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Long-term behavioral and pharmacodynamic effects of delta-9-tetrahydrocannabinol in female rats depend on ovarian hormone status

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Abstract

Abuse of Δ^9 -THC by females during adolescence may produce long-term deficits in complex behavioral processes such as learning, and these deficits may be affected by the presence of ovarian hormones. To assess this possibility, 40 injections of saline or 5.6 mg/kg of Δ^9 -THC were administered i.p. daily during adolescence to gonadally intact or ovariectomized (OVX) female rats, yielding 4 treatment groups (intact/saline, intact/THC, OVX/saline, and OVX/THC). Δ^9 -THC (0.56–10 mg/kg) was then re-administered to each of the 4 groups during adulthood to examine their sensitivity to its disruptive effects. The behavioral task required adult subjects to both learn (acquisition component) different response sequences and repeat a known response sequence (performance component) daily. During baseline (no injection) and control (saline injection) sessions, ovariectomized subjects had significantly higher response rates and lower percentages of error in both behavioral components than the intact groups irrespective of saline or Δ^9 -THC administration during adolescence; the intact group that received Δ^9 -THC had the lowest response rates in each component. Upon re-administration of Δ^9 -THC, the groups that received adolescent ovariectomy alone, adolescent Δ^9 -THC administration alone, or both treatments were found to be less sensitive to the rate-decreasing effects, and more sensitive to the error-increasing effects of Δ^9 -THC than the control group (i.e., intact subjects that received saline during adolescence). Neurochemical analyses of the brains from each adolescent-treated group indicated that there were also persistent effects on cannabinoid type-1 (CB-1) receptor levels in the hippocampus and striatum that depended on the brain region and the presence of ovarian hormones. In addition, autoradiographic analyses of the brains from adolescent-treated, but behaviorally-naïve, subjects indicated that ovariectomy and Δ^9 -THC administration produced effects on receptor coupling in some of the same brain regions. In summary, chronic administration of Δ^9 -THC during adolescence in female rats produced long-term effects on operant learning and performance tasks and on the cannabinoid system that were mediated by the presence of ovarian hormones, and that altered their sensitivity to Δ^9 -THC as adults.

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Keywords

adolescence; cannabinoid abuse; Δ^9 -tetrahydrocannabinol; CB-1 receptors; learning; behavior; rat

INTRODUCTION

Evidence that chronic cannabinoid administration can produce long-lasting behavioral effects has been mounting since the late 1970s when Fehr and colleagues (Fehr et al., 1976) used an extract of marijuana to show that 6 months of 20 mg/kg daily impaired maze learning and motor coordination for 2 months or more in adult rats after the last drug administration. More recently, studies demonstrating the persistent effects of the cannabinoids on brain function have focused on adolescence, as this is a time when many individuals engage in marijuana abuse (Gruber and Pope, Jr., 2002; Substance Abuse and Mental Health Services Administration, 2005) and the brain may be particularly vulnerable to toxic insults (Trezza et al., 2008). These studies involving adolescents have shown that chronic Δ^9 -THC administration, whether in humans or animals, can produce persistent behavioral and neurochemical effects that are different from the effects in adults. The most prominent effect reported in humans after early cannabis use is changes in verbal IQ (Ehrenreich et al., 1999; Pope, Jr. et al., 2003), while some of the behavioral effects reported in animal subjects after adolescent administration of Δ^9 -THC include disruptions of recognition memory (Schneider and Koch, 2003; Quinn et al., 2008), increases in anxiety as indicated by less time in the open arms of an elevated plus maze and reductions in social interactions (O'Shea et al., 2006; Quinn et al., 2008), changes in locomotor activity and rearing in a holeboard task (Bisicaia et al., 2003), changes in food intake and body weight (Manning et al., 1971; Bisicaia et al., 2003; Rubino et al., 2008), and disruption of pre-pulse inhibition in a startle apparatus (Schneider and Koch, 2003). Some of the long-term neurochemical effects that have been reported in rat brain after adolescent administration of Δ^9 -THC are decreases in receptor density (Rubino et al., 2008), decreases in GTP γ S binding (Rubino et al., 2008), and changes in hippocampal protein expression (Quinn et al., 2008).

Studies examining the effects of adolescent drug abuse have also pointed to the potential role of gonadal hormones on the effects of the cannabinoids. In humans, for example, marijuana abuse is more prevalent in males than females (National Institute of Justice, 2003; Substance Abuse and Mental Health Services Administration, 2005; Substance Abuse and Mental Health Services Administration, 2009), which suggests potential sex differences in both the reinforcing and subjective effects of Δ^9 -THC. In animals, both behavioral and pharmacodynamic data support the notion that there is an important interaction between the cannabinoids and the gonadal hormones (e.g., Bisicaia et al., 2003; O'Shea et al., 2004; Fattore et al., 2007; Rubino et al., 2008), although the data are not always consistent with epidemiological data from human studies. For example, Fattore et al. (2007) found that two strains of intact female rats had consistently higher rates of self administration for the cannabinoid agonist WIN 55,212-2 than males, and higher rates of intake than ovariectomized females. In terms of the cannabinoids' subjective effects, adolescent administration of Δ^9 -THC increased depressive-like behaviors such as floating in a forced-swim test more in female than male rats, 25 days after drug administration (Rubino et al., 2008). Peri-adolescent administration of the cannabinoid agonist CP 55,940 also selectively reduced the activity of females in a holeboard test when both sexes were tested 30 days after 11 daily injections (Bisicaia et al., 2003).

Although the interaction between the ovarian hormones and the cannabinoid system may occur at multiple levels (e.g., Rodriguez et al., 1994; Mize and Alper, 2000; Gonzalez et al., 2000), there are more neurochemical data than behavioral data to support this interaction. At

the receptor level, Rodriguez de Fonseca et al. (1994) found that the sex steroids in rats differentially affected the density and/or affinity of cannabinoid receptors in distinct brain regions. In the striatum, they found that cannabinoid receptor affinity increased after ovariectomy, suggesting ovarian hormones might inhibit or antagonize cannabinoid binding in this area. Similarly, Mize and Alper (2000) found that acute 17 β -estradiol administration in ovariectomized rats significantly decreased GTP γ S binding or coupling of the cannabinoid type-1 (CB-1) receptors to signal transduction pathways in the cortex and hippocampus. In the periphery, Gonzalez et al. (2000) demonstrated that estradiol administration in ovariectomized rats produced significantly lower CB-1 receptor mRNA levels in the anterior pituitary gland than in ovariectomized females without estradiol administration.

Interestingly, the pharmacodynamic effects obtained between estradiol and the cannabinoid system in brain regions such as the striatum and hippocampus would suggest that estradiol is capable of attenuating or reducing agonist-induced behaviors mediated by these brain regions. This interpretation is also consistent with a behavioral study by Daniel et al. (2002) showing that estradiol administration attenuated the disruptive effects of Δ^9 -THC in ovariectomized adult rats responding under an operant procedure requiring the acquisition and performance of response sequences. In this study, estradiol administration attenuated both the rate-decreasing and error-increasing effects of Δ^9 -THC. Although similar disruptive effects of Δ^9 -THC have been shown previously in rats (Brodkin and Moerschbaecher, 1997; Delatte et al., 2002), monkeys (Winsauer et al., 1999) and humans (Kamien et al., 1994), this was the first demonstration of the interaction of estrogen and Δ^9 -THC on nonspatial learning. Unfortunately, any assumed relationship between response rate in this task and striatum-mediated motor activity, or between response accuracy and hippocampally-mediated processes, would be highly oversimplified and correlational even though the cannabinoids are known to have effects in these areas (Landfield et al., 1988; Herkenham et al., 1991; Lawston et al., 2000; Coutts et al., 2001), and on locomotor activity (Davis et al., 1972; Navarro et al., 1994; Sanudo-Pena et al., 2000) and spatial learning processes (Lichtman et al., 1995; Hampson and Deadwyler, 2000).

In light of recent data indicating that the cannabinoids can produce persistent behavioral changes (e.g., O'Shea et al., 2006; Quinn et al., 2008), and that ovarian hormones may play an integral role in the responsiveness of the cannabinoid system in females (e.g., Rodriguez et al., 1994), the present study was undertaken to determine if ovarian hormones could attenuate the persistent effects produced by peri-adolescent THC administration in female rats. To examine this interaction, adolescent female rat pups were either ovariectomized or underwent a sham surgery on postnatal day (PD) 30 and then administered either Δ^9 -THC or saline for 40 days shortly thereafter. Following drug administration, all four groups of females were trained to respond under a behavioral procedure that assayed both learning and performance. Responding under the procedure was characterized by two separate dependent measures, response rate and accuracy. Stable responding by the subjects in each treatment group was followed by re-administration of Δ^9 -THC to establish dose-effect curves. Effects of the adolescent treatments were characterized during adulthood by alterations in baseline levels of each behavior (learning and performance) prior to the re-administration of Δ^9 -THC, and by differences in the dose-effect curves among groups. The groups were also sacrificed shortly after Δ^9 -THC re-administration to compare the behavioral effects of Δ^9 -THC with CB-1 receptor levels as determined by western blot. Subjects exposed to the adolescent treatments, but not to behavioral training or re-administration of Δ^9 -THC, were also sacrificed at the appropriate adult age to examine WIN 55,212-2 stimulated GTP γ S binding or receptor coupling to signal transduction pathways.

MATERIALS AND METHODS

Subjects

A total of forty-five female Long-Evans rats served as subjects for this experiment. Of these, thirty-three were used to determine the effects of hormone status and adolescent administration of Δ^9 -THC on behavior and on CB-1 receptor levels (Figure 1A). The remaining 12 were used to determine the effects of these two factors on WIN 55,212-2-stimulated GTP γ S binding, but did not receive any behavioral training or acute Δ^9 -THC determinations as adults (Figure 1B). All of these female rats were purchased from a commercial vendor (Harlan Sprague Dawley, Indianapolis, IN) as pups and arrived at the Animal Care facility on PD 21.

Following their arrival, the pups were group housed and provided a standard diet of rodent chow (Rodent Diet 5001, PMI Inc. St. Louis, MO) and water *ad libitum* until PD 30 when all the subjects were either ovariectomized or underwent a sham surgery (see Figure 1A). After these procedures, the subjects were individually housed in polypropylene plastic cages with hardwood chip bedding to allow for recovery. Food restriction was also instituted at this time to maintain the compatibility of the treated groups; in this case, subjects were maintained at approximately 90% of their free-feeding weights while allowing for a gain of 5 grams per week to control for normal growth.

Throughout testing, the colony room was maintained at $21 \pm 2^\circ$ C with $50 \pm 10\%$ relative humidity on a 14L:10D light/dark cycle (lights on 06:00 h, lights off 20:00 h). The subjects used in these studies were maintained in accordance with the Institutional Animal Care and Use Committee, Louisiana State University Health Sciences Center, and in compliance with the recommendations of the National Research Council in the Guide for the Care and Use of Laboratory Animals (National Research Council, 1996).

Adolescent Ovariectomies

Subjects were ovariectomized while under general anesthesia induced by i.p. injection of ketamine (50 mg/kg) and xylazine (10 mg/kg). During the ovariectomy procedure, ovaries were removed through bilateral flank incisions, ovarian blood vessels were tied off with 4-0 silk and muscle walls were closed with absorbable 3-0 vicryl suture (Ethicon, Inc., Somerville, NJ). Skin incisions were then closed with wound clips. Female subjects that were not ovariectomized underwent sham surgeries as a control for the ovariectomy. During sham surgeries, the subjects were anesthetized with ketamine/xylazine, shaved, and bilateral flank incisions were made, but the ovaries were not isolated or removed. Female rats generally recover fully within 2 days after surgery.

Adolescent Administration of Saline or Δ^9 -THC

From PD 35 to PD 75 (i.e., the beginning of adolescence to sexual maturity for most rats [Waynforth, 1992]), both the ovariectomized and intact (sham surgery) subjects received a single injection of either 5.6 mg/kg of Δ^9 -THC or saline intraperitoneally (i.p.) at the same time each day, yielding 4 treatment groups with respect to hormone status and chronic Δ^9 -THC administration (i.e., intact/saline, intact/THC, OVX/saline and OVX/THC). The Δ^9 -THC was obtained from National Institute on Drug Abuse (Research Technical Branch, Rockville, MD), and arrived in a 100% ethanol solution at a concentration of either 100 or 200 mg/ml. The Δ^9 -THC was stored at -20° C after these Δ^9 -THC solutions were partitioned into smaller aliquots and the ethanol was removed by high-speed vacuum. When needed, the aliquots of Δ^9 -THC were reconstituted for injection as an emulsion using ethanol, emulphor (Alkamuls EL-620, Rhodia, Inc., Cranbury, NJ), and saline in a proportion of 1:1:18. The volume for both saline and Δ^9 -THC injections was 0.1 ml/100 g body weight. On PD 76

(beginning of adulthood), all of the treatment groups began training to respond under a multiple schedule of repeated acquisition and performance of response sequences (Thompson and Moerschbaecher, 1978).

Apparatus for Behavioral Testing

Twelve identical modular test chambers (Coulbourn Instruments, Allentown, PA, Model E10-10TC) configured specifically for rodents were used. Located on the front wall of each chamber were a houselight, speaker, auditory feedback relay, pellet trough (5.5 cm above the floor and centered), and three response keys aligned horizontally (8 cm apart, center to center, and 14.5 cm above the floor). Each response key could be transilluminated by three Sylvania 28ESB indicator lamps, one with a red plastic cap, one with a yellow cap, and one with a green cap. Response keys required a minimum force of 0.15 N for activation and correct responses produced an audible click of the feedback relay. Each chamber was enclosed within a sound-attenuating cubicle equipped with a fan for ventilation and white noise to mask extraneous sounds. All test chambers were connected to a computer programmed in MED-PC for Windows, Version IV (MED Associates, Inc., St. Albans, VT), and to cumulative recorders (Gerbrands, Arlington, MA) located within the same room.

Behavioral Procedure for Behavioral Testing

Preliminary training of the subjects to nose press a key in the apparatus, and to acquire a 3-response sequence on a daily basis (i.e., repeated acquisition), has been described previously (Gerak et al., 2004). After this preliminary training was completed, a multiple schedule with repeated acquisition and performance components was instituted. During the acquisition components, the three response keys in the apparatus were illuminated at the same time with one of three colors: green, red or yellow. Each subject's task was to respond on the correct key in the presence of each color, such that a correct response in the presence of one color changed the color of the key lights as well as the position for the next correct response (e.g., keys green, center correct; keys red, left correct; keys yellow, right correct). When the response sequence was completed by emitting three correct responses (i.e., one correct response in the presence of each color), the key lights were extinguished and the stimulus light in the pellet trough was illuminated for 0.4 s. Subsequently, the response keys were illuminated with the first stimulus (i.e., green) and the sequence was reset. Within a given session, the correct response that was associated with a particular color did not change, and the same sequence (in this case, center-left-right or C-L-R) was repeated during all acquisition components of a given session. Responding on this sequence was maintained by food presentation under a second-order fixed-ratio (FR) 3 schedule such that every three completions of the sequence resulted in the presentation of a 45-mg food pellet (Purina Mills TestDiet, Richmond, VA). When rats responded on an incorrect key (in the example, the left or right key when the green lights were illuminated), the error was followed by a 5-s timeout. An incorrect response did not reset the three response sequence (i.e., the stimuli and the position of the correct response were the same before and after a timeout).

To establish a steady state of repeated acquisition, the sequence was changed from session to session (i.e., daily). An example of sequences for five consecutive sessions was: C-L-R, L-R-C, C-R-L, R-L-C, and L-C-R. The sequences were carefully selected to be equivalent in several ways, and there were restrictions on their ordering across sessions. Briefly, each sequence was scheduled with equal frequency and consecutive correct responses within a sequence were scheduled on different keys. Occasionally, a correct sequence position for a given color was the same for two consecutive sessions (as in the list of sequences above, L-R-C and C-R-L).

During performance components, the houselight and the response keys were illuminated. The houselight served as a discriminative stimulus for responding during this component, and unlike the acquisition component, the sequence in this component remained the same from session to session (i.e., R-C-L). In all other aspects (colored stimuli for each response in the sequence, second-order FR 3 schedule of food presentation, 5-s timeout, etc.), the performance components were identical to the acquisition components. Experimental sessions always began with an acquisition component, which then alternated with a performance component after 40 reinforcers or 20 min, whichever occurred first. Each session terminated after 150 reinforcers or 80 min, whichever occurred first. Throughout testing, sessions were generally conducted 5 days per week, Monday through Friday.

Post Training Δ^9 -THC Administration during Adulthood

As diagramed in Figure 1, the groups involved in behavioral testing were trained to respond under the operant procedure between PD 76-160, whereas each of the groups were re-administered Δ^9 -THC acutely between during PD 161-201. Essentially, during these 40 days of acute testing, doses of Δ^9 -THC were administered 30 minutes prior to the start of the session and in a mixed order every 3 or 4 days in order to establish full dose-effect curves for response rate and the percentage of errors. The dose-effect curves for each group ranged from an ineffective dose to a dose that substantially decreased overall response rate or eliminated responding entirely. As a control for these acute Δ^9 -THC injections, subjects in every group received saline or vehicle injections every 4 or 5 days, 30 minutes prior to the start of the session. On days when the subjects were not receiving Δ^9 -THC or saline, baseline sessions (no injections) were conducted to maintain stable responding under the behavioral procedure.

Western Blot Analysis

Upon completing the Δ^9 -THC dose-effect curves for each treatment group, subjects from each treatment group were sacrificed in pairs for analysis of CB-1 receptor protein levels in specific brain regions. To control for the effects of circulating levels of endogenous hormones across the estrous cycle, each gonadally intact female was sacrificed with one ovariectomized female from the respective adolescent treatment groups (saline or Δ^9 -THC) when the gonadally intact member of the pair was in proestrus (i.e., every intact/THC was paired with a OVX/THC, and every intact/saline was paired with a OVX/saline). The levels of CB-1 receptor were determined by western blot of brain homogenate. The areas that were homogenated prior to protein extraction were separated using the procedure described by Glowinski and Iversen (Glowinski and Iversen, 1966). The brain regions dissected were the cerebellum, pons/medulla, striatum, hypothalamus, midbrain, hippocampus, and cortex; however, only the striatum and hippocampus were analyzed for CB-1 receptor levels in this experiment. For these analyses, 100 μ g of tissue from each specific brain area from each female rat was resuspended in a lysis buffer (20 mM Tris pH 8.0, 137 mM NaCl, 0.5 mM sodium orthovanadate, 2 mM okadaic acid, 10% glycerol, 1% Nonidet P40, 2% protease inhibitor) and processed for protein extraction using MicroRotor Lysis Kit (Bio-Rad, Hercules, CA) following the manufacturer's protocol. The protein concentration was determined using the Bradford Method (Bradford, 1976) and diluted with 1xSDS buffer to 2 μ g/ μ L. Equal amounts of protein from each area (100 μ g) were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE 12%) and transferred to nitrocellulose PDVF membranes (Amersham Biosciences, Piscataway, NJ). The membranes were subsequently immunoblotted for two hours at room temperature with two specific antibodies, a rabbit anti-CB-1 receptor diluted in a proportion 1:500 from Biomol International (Enzo Life Sciences, Plymouth Meeting, PA) and a mouse anti- β -actin diluted in a proportion of 1:2000 from Santa Cruz Biotechnology (Santa Cruz, CA). These antibodies were then followed by specific secondary antibodies (1:2000 for 90 min) and

visualized using ECL Plus (PerkinElmer Life Sciences, Waltham, MA) and a Fuji Film luminescent image analyzer (LAS-1000 Plus). The images were quantified by densitometry using the Image Gauge program (Filipeanu et al., 2004). For each sample, the value of CB-1 receptor expression was normalized to β -actin values.

WIN 55,212-2-stimulated [35 S]GTP γ S binding

WIN 55,212-2-stimulated [35 S]GTP γ S binding was determined in brain slices from the 12 subjects that were maintained in parallel with the subjects that received behavioral training; however, these subjects only received the various adolescent treatments. For these analyses, frozen coronal sections, 20 μ m thick, were cut on a microtome cryostat, thaw-mounted on gelatinized slides, and stored at -70° C. Ten consecutive sections (alternating across two slides) were taken from each of six levels of each brain corresponding to bregma 4.20, 1.60, 0.48, -0.92, -2.80, -5.60 (Paxino and Watson, 1998). Brain regions in which measures were taken are illustrated in Figure 2. Slide-mounted sections were thawed, dried and preincubated in slide mailers containing 2 mM GDP (Sigma, St. Louis, MO) in assay buffer (50 mM Tris, 3 mM MgCl₂, 0.2 mM EGTA, 100 mM NaCl, and 0.5% bovine serum albumin fatty acid-free, pH 7.4) at 25°C for 20 min to reduce basal levels of [35 S]GTP γ S binding. Sections were incubated in slide mailers for 2 h at 25°C in assay buffer with 2 mM GDP and 0.05 nM [35 S]GTP γ S (Amersham Biosciences, Piscataway, NJ) with the CB-1 agonist WIN 55,212-2 (50 μ M, Sigma) to determine stimulated levels of binding. Alternate sections were incubated in assay buffer with GDP and [35 S]GTP γ S without WIN 55,212-2 to determine basal levels of binding. In order to confirm that WIN 55,212-2-stimulated [35 S]GTP γ S binding was a result of stimulation of CB-1 cannabinoid receptors, additional sections were incubated in assay buffer with GDP, [35 S]GTP γ S, and WIN 55,212-2 in the presence of the CB-1 receptor antagonist SR141716A (10 μ M). Nonspecific binding was determined in the presence of 10 μ M unlabeled GTP γ S (Sigma, St. Louis, MO) without WIN 55,212-2. Tissue sections were exposed to film (Bmax, Amersham Biosciences) for 48 hours. Films were developed for 5 minutes in Kodak GBX developer and fixed for 5 minutes in GBX Fixer. Relative optical densities for regions of interest were determined using MCID software, and the experimenter was blind to treatment conditions during imaging procedures. Values were averaged across hemispheres and across sections for each brain. Data were expressed as a percent increase in the optical density of stimulated sections from basal sections.

Data Analyses

The weight data from the 4 behavioral groups were compared initially using a three-way ANOVA with hormone status and Δ^9 -THC considered to be between-group factors and the phase of the experiment as a repeated measure (SigmaStat Statistical Software, SYSTAT Software, Inc. Point Richmond, CA, USA). When a significant interaction occurred, two-way ANOVA tests for each experimental phase (i.e., pre-THC, post-THC, final) were conducted and followed by post-hoc Holm-Sidak tests to determine differences between the treated groups and the untreated control group. Significance was accepted at α level ≤ 0.05 for all statistical tests. Two-way ANOVA tests with drug and hormone status as factors comprised the analyses for CB-1 receptor levels and WIN 55,212-2-stimulated GTP γ S binding. When a significant interaction was obtained, one-way ANOVA tests along with the appropriate post-hoc tests were also conducted.

The data collected for the acquisition and performance components of the behavioral procedure were analyzed in terms of: (1) the overall response rate (total responses/min, excluding timeouts), and (2) the overall accuracy, expressed as the percentage of errors [(incorrect responses/total responses) $\times$ 100]. However, when the overall response rate was less than 5 responses/min, data were excluded from the analyses for percent errors because

of the small number of responses involved. Significant differences in response rate or the percentage of errors in each behavioral task between the treated groups and the untreated control group (i.e., acquisition and performance) after acute saline administration were determined using a two-way ANOVA (i.e., acquisition and performance) with drug and hormone status serving as factors. Significant main effects or interactions were determined by Holm-Sidak post-hoc tests. Given the differences in control levels of responding obtained, the differences in sensitivity of all of the groups to the rate-decreasing effects of Δ^9 -THC, and the resulting differences in animal number for each acute dose administered during adulthood, the dose-effect data for both dependent measures were expressed as a percent of control and significant differences were indicated by doses of Δ^9 -THC that produced differences from control levels that were greater than 20%.

Persistent or long-term changes in the sensitivity of each of the 4 groups to the effects of Δ^9 -THC as a result of adolescent treatment were also quantified by comparing the ED50 values of the dose-effect curves established during adulthood. These ED50 values were determined by linear regression using two or more data points reflecting the slopes of the descending curve for response rate or the ascending curve for the percentage of errors. For response rate, the ED50 represented the estimated dose of Δ^9 -THC that decreased responding from control levels by 50%. For the percentage of errors, the ED50 represented the estimated dose of Δ^9 -THC that increased the percentage of errors from control levels by 50% (i.e., ED150).

RESULTS

Effects of Adolescent Treatments on Body Weight

As shown in Table 3, the mean weights for the groups involved in behavioral testing increased during the experiment and there were significant differences among the groups during the experiment as indicated by a significant interaction of hormone status and the phase of the experiment ($F(2,72)=5.33$, $p=0.007$). Immediately after recovery from the ovariectomies or sham surgeries, and prior to the initiation of Δ^9 -THC or saline administration on PD 35, the weights of the four groups were not significantly different. The average weight of all of the pups at this time was 120.6 ± 1.2 g (mean $\pm$ SEM), and the grand mean for the 4 groups was 120.7 g. On PD 75 after Δ^9 -THC or saline administration, however, there was a main effect of hormone status as the mean weights for the two ovariectomized groups were significantly higher than those for the two intact groups ($F(1,27)=11.84$, $p=0.002$) by an average of 13 g. There was also a main effect of hormone status after all of the experimental manipulations had been completed ($F(1,27)=13.19$, $p=0.001$). The mean weights and SEM for each of the groups at the end of the experiment were: 259.9 ± 7.0 g for the intact/saline group, 259.1 ± 5.1 g for the intact/THC group, 283.1 ± 5.4 g for the OVX /saline group and 286.9 ± 4 g for the OVX/THC group.

Effects of Adolescent Treatments on the Baseline of Behavioral Responding as Adults

Each of the groups was successfully trained to respond under the behavioral procedure, which included both learning and performance components, after the adolescent treatments. In general, responding in each component was considered stable when the response rate and the percentage of errors was less than 20% for 10 consecutive days. The pattern of responding in the acquisition or learning component also had to be characterized by a decrease in the number of errors and an increase in consecutive correct completions of the response sequence each day, as within-session error reduction essentially defined sequence learning. The pattern of responding in the acquisition components also had to differ from the pattern in the performance component as sequence acquisition (learning) was not required for reinforcement in the performance component. The errors emitted during sequence

acquisition also accounted for the fact that the percentage of errors in this component was typically larger than the percentage of errors in the performance component. The mean number of days required to establish stable responding under the behavioral procedure prior to re-administering Δ^9 -THC as an adult averaged 114 days, and the mean number of days of training for each group did not differ significantly ($F(3,27)=1.46$, $p>0.05$).

Although the average number of days required to achieve stable responding in each behavioral component did not vary significantly among the groups, both the rate and accuracy of responding attained by the groups under baseline (no injections) and control (saline injection) conditions was significantly different. As shown in Figure 3, rate and accuracy were dependent on hormonal status, and hormonal status alone, even though the group that received Δ^9 -THC as adolescents (intact/THC) had the lowest mean response rate of the 4 groups in both behavioral components. This was verified by a significant main effect of ovariectomy on response rate and the percentage of errors in both the acquisition (rate, $F(1,27)=7.57$, $p=0.01$; error, $F(1,27)=7.51$, $p=0.011$) and performance (rate, $F(1,27)=6.63$, $p=0.016$; error, $F(1,27)=4.63$, $p=0.041$) components, and the absence of significance for the effect of Δ^9 -THC administration and for the interaction of hormonal status and Δ^9 -THC administration ($p>0.05$). Response rates for the intact groups averaged 37 ± 5.19 (adolescent saline) and 19 ± 3.08 (adolescent Δ^9 -THC) responses per minute in the acquisition components and 35 ± 5.55 (adolescent saline) and 19 ± 2.56 (adolescent Δ^9 -THC) responses per minute in the performance components. In the ovariectomized groups, response rates averaged 45 ± 6.38 (adolescent saline) and 43 ± 6.90 (adolescent Δ^9 -THC) responses per minute in the acquisition components and 44 ± 7.33 (adolescent saline) and 39 ± 6.56 (adolescent Δ^9 -THC) responses per minute in the performance components.

With respect to the percentage of errors, ovariectomized groups had significantly lower percentages of error in each component of the task than the intact groups. For example, the percentage of errors in the intact groups averaged $23 \pm 2.09\%$ (adolescent saline) and $25 \pm 2.22\%$ (adolescent Δ^9 -THC) in the acquisition components and $10 \pm 1.58\%$ (adolescent saline) and $10 \pm 1.05\%$ (adolescent Δ^9 -THC) in the performance components. In the ovariectomized groups, the percentage of errors averaged $17 \pm 1.83\%$ (adolescent saline) and $18 \pm 2.87\%$ (adolescent Δ^9 -THC) in the acquisition components and $7 \pm 1.34\%$ (adolescent saline) and $7 \pm 0.45\%$ (adolescent Δ^9 -THC) in the performance components.

Effects of Adolescent Δ^9 -THC on Adult Sensitivity to Δ^9 -THC

As shown in Figure 4A, adult subjects that received Δ^9 -THC during adolescence were less sensitive to its rate-decreasing effects and more sensitive to its error-increasing effects in the acquisition and performance components than the intact/saline (control) group. That is, when the dose-effect curves for these two groups were plotted as a percent of control to normalize the data with respect to their differences in responding under control conditions, larger doses were required to produce rate-decreasing effects and smaller doses were required to produce error-increasing effects in the intact/ Δ^9 -THC group. The differences in adult sensitivity to Δ^9 -THC were also reflected in the ED50s for both adolescent-treated groups, which are shown in Table 1. For example, the ED50 for the control group was 2.02 mg/kg for response rate in the acquisition component, whereas the ED50 for the intact/ Δ^9 -THC group was 4.22 mg/kg for response rate in the same component, a greater than two-fold difference indicating that the latter group was less sensitive to the rate-decreasing effects as adults. The difference in ED50s for response rate in the performance components was greater than three-fold (see Table 1). Unlike the ED50 values for response rate, the ED50 values for Δ^9 -THC's error-increasing effects in the acquisition components could not be calculated for the control group because Δ^9 -THC did not increase the percentage of errors by more than 50% prior to eliminating responding; the ED50 for Δ^9 -THC's error-increasing

effects in the performance component was 2.34. In the intact/THC group, the ED50s were 1.07 and 0.98 mg/kg for the acquisition and performance components, respectively.

Effects of Adolescent Ovariectomy on Adult Sensitivity to Δ^9 -THC

Figure 4B compares the sensitivity of the OVX/saline group to that of the control group after adult Δ^9 -THC administration. The changes in sensitivity for the OVX/saline group were similar to those for the intact/ Δ^9 -THC group in that the OVX/saline group was also less sensitive to the rate-decreasing effects of Δ^9 -THC and more sensitive to its error-increasing effects in both the acquisition and performance components than the control group.

Moreover, the ED50 for Δ^9 -THC's rate-decreasing effect in the acquisition components was two-fold higher in the OVX/saline group than in the control group, and greater than two-fold higher in the performance components (see Table 1). The ED50 values for Δ^9 -THC's error-increasing effects in the acquisition and performance components were 1.18 and 1.10 mg/kg, respectively.

Effects of Ovariectomy and Adolescent THC on Adult Sensitivity to Δ^9 -THC

The dose-effect curves in Figure 4C show that the OVX/ Δ^9 -THC group was less sensitive to the rate-decreasing effects of Δ^9 -THC and more sensitive to its error-increasing effects in both behavioral components than the control group; the same relationship of effects that was found with the intact/THC and OVX/saline groups. Furthermore, the ED50s of the dose-effect curves for response rate in OVX/ Δ^9 -THC group were the highest of all of the treatment groups (i.e., 11 and 12.90 mg/kg for the acquisition and performance components, respectively), indicating that this group was the least sensitive to Δ^9 -THC's rate-decreasing effects. In contrast, the ED50s of the dose-effect curves for the percentage of errors indicated that this group was the most sensitive to Δ^9 -THC's error-increasing effects in the performance components, and the least sensitive in the acquisition components of all the groups (see Table 1).

Effects of Adolescent Treatments on CB-1 Receptor Levels

When CB-1 receptor protein levels were determined for each of the treatment groups by western blot, CB-1 receptor could be readily detected in both hippocampus and striatum as a major band of approximately 50 kDa, similar to the predicted molecular weight for the mouse CB-1 receptor of 53 kDa (Figure 5A). A few additional bands with higher molecular weight were detected occasionally, indicating post-translational receptor modifications (i.e., glycosylation). None of these bands were observed after preadsorption of the antibody with the control antibody, proving the identity of the protein. Following normalization to β -actin, an internal control for the overall amount of protein, significant differences in CB-1 receptor levels among the different treatment groups were apparent in both the hippocampus and striatum (Figure 5B). In the hippocampus, there was a significant interaction of ovariectomy and Δ^9 -THC administration during adolescence ($F(1,20)=7.12$, $p=0.015$). Subsequent one-way ANOVA tests that compared the three treatment groups with the control group indicated that CB-1 receptor levels in the intact/THC group were significantly higher ($F(1,10)=8.15$, $p=0.017$) than levels in the control group. The effects in the striatum were different from those in the hippocampus in that there was a significant main effect of ovariectomy on CB-1 receptor levels ($F(1,1)=14.21$, $p=0.001$), but no significant effect of Δ^9 -THC administration or interaction of the two factors ($p>0.05$). More specifically, in the striatum, CB-1 receptor levels in both ovariectomized groups were lower than those in both intact groups.

Effects of Adolescent Treatments on WIN 55,212-2-stimulated activation of [³⁵S]GTPγS binding

The specificity of the assay is illustrated in Figure 6. WIN 55,212-2-activation of G-proteins was increased when compared to basal levels of activation. This increase was blocked by rimonabant (SR141716A), indicating that the increase over basal levels was due to activation of CB-1 receptors. Additionally, little nonspecific binding in the presence of excess unlabeled GTPγS was evident. Values obtained for the intact, saline-treated females indicate the relative amount of stimulation across brain areas (see Table 2).

In the 12 subjects that did not receive behavioral training or acute doses of Δ⁹-THC during adulthood to establish dose-effect curves (i.e., n=3 per treatment group), the effects of adolescent Δ⁹-THC treatment on WIN 55,212-2-stimulated activation of [³⁵S]GTPγS binding varied depending upon brain area, ovarian hormonal status, and Δ⁹-THC administration during adolescence. As shown in Figure 7A, a two-way ANOVA revealed a significant main effect of ovariectomy for two areas of the hippocampus (i.e., dentate gyrus (F(1,8)=6.70, p=0.032) and CA3 (F(1,8)=6.41, p=0.035)) and a significant main effect in the globus pallidus (F(1,8)=5.86, p=0.042), respectively. There were no significant main effects or interactions for any of the other brain regions examined (see Table 2).

With respect to the main effects in the hippocampus, CB-1 activation by WIN 55,212-2 was significantly reduced in the ovariectomized groups compared to the intact groups irrespective of Δ⁹-THC or saline administration during adolescence (see Figure 7A). The pattern of effects in CA1 region of the hippocampus was similar to that for the DG and CA3 regions, although the effect of ovariectomy did not reach levels of statistical significance (i.e., p=0.054). In contrast to the main effect observed in the hippocampus, there was a significant main effect of Δ⁹-THC administration during adolescence on WIN 55,212-2-stimulated activation of [³⁵S]GTPγS binding in the globus pallidus and this effect occurred irrespective of hormone status (F(1,8)=5.85, p=0.042). For example, in the intact groups (Figure 7B), Δ⁹-THC administration during adolescence decreased activation from 140% above basal levels to 82% above basal levels. In the ovariectomized groups, Δ⁹-THC administration during adolescence decreased activation from 103% above basal levels to 94% above basal levels.

Uterine Weights

A two-way ANOVA comparing the uterine weights from each of the 4 treatment groups (including those subjects that had no behavioral training or adult administration of Δ⁹-THC) indicated that there was a main effect of ovariectomy (F(1,39)=32.06, p<0.001), but no effect of adolescent Δ⁹-THC administration and no significant interaction of these factors. The mean weight and SEM for the uteri of the gonadally intact females was 100 ± 10 mg, whereas the mean weight and SEM for the uteri of the ovariectomized females was 25 ± 10 mg, confirming the efficacy of hormone manipulations.

DISCUSSION

The primary findings from the present study involving female rats were that chronic Δ⁹-THC administration during adolescence can have long-term effects on complex operant processes such as learning, and on the sensitivity of adults to the disruptive effects of Δ⁹-THC. Along with these behavioral effects, adolescent administration of Δ⁹-THC also produced long-term, region specific alterations in the density and function of CB-1 receptors in the brain. These findings complement and extend data in the literature on the persistent behavioral and pharmacodynamic effects of Δ⁹-THC in both sexes (Stiglick and Kalant, 1983; Schneider and Koch, 2003; Biscaia et al., 2003; O'Shea et al., 2004; Quinn et al.,

2008). The present data also strongly support an increasing amount of data in the literature indicating that ovarian hormones likely play an integral role in the development and maintenance of these long-term effects and that the untoward consequences of chronic adolescent cannabinoid use may be greater in females than males (Fattore et al., 2007; Rubino et al., 2008).

While both Δ^9 -THC and estrogen are known to have effects on learning and memory in mammals (Hampson and Deadwyler, 1999; Daniel, 2006), there is comparatively little data on the effects of these substances during adolescence or the extent to which the effects might differ between adolescents and adults. Furthermore, only limited data on the interactive effects of Δ^9 -THC and estrogen on nonspatial learning and performance tasks exist, as most of the published studies have used spatial tasks (see Daniel, 2006). In the present study where subjects were required to learn (acquisition) or repeat (performance) response sequences, chronic administration of Δ^9 -THC during adolescence produced relatively permanent changes in the baseline levels of responding in each behavioral component, and persistent changes in Δ^9 -THC sensitivity that were opposite in direction for the two dependent measures. The relatively permanent changes in baseline levels of responding consisted of the significantly low rates of responding obtained for the intact groups in each component compared to the ovariectomized (OVX) groups, particularly the intact/ Δ^9 -THC group. The persistent changes observed during adulthood were a reduced sensitivity to the rate-decreasing effects and enhanced sensitivity to the error-increasing effects of Δ^9 -THC in both components. The changes in adult sensitivity for the intact/ Δ^9 -THC group were also evident in the ED50 values of the dose-effect curves for both response rate and the percentage of errors. Compared to the control group, the ED50 values for the intact/ Δ^9 -THC group were shifted rightward by two- to four-fold depending on the treatment and dependent measure.

Unlike adolescent administration of Δ^9 -THC in intact females, which tended to decrease response rates in each behavioral component, ovariectomy during adolescence significantly increased baseline levels of responding in the two behavioral components compared to the control group. The OVX/saline group was also less sensitive to the rate-decreasing effects and more sensitive to the error-increasing effects of Δ^9 -THC when it was administered during adulthood. These changes in sensitivity were not surprising with regard to the error-increasing effects of Δ^9 -THC, but they were surprising with regard to its rate-decreasing effects, because Daniel et al. (2002) found that estradiol administration in ovariectomized subjects could attenuate both the rate-decreasing and error-increasing effects of Δ^9 -THC. The fact that adolescent ovariectomy and the resulting loss of estrogen produced an attenuation of Δ^9 -THC's rate-decreasing effects in this study would, therefore, suggest that the interactive effects of estrogen and Δ^9 -THC may be age-dependent, and that the loss of estrogen during adolescence may permanently affect the brain areas that mediate the rate-decreasing effects of Δ^9 -THC. Certainly, one of those areas could be the striatum, as this area had a large basal response following WIN 55,212-2 stimulation (Table 2) and it is an area where the gonadal hormones are known to interact with cannabinoid receptors (Rodriguez et al., 1994). For example, Rodriguez de Fonseca et al. (1994) found that cannabinoid receptor affinity increased in the striatum after ovariectomy and that the gonadal hormones could inhibit cannabinoid binding in this area. Given these data, gonadal hormones should reduce agonist-mediated effects in this area or attenuate cannabinoid-mediated effects in a manner similar to that observed in a previous study examining the interaction of estrogen and Δ^9 -THC (Daniel et al., 2002). In the present study, however, an absence of ovarian hormone (i.e., ovariectomy) produced pharmacodynamic effects that were consistent with inhibition of the cannabinoid system. Moreover, analyses of the striatum from ovariectomized subjects indicated that there was reduced CB-1 receptor density in this area compared with intact subjects, which would be consistent with the

reduced sensitivity to Δ^9 -THC's rate-decreasing effects and with the notion that there may be age-dependent effects of the ovarian hormones.

Although ovariectomized adult females (i.e., OVX/saline) were more sensitive to the error-increasing effects of Δ^9 -THC, CB-1 receptor levels in the hippocampus were not significantly increased for this group. There was, however, a trend in that mean receptor levels for this group were higher than that for the control group and one subject within this group had the highest levels of all of the subjects. With respect the neurochemical data for the OVX/saline group, adolescent ovariectomy significantly decreased agonist-stimulated GTP γ S binding or receptor coupling with signal transduction pathways in the dentate gyrus and CA3 regions of the hippocampus. Interestingly, a relatively large number of CB-1 receptors with reduced coupling could result in an increased, yet tempered, agonist-derived signal such as the one observed for the OVX/saline group (i.e., an error-increasing effect that was greater than that for the control group, but less than the one for the intact/ Δ^9 -THC group). Similar to the data from Rodriguez de Fonseca et al. (1994), the neurochemical data from this study indicate that the gonadal hormones can produce subtle, yet sometimes pronounced, effects on the density and/or affinity of ligands for cannabinoid receptors in different brain regions. As reported in the literature, the extent of these effects can vary widely depending upon the brain area (Sim et al., 1996), dose of drug administered (McKinney et al., 2008), and the length of the recovery period (McKinney et al., 2008; Rubino et al., 2008). The present study would suggest that the age of the subjects may also be critical when females are involved.

Some of the most interesting behavioral and neurochemical data in the present study came from the OVX/ Δ^9 -THC group. The data from this group indicated that an ovariectomy could attenuate the persistent decrease in baseline rates of responding produced by adolescent administration of Δ^9 -THC in both behavioral tasks (Figure 3), while the combination of treatments still altered adult sensitivity to the rate-decreasing and error-increasing effects of Δ^9 -THC, some 130 days after drug cessation. The absence of a significant effect on baseline rates of responding in this group (see Figure 3) would also suggest that the ovarian hormones must be present for adolescent administration of Δ^9 -THC to produce the low baseline levels of responding observed, or that the rate-increasing effects produced by the removal of the ovarian hormones effectively opposed the decreases in response rate produced by adolescent administration of Δ^9 -THC. In fact, the baseline rates of responding for the OVX/ Δ^9 -THC group were similar to those for the OVX/saline group, and the rates for both of these groups were significantly higher than those for the intact groups. From a behavioral standpoint, the increases in response rate in these food-motivated tasks suggest the possibility that the removal of estradiol may have increased the deprivation level of the subjects in the ovariectomized groups. Clearly, the ovariectomized groups weighed significantly more than the subjects in the intact groups (even though all of the groups were comparably fed), and this effect likely occurred because ovariectomy is known to increase fat stores and body weight (Couse and Korach, 1999). However, an increase in body weight does not necessarily indicate that the ovariectomized groups had higher deprivation levels, and the mechanisms by which estrogen might increase food intake (e.g., meal size) are not as well understood. If, for example, estrogen can enhance satiety signals as reported in the literature (e.g., Asarian and Geary, 2007; Thammacharoen et al., 2008), then the removal of estradiol by ovariectomy could have increased deprivation level through these signals.

One of the simplest pharmacodynamic explanations for the difference between the intact/ Δ^9 -THC group and the OVX/ Δ^9 -THC group would be that ovariectomy reduced the number of CB-1 receptors in the striatum as indicated by the main effect of hormone in this brain region (see Figure 5), and thereby, minimized the effectiveness or capacity of chronic Δ^9 -THC to modify the cannabinoid system in this region of the brain. For example, if the

primary effect of chronic adolescent administration of Δ^9 -THC is to decrease GTP γ S binding (i.e., coupling) in this brain region, then this effect might be diminished if the relative number of receptors was substantially reduced. Another possibility is that ovariectomy and chronic Δ^9 -THC administration during adolescence produced the same pharmacodynamic effects in some brain regions. Obviously, this hypothesis remains to be tested experimentally, but it would be consistent with the GTP γ S binding data obtained for the globus pallidus (Figure 7B). These data indicated that adolescent administration of Δ^9 -THC alone can produce marked reductions in GTP γ S binding or receptor coupling in intact subjects, but not in ovariectomized subjects because ovariectomy alone markedly reduced CB-1 receptor coupling.

Unfortunately, none of the above explanations are appropriate for the neurochemical data obtained from the hippocampus, or would fit with the increases in sensitivity that occurred behaviorally. This is because CB-1 receptor levels were not significantly different between the OVX/ Δ^9 -THC and control group (Figure 5), and significant reductions in GTP γ S binding or coupling in the hippocampus occurred with ovariectomy alone (Figure 7) and not with adolescent administration of Δ^9 -THC. Furthermore, even though reductions in GTP γ S binding in this area after chronic Δ^9 -THC administration would be consistent with published data from rodents showing that chronic Δ^9 -THC administration can produce this type of adaptive or “desensitizing” effect in both males (Sim et al., 1996; McKinney et al., 2008; Rubino et al., 2008) and females (Rubino et al., 2008), this type of adaptive response is not consistent with the increased behavioral sensitivity obtained for Δ^9 -THC-induced disruptions in response accuracy. Pharmacokinetic explanations for this brain-behavior inconsistency may also have limited applicability because there seems to be few circumstances under which changes in the effective concentrations of Δ^9 -THC, at any age, could produce similar pharmacodynamic effects in two different brain regions, while producing opposite effects on two behavioral measures of responding (i.e., a decrease in sensitivity and an increase in sensitivity). In general, chronic administration of Δ^9 -THC generally produces a dose-dependent CB-1 receptor downregulation together with tolerance to a variety of Δ^9 -THC-induced behaviors (McKinney et al., 2008). Using an operant schedule similar to the one used in this study, Delatte et al. (2002) also found that repeated administration of Δ^9 -THC in male rats reduced both its rate-decreasing and error-increasing effects in both components of the task rather than sensitizing subjects to its error-increasing effects as was shown in this study.

What remains then to explain the increase in sensitivity to the error-increasing effects of Δ^9 -THC are a variety of possibilities that need to be investigated further. For example, although the level of CB-1 receptors in the OVX/saline and OVX/ Δ^9 -THC groups was not significantly different from that for the control group in the hippocampus (Figure 8B), the western blot determinations were evaluating the total cellular levels of CB-1 receptor and do not differentiate between receptors on the cell surface and those that are localized intracellularly. The ratio of cell surface receptors to intracellular receptors would determine the cellular response to a cannabinoid agonist. Further, recent evidence in this field indicates that the cellular effects of GPCR stimulation may occur through G-protein independent pathways, involving the scaffold activities of β -arrestin (DeWire et al., 2007; Breivogel et al., 2008). For example, the antinociceptive and body-temperature effects of CB-1 agonists were enhanced in β -arrestin mice (Breivogel et al., 1999). Whether or not these mechanisms can explain the discrepancies concerning the accuracy data is currently under investigation.

One explanation that blends both the existing neurochemical and behavioral data, is that the error-increasing effects of Δ^9 -THC were unmasked by the development of tolerance to its rate-decreasing effects. In other words, both adolescent ovariectomy and Δ^9 -THC administration independently reduced the sensitivity of subjects to the rate-decreasing

effects of Δ^9 -THC, and this allowed the subjects to continue responding and emit errors that would not otherwise have been emitted. Although data from McKinney et al. (2008) and others (Breivogel et al., 1999) have suggested that the striatum is slow to adapt to chronic treatment with Δ^9 -THC, the subjects were not treated during adolescence in these studies. In addition, neurochemical data from the present study showed clear reductions of receptor levels in the striatum and decreases in coupling in the globus pallidus almost 130 days after the last Δ^9 -THC injection. Chronic Δ^9 -THC treatment in the present study was also substantially longer than in either of those studies and may have contributed to the observed reductions in this brain region.

In summary, chronic Δ^9 -THC administration during adolescence interacted with ovarian hormones to produce behavioral disruptions that persisted well into adulthood in female rats responding under an operant task with both learning and performance components. These effects were also concomitant with persistent alterations in receptor levels and receptor coupling in the striatum and hippocampus as measured by western blot and agonist-stimulated GTP γ S binding. Finally, both the long-term behavioral and pharmacodynamic effects of Δ^9 -THC administration during adolescence in these female subjects were dependent on ovarian hormones. A primary example of these interactive effects occurred in the hippocampus where CB-1 receptor density was significantly increased by Δ^9 -THC administration during adolescence, but not by ovariectomy and Δ^9 -THC administration during the same period.

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Fig. 1.

Diagrams showing the timeline of manipulations for the subjects in the four adolescent treatment groups that received behavioral training and re-administration of Δ^9 -THC as adults (A), and the timeline for the subjects that only received the adolescent treatments (B).

Fig. 2.

Autoradiography of WIN 55,212-2-stimulated [³⁵S]GTPγS binding in selected coronal sections of female rat brain showing the 14 distinct regions examined approximately 130 days after adolescent treatment (see Table 2 for full name of region and its abbreviation).

Fig. 3.

Bar graph showing the effects of each adolescent treatment on response rate and the percentage of errors in the acquisition and performance components of an operant procedure under control conditions where the subjects in each group received acute injections of saline. Asterisks indicate a main effect of ovariectomy on each measure as determined by a two-way ANOVA.

Fig. 4. Δ^9 -THC dose-effect curves for response rate and the percentage of errors (plotted as a percent of control) in each behavioral component of an operant task established in adult female rats after adolescent ovariectomy (OVX) or sham surgery (Intact) and administration of either saline or Δ^9 -THC. Panel A shows the overall effects of adolescent Δ^9 -THC (unfilled and filled squares), panel B shows the effects of adolescent ovariectomy (unfilled and filled triangles), and panel C shows the effects of adolescent ovariectomy and Δ^9 -THC (unfilled and filled diamonds). The data obtained in the control group, or the intact group that received saline during adolescence, is replotted in each panel for comparison and represented by the unfilled (acquisition component) and filled (performance component) circles. The data points and vertical lines in the dose-effect curves for each group in each panel represent a grand mean and standard error of the mean (SEM) for each dose, as there was a mean for each dose in each subject because the doses in the dose-effect curves were determined multiple times. Asterisks indicate a change of greater than 20% from control. Numerical values in parentheses and adjacent to a data point indicate the number of subjects represented by that point when it differed from the total number of subjects for that group.

Fig. 5.

Panel A shows the effects of ovariectomy and Δ^9 -THC administration during adolescence on CB-1 receptor protein expression in the hippocampus and striatum in the same subjects as adults. Panel B shows a bar graph representing the quantification of the effects of the different adolescent treatments in these brain regions. The asterisk in the left-hand graph for the hippocampal levels of CB-1 receptor protein indicates a significant difference from the control group (i.e., the intact group that received adolescent saline), whereas the asterisk in the right-hand graph for the striatal levels of CB-1 receptor protein indicates a significant main effect of ovariectomy.

Fig. 6.

Autoradiography in a selected coronal section of female rat brain showing the level of nonspecific WIN 55,212-2-stimulated [35 S]GTP γ S binding and the antagonism of binding by the cannabinoid antagonist SR141716A (rimonabant). These sections were taken from female rats that received the adolescent treatments, but were not sacrificed until approximately 130 days after adolescent treatment; these female subjects received no behavioral training or re-administration of Δ^9 -THC as an adult.

Fig. 7.

Bar graphs representing the effect of the different adolescent treatments on WIN 55,212-2-stimulated [35 S]GTP γ S binding in three subregions of the hippocampus (A) and the globus pallidus (B). The asterisks in the graphs for the dentate gyrus (DG) and CA3 subregions indicate a significant main effect of ovariectomy as determined by a two-way ANOVA. The letters in the graph for the globus pallidus indicate there was a main effect of adolescent Δ^9 -THC administration.

Table 1

Effective dose for decreasing response rate by 50% or increasing the percentage of errors by 50% in female rats responding under a behavioral procedure with both acquisition and performance components (i.e., ED50s).

ED50 (mg/kg)	Acquisition Component		Performance Component	
Group	Resp. Rate	Percent Error	Resp. Rate	Percent Error
Intact/Saline	2.02	-	1.99	2.34
Intact/THC	4.22	1.07	7.38	0.98
OVX/Saline	4.50	1.18	5.86	1.10
OVX/THC	11.00	2.79	12.90	0.70

Table 2Levels of WIN 55,212-2-stimulated [35 S]GTP γ S binding in female rat brain.

Brain Area	Percent Increase from Basal
primary motor area (MI)	43.07 $\pm$ 5.22
prefrontal cortex, medial (mPFC)	52.58 $\pm$ 2.64
prefrontal cortex, orbital (PFC-o)	48.15 $\pm$ 6.20
cingulate cortex (Cg)	51.53 $\pm$ 2.94
nucleus accumbens (NAc)	33.63 $\pm$ 0.86
primary sensory area (S1)	40.32 $\pm$ 18.59
striatum, lateral (Str-L)	115.51 $\pm$ 43.58
striatum, medial (Str-M)	53.90 $\pm$ 2.82
globus pallidus (GP)	140.29 $\pm$ 21.38
hippocampus, dentate gyrus (DG)	34.37 $\pm$ 4.77
hippocampus, CA3 (CA3)	37.40 $\pm$ 4.83
hippocampus, CA1 (CA1)	33.20 $\pm$ 3.93
basolateral amygdale (BLA)	35.87 $\pm$ 10.94
substantia nigra (SN)	46.61 $\pm$ 27.56

Table 3

Mean body weight for each treatment group at three different time points during the study.

Treatment Group	Pre-THC	Post-THC	Final
Intact/Saline	119.5 ± 2.7	209.9 ± 1.8 ^a	259.9 ± 8.1 ^a
Intact/THC	120.2 ± 2.2	206.1 ± 4 ^a	259.1 ± 6.7 ^a
OVX/Saline	125.8 ± 2.5	222.1 ± 2.8 ^b	283.1 ± 6.3 ^b
OVX/THC	118.4 ± 1.6	214.4 ± 2.5 ^b	286.9 ± 4.6 ^b

Superscript letters indicate significant differences