least 20 active NP responses. There was a difference in the number of rats in each group to meet criteria for acquisition: 6/12 rats in the vehicle-treated group and 2/11 rats in the THC-treated group never acquired. Of the rats that actually acquired self-administration of 0.1 mg/kg/inj cocaine, there was no significant difference in the mean ($\pm$ S.E.M.) number of sessions to reach acquisition between rats treated with vehicle (11.33 $\pm$ 3.432) and THC (9.33 $\pm$ 1.434).

Acquisition of responding for sugar pellets. Figure 6 shows that adolescent exposure to THC did not affect the acquisition of sugar pellets. There was no significant effect of adolescent treatment on the number of active NP responses [$F(1,10)=1.049$, $p=0.3298$, Fig. 6a, d] or the number of reinforcers earned [$F(1,10)=0.6477$, $p=0.4397$, Fig. 6c, f]. There was a significant main effect of session on number of reinforcers earned [$F(29, 290)=23.89$, $p<0.0001$, Fig. 6c, f], with the number of active NP responses increasing as the FR requirement increased (Fig. 6a). The number of sugar pellets earned increased over consecutive sessions for rats treated with vehicle and THC, until stable responding was reached with FR5. During the last 15 consecutive sessions, there was no significant difference in number of reinforcers earned across sessions or between

treatment groups. Inactive NP responses remained low in all sessions (Fig. 6b,e), with no significant main effect of adolescent treatment [$F(1,10)=0.2098$, $p=0.6567$], and a significant main effect of session [$F(29,290)=0.9164$, $p<0.0001$] as the inactive responses decreased across consecutive sessions.

Effect of adolescent THC treatment on breakpoint. Animals acquired cocaine self-administration behavior for 0.32 mg/kg/inj cocaine on an FR1 schedule within 7 days of self-administration (data not shown). Figure 7 illustrates that there was no difference in the mean breakpoints during the 7 days of self-administration on a PR schedule of reinforcement with either 0.32 (Fig. 7a) or 0.1 (Fig. 7b) mg/kg/inj cocaine between groups of rats treated with vehicle or THC during adolescence. The breakpoint was higher for all rats self-administering 0.32 mg/kg/inj cocaine compared to rats self-administering 0.1 mg/kg/inj cocaine. There was a main effect of session on breakpoint for self-administration of 0.32 mg/kg/inj cocaine [$F(6, 54)=9.868$, $p<0.0001$] and 0.1 mg/kg/inj cocaine [$F(6, 54)=3.4$, $p=0.0064$]. Interestingly, breakpoint increased across sessions in rats self-administering 0.32 mg/kg/inj cocaine, but decreased across sessions in rats self-administering 0.1 mg/kg/inj cocaine. Post hoc tests revealed a significant increase in breakpoint from session 1 to session 7 in rats self-administering 0.32 mg/kg/inj cocaine that were treated with vehicle ($p<0.001$) and THC ($p<0.001$). In vehicle-treated rats self-administering 0.1 mg/kg/inj cocaine, there was a significant decrease in breakpoint from session 1 to session 7 ($p<0.05$), but no significant effect in THC-treated rats.

Effect of adolescent THC treatment on locomotor sensitization. Figure 8a shows that locomotor activity counts increased as the dose of cocaine increased in rats treated with

1 mg/kg THC and vehicle during adolescence. Both treatment groups showed locomotor sensitization to cocaine on day 16 compared to day 1 of testing. Repeated measures ANOVA revealed a main effect of day [$F(1,14)=27.660$, $p<0.001$] and cocaine dose [$F(3,42)=84.233$, $p<0.001$] on locomotor activity. There was a significant interaction of day by cocaine dose [$F(3,42)=2.790$, $p=0.006$] and a trend of cocaine dose by adolescent treatment [$F(3,42)=2.46$, $p=0.084$]. Figure 8b shows that rats treated with vehicle and 1 mg/kg THC during adolescence showed similar locomotor activity sensitization to cocaine. Repeated measures ANOVA of locomotor activity counts with 18 mg/kg cocaine revealed a significant effect of day [$F(1, 14)=19.34$, $p=0.0006$] and adolescent treatment [$F(1,14)=6.233$, $p=0.0256$], suggesting that THC-treated rats showed an overall greater locomotor response to the psychomotor stimulating effects of cocaine. There was no significant difference in locomotor activity between rats treated with vehicle and THC on day 1 or on day 16. There was a significant increase in locomotor activity between day 1 and day 16 for vehicle-treated ($p<0.05$) and THC-treated rats ($p<0.01$). These results suggest that both treatment groups showed sensitization to cocaine, with no difference in the degree of sensitization between THC and vehicle-treated rats.

DISCUSSION

This study provides evidence that adolescent exposure to THC has long-lasting effects on behavior in adulthood. Daily exposure to 1 mg/kg THC during adolescence decreased weight gain during treatment, but body weight recovered to nearly the same as vehicle-treated rats during the post-injection period before the start of behavioral experiments. This suggests that adolescent exposure to small doses of THC during

adolescence may have an effect on normal growth and development in rats; however, it is unknown at this point whether THC treatment during adolescence alters food consumption or metabolic rate. Although there is little research on the effect of adolescent THC use on body weight in humans, this is consistent with an epidemiological study in young adults, showing that previous or current cannabis users at age 21 had a lower prevalence of obesity than non-cannabis users (Hayatbakhsh *et al*, 2010). However, in another study of adult males between the ages of 19-30 who smoked marijuana containing 2-3% THC, caloric intake was increased by 40% and showed increases in body weight (Foltin *et al*, 1988). This may suggest different effects of THC on body weight during adolescence and adulthood, but further research is needed.

The present study evaluated the long-term effects of chronic adolescent exposure to THC on cocaine-induced behaviors in adulthood. During acquisition of 0.32 mg/kg/inj cocaine self-administration with increasing FR, there was no difference in the acquisition pattern of rats treated with vehicle or THC during adolescence. However, during daily evaluations of the within-session cocaine dose-effect curve, there were significant differences between rats treated with vehicle and THC, with THC-treated rats responding for more cocaine at lower doses. This effect became more evident across consecutive sessions, especially during the last 5 sessions. In both treatment groups, the cocaine dose-effect curve shifted upwards over time, which could be seen by the increase in the AUC of the dose-effect functions across sessions. Specifically, THC exposure potentiated the reinforcing effects of cocaine at least at small cocaine doses, seen in the elevation of the ascending limb of the dose-effect curves. Future studies will

determine if this is actually a leftward shift in the cocaine dose-effect curve. Also, in the absence of drug, there was no change in the number of responses made on the active nose-poke. This suggests that THC exposure does not alter responding for cocaine-paired stimuli in the absence of drug; however, this needs to be further evaluated through an extinction-reinstatement paradigm.

As further evidence that THC exposure during adolescence enhances the rewarding effects of low doses of cocaine, rats treated with THC showed significant increases in the pattern of self-administration of a small cocaine dose (0.1 mg/kg/inj) as compared with rats treated with vehicle. THC-treated rats were also more likely to acquire self-administration of the low dose of cocaine compared to vehicle-treated rats. This is consistent with our results from the multiple dose procedure, demonstrating that small doses of cocaine were more reinforcing in THC-treated rats.

However, the present study found no difference in breakpoint for cocaine between rats treated with THC and vehicle. As the work requirement to obtain the drug increased, THC-treated rats did not differ from vehicle-treated rats. This suggests that adolescent exposure to THC does not alter motivation for cocaine during adulthood, despite the increased reinforcing effects of small doses of cocaine in THC-treated rats.

We also showed that there were no differences in the self-administration of sugar pellets between rats treated with vehicle or THC. These data suggest that THC exposure during adolescence does not affect the ability to learn operant tasks and does not alter the reinforcing effects of all rewards, but the effect may be specific for drug reinforcers.

The effect of THC exposure on the reinforcing effects of cocaine may be specific to exposure during the critical period of adolescence. Studies have shown that short-term exposure to THC during adulthood has different effects on adult behavior than exposure to THC during adolescence. Panlilio *et al* (2007) demonstrate that after a 3-day THC exposure regimen, adult rats showed a decreased breakpoint in progressive ratio testing, which was interpreted as decreased reinforcing efficacy of cocaine. These findings are not consistent with results of the effects of adolescent THC exposure, emphasizing the importance of studying the effects of cannabinoid exposure while the brain is developing. When the brain is undergoing rapid developmental changes, it may be more sensitive to the effects of THC. Additional studies showed that intermittent THC exposure during adolescence led to increased heroin self-administration in rats (Ellgren *et al*, 2007), and chronic adolescent exposure to a cannabinoid receptor 1 and 2 agonist CP 55,940 can induce long-term behavioral and neural changes, including increased cocaine self-administration (Higuera-Matas *et al*, 2008). These studies are consistent with our findings that exposure to THC during the vulnerable period of adolescence can cause changes in adulthood, as shown by increased cocaine self-administration behavior at low doses of cocaine.

Additionally, our results also showed that chronic THC exposure during adolescence did not increase locomotor sensitization to cocaine, but did increase the overall response to cocaine. However, previous studies showed that chronic THC administration during adolescence increased locomotor activity sensitization to cocaine when tested during adolescence (Dow-Edwards and Izenwasser, 2012). This effect was not seen in studies that exposed adult rats to chronic THC (Panlilio *et al*, 2007) or the

cannabinoid agonist CP 55,940 (Arnold *et al*, 1998). In both of these adult studies, there was no effect on the acute locomotor effects of cocaine or cocaine sensitization. Given our results of increased overall locomotor response to cocaine and evidence of increased locomotor sensitization in previous research (Dow-Edwards and Izenwasser, 2012), there is evidence that the effects of cannabinoid exposure may be different during the critical period of adolescence.

In conclusion, our findings indicate that chronic THC exposure during adolescence may have long-term effects on behavior in adulthood, including increased cocaine self-administration for low doses of cocaine and increased psychomotor effects of cocaine. Therefore, exposure to THC during adolescence may alter normal brain development and maturation, sensitizing the reward- and addiction-related pathways in the brain.

ACKNOWLEDGMENTS

This research was supported in part by a grant from the University of Michigan Substance Abuse Research Center (Innovative Approaches to Investigate Aspects of Drug Use and Abuse Program). Special thanks to Dr. Emily Jutkiewicz for her continued support, invaluable feedback, and outstanding mentorship.

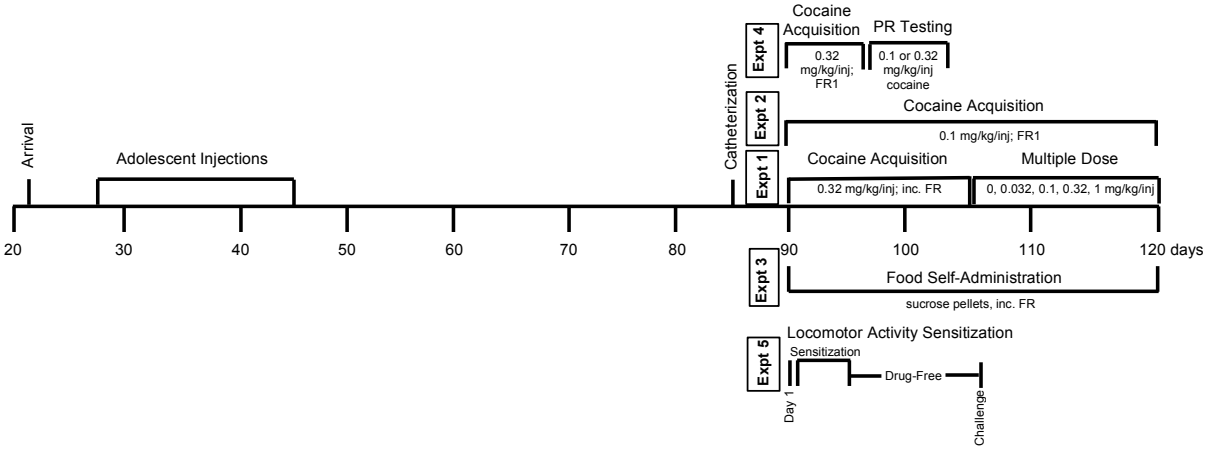

Figure 1. Timeline of experiments

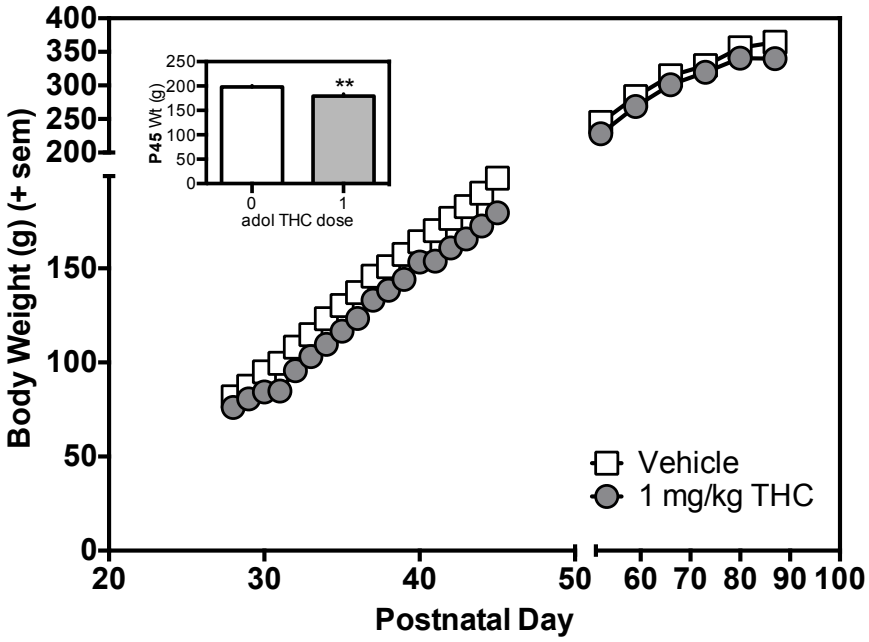

Figure 2. Body weight of rats treated with vehicle or 1 mg/kg THC during adolescence. Data are presented as the mean body weight (g) ± S.E.M. (vehicle N=19, 1 mg/kg THC N=10) through postnatal day 90. **p<0.01 as compared with vehicle treatment.

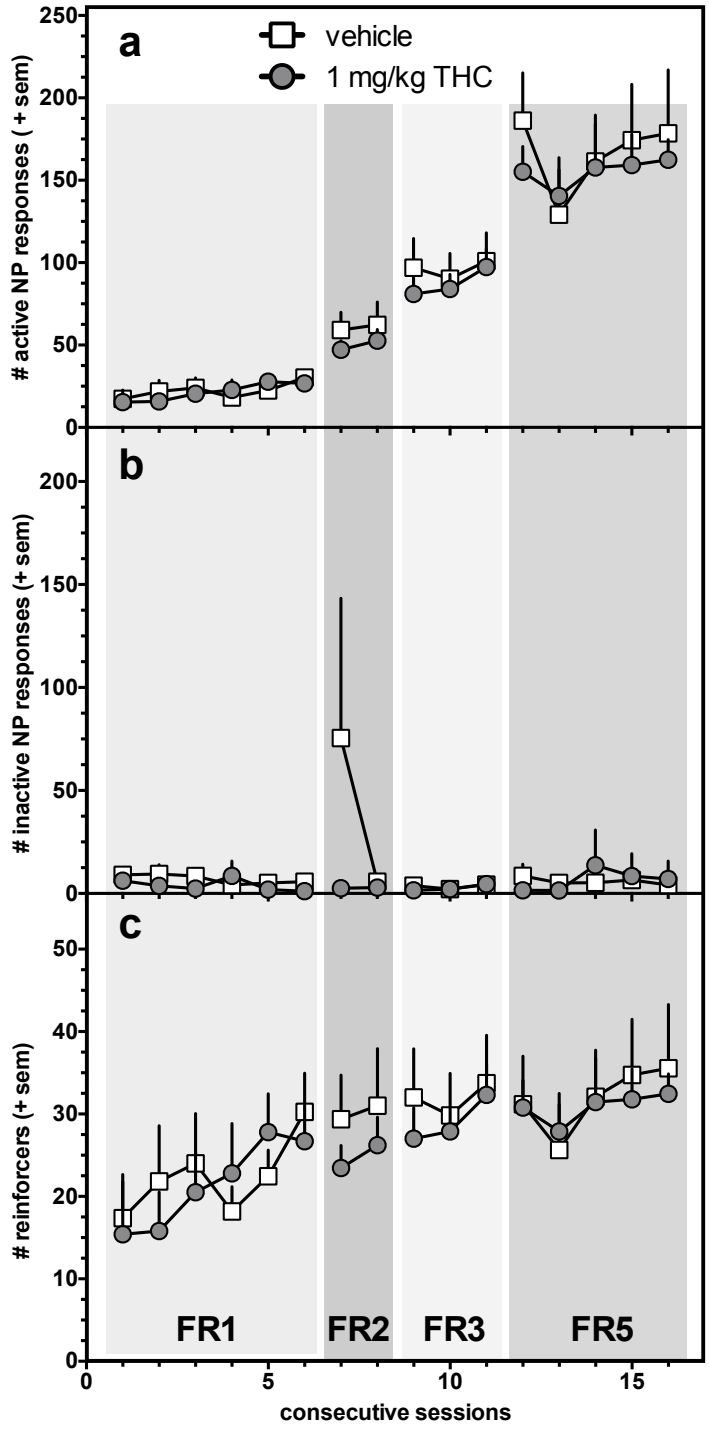

Figure 3. Acquisition 0.32 mg/kg/inj cocaine by rats treated with vehicle or 1 mg/kg THC during adolescence. Data are presented as the mean ± S.E.M. (N=9-11) number of active NP responses (a), number of inactive NP responses (b), and number of reinforcers delivered (c) over 16 consecutive sessions.

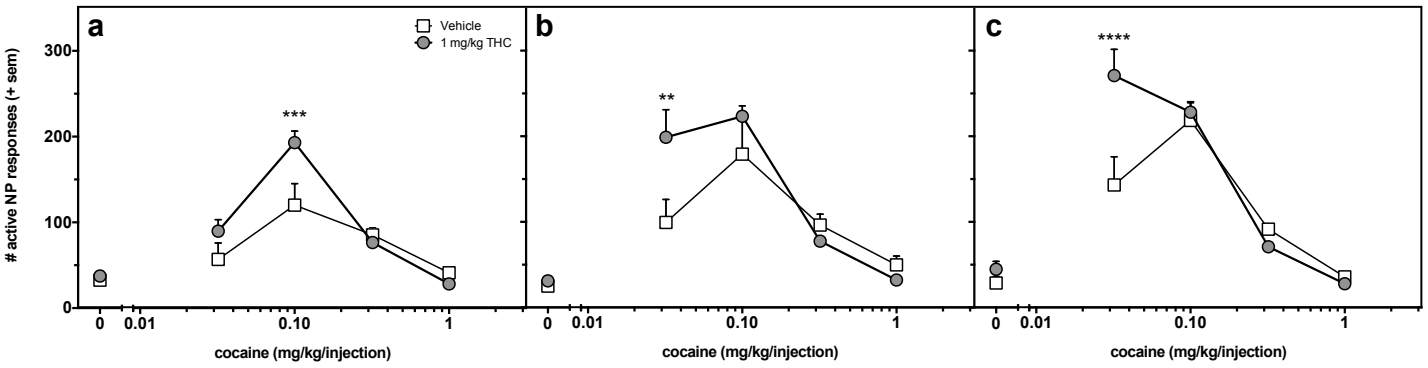

Figure 4. Dose-effect curves for the number of active NP responses for rats treated with vehicle (N=9) or 1 mg/kg THC (N=7) during adolescence presented as mean ± S.E.M. averaged over (a) days 1-5, (b) days 6-10, and (c) days 11-15 of self-administration on the multiple dose schedule of reinforcement (FR5). **p<0.01, ***p<0.001, ****p<0.0001 as compared with vehicle-treated rats at same cocaine dose on same day.

Table 1. Area under curve (AUC) for cocaine dose-effect curves obtained in daily self-administration sessions

	Adolescent Treatment	
Day	**Vehicle**	**1 mg/kg THC**
1	25.5 (6.5)	33.9 (8.2)
2	39.2 (10.5)	61.4 (6.4)
3	45.3 (9.8)	70.9 (5.7)
4	51.5 (10.2)	78.8 (5.2)
5	60.8 (11.5)	81.8 (4.7)
6	57.7 (10.5)	81.2 (6.8)
7	56.7 (12.6)	76.3 (7.3)
8	57.4 (12.1)	77.9 (6.6)
9	44.7 (12.7)	86.7 (6.5)**
10	64.7 (9.8)	93.2 (7.6)
11	69.8 (7.7)	95.5 (5.5)
12	72.9 (12)	84.3 (4.2)
13	71.4 (7.1)	83.1 (5.6)
14	73.8 (8.6)	89.8 (4.9)
15	72.2 (7.9)	85.8 (5)

Data are expressed as averages (± S.E.M.) of individual rat AUCs within each day and adolescent treatment group.
** $p<0.01$ as compared with vehicle treatment

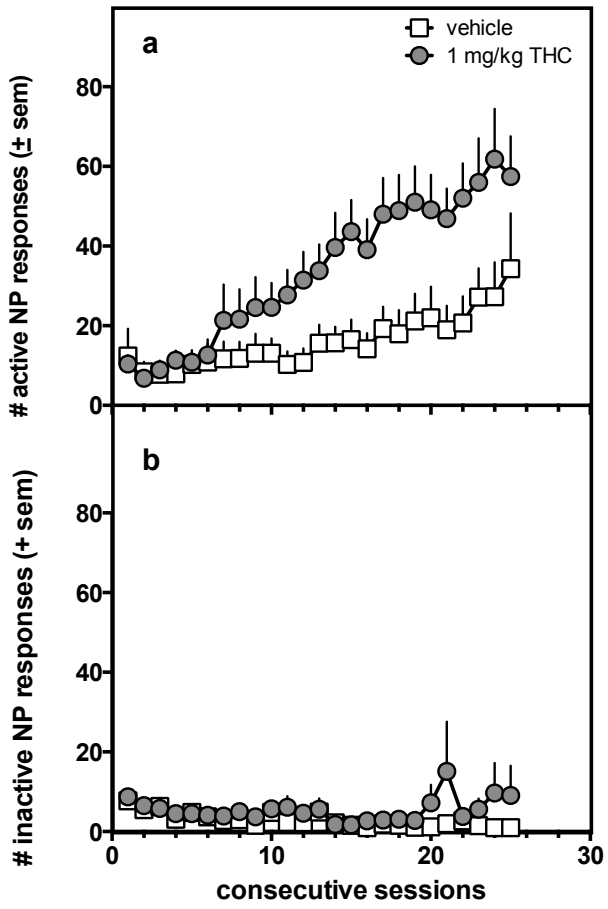

Figure 5. Acquisition of 0.1 mg/kg/inj cocaine by rats treated with vehicle or 1 mg/kg THC during adolescence. Data are presented as the mean ± S.E.M. (N=11) number of active NP responses (a) and number of inactive NP responses (b) over 25 consecutive sessions.

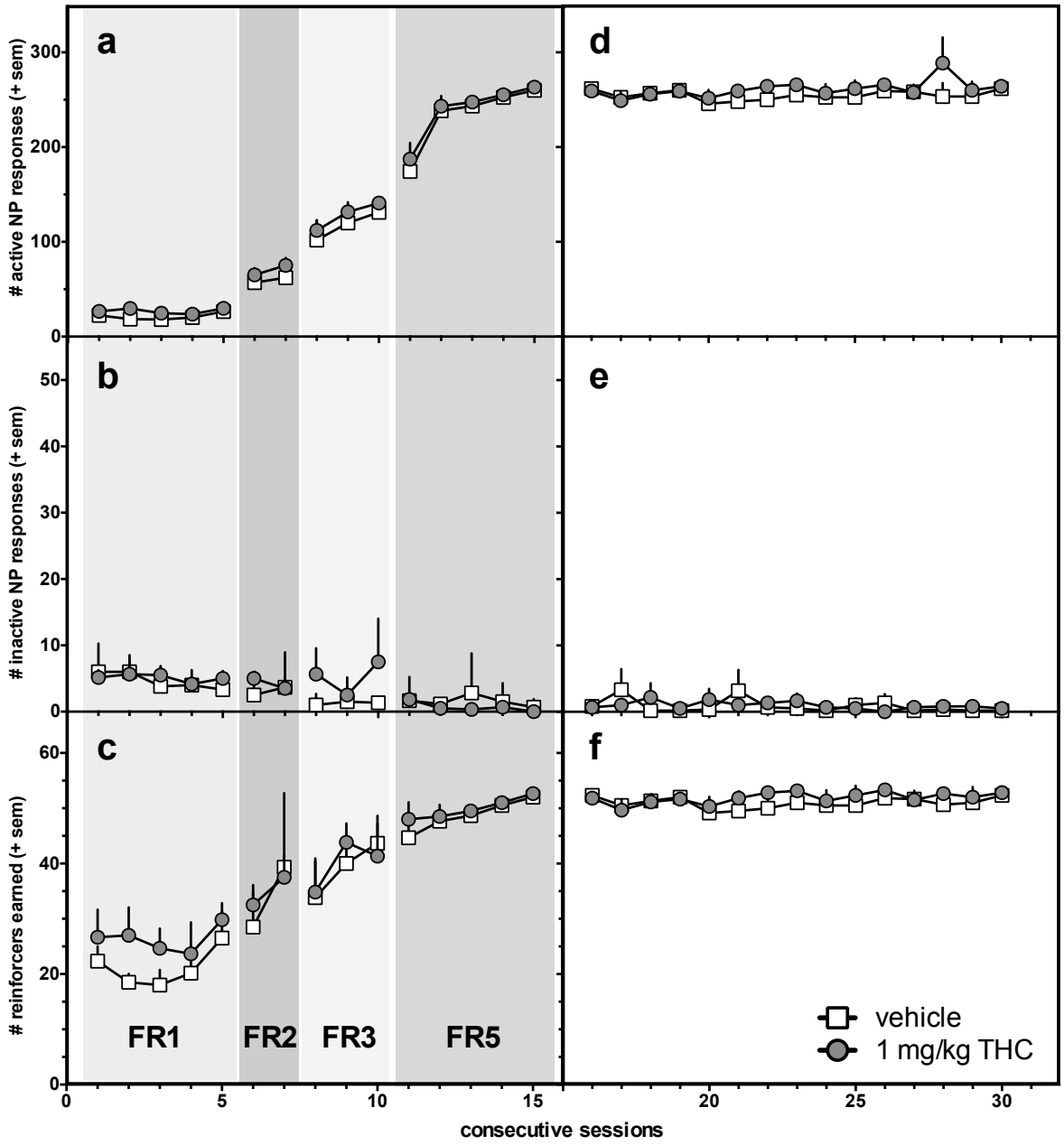

Figure 6. Acquisition responding for sugar pellets by rats treated with vehicle or 1 mg/kg THC during adolescence. Data are presented as the mean ± S.E.M. (N=6) number of active NP responses (a, d), number of inactive NP responses (b, e), and number of reinforcers delivered (c, f) over 30 consecutive sessions.

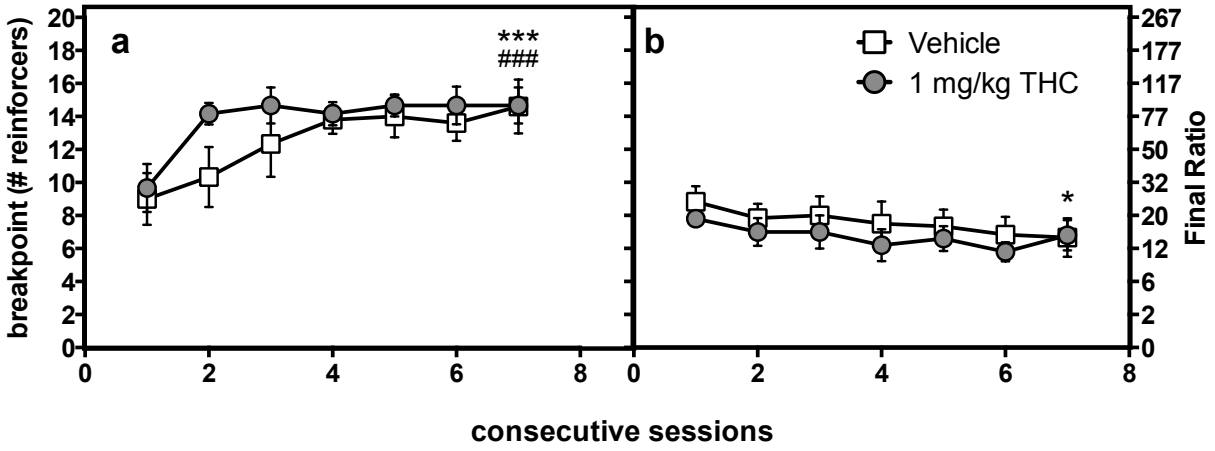

Figure 7. Cocaine self-administration reinforced on a PR schedule of rats treated with vehicle or 1 mg/kg THC during adolescence. Data presented as the mean ±S.E.M. (N=5-6) breakpoints measured in groups of animals self-administering either (a) 0.32 mg/kg/inj cocaine or (b) 0.1 mg/kg/inj cocaine. The final ratio values corresponding to breakpoint are represented on the right *y* axis. *p<0.05, ***p<0.001 as compared with vehicle-treated session 1; ###p<0.001 as compared with THC-treated session 1.

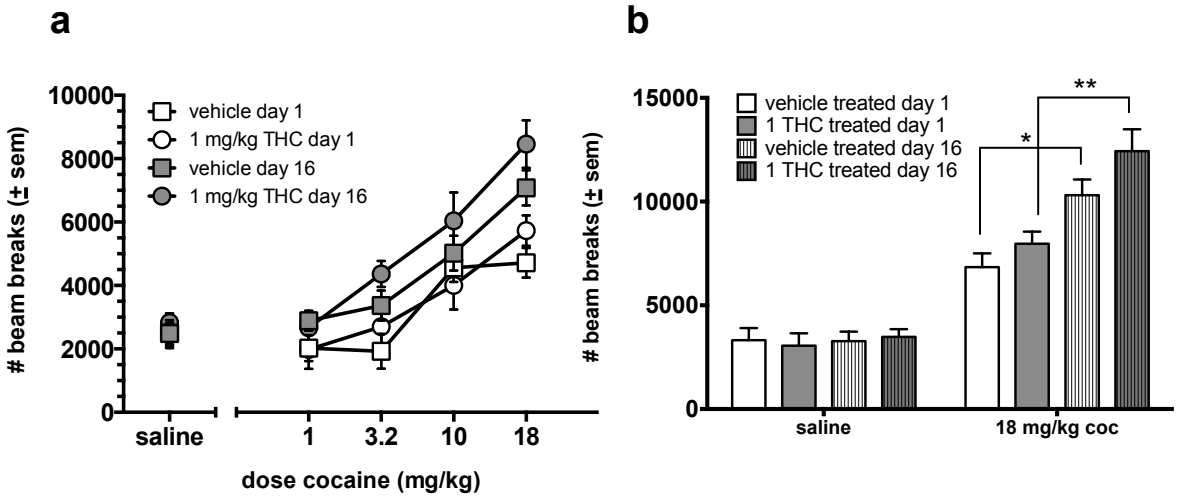

Figure 8. Locomotor activity counts (XY total + Z total beam breaks) for rats treated with vehicle or 1 mg/kg THC during adolescence presented as mean ± S.E.M. (N=8) summed over the first 20 min following each injection of either saline or cocaine on days 1 and 16 (a). (b) shows the mean ± S.E.M. (N=8) number of beam breaks in the first 30 min after saline or 18 mg/kg cocaine injection on days 1 and 16 for rats treated with vehicle or 1 mg/kg THC during adolescence. *p<0.05, **p<0.01

REFERENCES

Arnold JC, Topple AN, Hunt GE, McGregor IS (1998). Effects of pre-exposure and co-administration of the cannabinoid receptor agonist CP 55,940 on behavioral sensitization to cocaine. *Eur J Pharmacol* **354**: 9–16.

Collins GT, Woods JH (2007). Drug and reinforcement history as determinants of the response-maintaining effects of quinpirole in the rat. *J Pharmacol Exp Ther* **3232**: 599–605.

Bossong MG, Niesink RJM (2010). Adolescent brain maturation, the endogenous cannabinoid system and the neurobiology of cannabis-induced schizophrenia. *Prog Neurobiol* **92**: 370–85.

Doherty J, Ogbomnwan Y, Williams B, Frantz K (2009). Age-dependent morphine intake and cue-induced reinstatement, but not escalation in intake, by adolescent and adult male rats. *Pharmacol Biochem Behav* **92**: 164–72.

Doremus-Fitzwater TL, Varlinskaya EI, Spear LP (2010). Motivational systems in adolescence: possible implications for age differences in substance abuse and other risk-taking behaviors. *Brain Cogn* **72**: 114–23.

Dow-Edwards D, Izenwasser S (2012). Pretreatment with Δ9-tetrahydrocannabinol (THC) increases cocaine-stimulated activity in adolescent but not adult male rats. *Pharmacol Biochem Behav* **100**: 587–91.

Ellgren M, Artmann A, Tkalych O, Gupta A, Hansen HS, Hansen SH, *et al* (2008). Dynamic changes of the endogenous cannabinoid and opioid mesocorticolimbic systems during adolescence: THC effects. *Eur Neuropsychopharmacol* **18**: 826–34.

Ellgren M, Spano SM, Hurd YL (2007). Adolescent cannabis exposure alters opiate intake and opioid limbic neuronal populations in adult rats. *Neuropsychopharmacology* **32**: 607–15.

Foltin RW, Fischman MW, Byrne MF (1988). Effects of smoked marijuana on food intake and body weight of humans living in a residential laboratory. *Appetite* **11**: 1–14.

Gfroerer, J. C., Wu, L.-T., & Penne, M. A. (2002). *Initiation of Marijuana Use: Trends, Patterns, and Implications* (Analytic Series: A-17, DHHS Publication No. SMA 02-3711). Rockville, MD: Substance Abuse and Mental Health Services Administration, Office of Applied Studies.

Gruber S a, Dahlgren MK, Sagar K a, Gönenç A, Lukas SE (2014). Worth the wait: effects of age of onset of marijuana use on white matter and impulsivity. *Psychopharmacology (Berl)* **231**: 1455–65.

Hayatbakhsh MR, O'Callaghan MJ, Mamun A a, Williams GM, Clavarino A, Najman JM (2010). Cannabis use and obesity and young adults. *Am J Drug Alcohol Abuse* **36**: 350–6.

Higuera-Matas A, Soto-Montenegro ML, Olmo N del, Miguéns M, Torres I, Vaquero JJ, *et al* (2008). Augmented acquisition of cocaine self-administration and altered brain glucose metabolism in adult female but not male rats exposed to a cannabinoid agonist during adolescence. *Neuropsychopharmacology* **33**: 806–13.

Hoffman AF, Oz M, Yang R, Lichtman AH, Lupica CR (2007). Opposing actions of chronic Delta9-tetrahydrocannabinol and cannabinoid antagonists on hippocampal long-term potentiation. *Learn Mem* **14**: 63–74.

Kota D, Robinson SE, Imad Damaj M (2009). Enhanced nicotine reward in adulthood after exposure to nicotine during early adolescence in mice. *Biochem Pharmacol* **78**: 873–9.

LaLumiere RT, Kalivas PW (2008). Glutamate release in the nucleus accumbens core is necessary for heroin seeking. *J Neurosci* **28**: 3170–7.

McFarland K, Davidge SB, Lapish CC, Kalivas PW (2004). Limbic and motor circuitry underlying footshock-induced reinstatement of cocaine-seeking behavior. *J Neurosci* **24**: 1551–60.

National Institute on Drug Abuse (2013). Monitoring the future study: Trends in prevalence of marijuana/hashish for 8th-graders, 10th-graders, and 12th-graders. Retrieved from http://www.drugabuse.gov/drugs-abuse/marijuana

Panlilio L V, Solinas M, Matthews SA, Goldberg SR (2007). Previous exposure to THC alters the reinforcing efficacy and anxiety-related effects of cocaine in rats. *Neuropsychopharmacology* **32**: 646–57.

Rice D, Barone Jr. S (2000). Critical Periods of Vulnerability for the Developing Nervous System : Evidence from Humans and Animal Models. *Environ Health Perspect* **108**: 511–533.

Roberts DCS, Bennett SAL (1993). Heroin self-administration in rats under a progressive ratio schedule of reinforcement. *Psychopharmacology (Berl)* **111**: 215–218.

Rodríguez De Fonseca F, Gorriti MA, Bilbao A, Escuredo L, García-Segura LM, Piomelli D, *et al* (2001). Role of the endogenous cannabinoid system as a modulator of dopamine transmission: implications for Parkinson's disease and schizophrenia. *Neurotox Res* **3**: 23–35.

Rodríguez De Fonseca F, Ramos J, Bonnin A, Fernandez-Ruiz J (1993). Presence of cannabinoid binding sites in the brain from early postnatal ages. *Neuroreport* **4**: 135–138.

Shram MJ, Lê AD (2010). Adolescent male Wistar rats are more responsive than adult rats to the conditioned rewarding effects of intravenously administered nicotine in the place conditioning procedure. *Behav Brain Res* **206**: 240–4.

Spear LP (2000). The adolescent brain and age-related behavioral manifestations. *Neurosci Biobehav Rev* **24**: 417–463.

Substance Abuse and Mental Health Services Administration, *Results from the 2012 National Survey on Drug Use and Health: Summary of National Findings*, NSDUH Series H-46, HHS Publication No. (SMA) 13-4795. Rockville, MD: Substance Abuse and Mental Health Services Administration, 2013.