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RESEARCH PAPER

Regional enhancement of cannabinoid CB_1 receptor desensitization in female adolescent rats following repeated Δ^9-tetrahydrocannabinol exposure

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BACKGROUND AND PURPOSE
Disruption of the substantial re-organization of the brain during adolescence may be induced by persistent abuse of marijuana. The aim of this study was to determine whether adolescent and adult rats exhibit differential adaptation of brain cannabinoid (CB_1) receptors after repeated exposure to Δ^9-tetrahydrocannabinol (THC).

EXPERIMENTAL APPROACH
Rats of both ages and sexes were dosed with 10 mg kg^{-1} THC or vehicle twice daily for 9.5 days. Subsequently, CB_1 receptor function and density were assessed.

KEY RESULTS
In all brain regions, THC treatment produced desensitization and down-regulation of CB_1 receptors. While the magnitude of down-regulation did not differ across groups, greater desensitization was evident in the brains of THC-treated female adolescent rats for most regions. Adolescent females showed greater desensitization than adult females in the prefrontal cortex, hippocampus, periaqueductal gray (PAG) and ventral midbrain. In contrast, adolescent males exhibited less desensitization in the prefrontal cortex, hippocampus and PAG, an effect opposite to that seen in females. With the exception of the PAG, sex differences were seen only in adolescents, with greater desensitization in the prefrontal cortex, striatum, hippocampus, PAG, and ventral midbrain of females.

CONCLUSIONS AND IMPLICATIONS
These results suggest that the brains of adolescent females may be particularly vulnerable to disruption of CB_1 receptor signalling by marijuana abuse. Alternatively, increased desensitization may reflect protective adaptation. Given the extensive re-organization of the brain during adolescence, this disruption has potential long-term consequences for maturation of the endocannabinoid system.

Abbreviations
2-AG, 2-arachidonylglycerol; CP55,940, (-)-cis-3-[2-hydroxy-4(1,1-dimethyl-heptyl)phenyl]-trans-4-(3-hydroxy-propyl)cyclohexanol; PAG, periaqueductal gray; PN, postnatal day; SR141716A, N-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide HCl; THC, Δ^9-tetrahydrocannabinol; VTA, ventral tegmental area

Introduction

Marijuana, one of the most commonly used illicit drugs (Copeland and Swift, 2009), is widely used by adolescents and young adults, but its effects on the adolescent brain are not well understood. Use of marijuana and other psychoactive drugs during adolescence is of particular concern due to possible drug-induced disruption of the maturation of the CNS that occurs in this important developmental window (Jager and Ramsey, 2008). Previous studies have indicated that frequent marijuana use during adolescence led to later deficits in cognition (see Schweinsburg *et al.*, 2008) and visual attention (Ehrenreich *et al.*, 1999), and was associated with decreases in educational achievement (Brook *et al.*, 2008). Furthermore, abstinent adolescent marijuana users exhibited deficits in sustained attention and working memory that were associated with altered hippocampal function as measured by functional magnetic resonance imaging (Jacobsen *et al.*, 2004). Similarly, treatment of rodents during adolescence with Δ^9-tetrahydrocannabinol (THC) [the primary psychoactive substituent of marijuana; (Gaoni and Mechoulam, 1964)] produced altered cognition and emotionality in adulthood (Rubino *et al.*, 2008; Rubino *et al.*, 2009). Working memory and social interaction were also impaired in adolescent rats following 21 days of treatment with the cannabinoid agonist CP55,940, whereas adult animals showed no effect (O'Shea *et al.*, 2004). These data suggest that marijuana use during adolescence could produce immediate and long-term behavioural alterations, but do not provide a neural basis for these observations.

THC produces its CNS effects by binding to G-protein coupled cannabinoid type 1 (CB_1) receptors (Howlett *et al.*, 2002; receptor nomenclature follows Alexander *et al.*, 2009). While CB_1 receptors are widely distributed in the brain at high density (Herkenham *et al.*, 1991), their expression can vary throughout life. For example, the expression of CB_1 receptors and mRNA increase from birth until adolescence whereupon they decrease to adult levels (Rodriguez de Fonseca *et al.*, 1993; McLaughlin *et al.*, 1994; Belue *et al.*, 1995). Consistent with these findings, the acute effects of THC differ somewhat between adolescents and adults for certain *in vivo* measures (Cha *et al.*, 2006; Wiley *et al.*, 2007). The issue is further complicated by reports that adult female rats are more sensitive to the locomotor and antinociceptive effects of cannabinoids than male rats (Tseng and Craft, 2001; Wiley, 2003). Taken together, these findings demonstrate the importance of considering both age and sex in evaluating the effect of cannabinoids on CB_1 receptor function.

CB_1 receptor density and G-protein activation are further altered by repeated cannabinoid treatment. Animal studies have shown that repeated THC administration results in region-specific down-regulation (loss of receptor number) and desensitization (attenuated receptor-mediated G-protein activity) of CB_1 receptors (Sim-Selley, 2003). The potential impact of these adaptations is supported by findings that repeated marijuana use also produces CB_1 receptor down-regulation in brains from human marijuana users (Villares, 2007). Surprisingly, little is known about the neurobiological changes that occur following THC exposure during adolescence, despite the prevalence of marijuana use among adolescents. Adolescence in rodents is typically defined as a 2 week period that occurs between postnatal day (PN) 28–42 (Spear, 2000), during which changes in both physical (growth spurt, puberty) and behavioural (increased risk taking, increased social interaction with same-aged rodents) domains are similar to those observed in human adolescents. The few studies that have examined the effect of THC treatment during adolescence on CB_1 receptors have provided conflicting results, showing reduced CB_1 receptor density and G-protein activity (Rubino *et al.*, 2008) or no effect (Ellgren *et al.*, 2007). However, differences in rat strains and treatment regimen complicate comparison of results. Moreover, these studies did not compare the effects of THC in adolescents directly with the effects in adult animals. Therefore, the purpose of this study was to determine the consequences of repeated THC administration on CB_1 receptor density and receptor-mediated G-protein activity in various brain regions from adolescent and adult, male and female Long-Evans rats. This is an important question because CB_1 receptor desensitization/down-regulation might disrupt receptor-mediated effects of the endocannabinoid system in adolescents using marijuana and inappropriate modulation of this highly adapting system during adolescence could have profound consequences in later life, especially if chronic exposure to THC disrupts normal CNS maturation.

Methods

Animals

All animal care and experimental procedures reported in this manuscript were in accordance with guidelines published in the *Guide for the Care and Use of Laboratory Animals* (National Research Council, 1996) and were approved by the Institutional Animal Care and Use Committee at Virginia Commonwealth University. Adult (PN > 60) and

adolescent (PN 25) male and female Long-Evans rats, purchased from Harlan (Indianapolis, IN, USA), were housed one per cage. They were allowed to habituate to the animal facilities for at least 3 days before injections began. All animals were kept in a temperature-controlled (23°C) environment with a 12 h light/dark cycle (lights on at 0700 h). Rats were free fed and had free access to water.

Chronic THC administration

THC was dissolved in a solution of Tween 80/saline (8: 92, v/v). Rats were injected i.p. twice daily (7:00 and 14:00) with THC (10 mg kg^{-1}) or vehicle for 9.5 days (PN 30–39 and PN ~ 65–74 for adolescents and adults, respectively). Rats were killed by decapitation twenty-four hours after the final injection (PN 40 for adolescents and ~PN 75 for adults) and various brain regions were dissected, including prefrontal cortex, striatum (includes caudate putamen, nucleus accumbens and rostral globus pallidus), hypothalamus, hippocampus, ventral midbrain (includes ventral tegmental area and substantia nigra), periaqueductal gray (PAG) and cerebellum. Tissue was stored at –80°C until use.

Agonist-stimulated [^{35}S]GTPγS binding

Tissue was placed in 5 mL of cold membrane buffer (50 mM Tris-HCl, 3 mM $MgCl_2$, 1 mM EGTA, pH 7.4) and homogenized. The samples were then centrifuged at 50 000× *g* at 5°C for 10 min. Supernatant was removed, and samples were resuspended in 5 mL of assay buffer A (100 mM NaCl, 3 mM $MgCl_2$, 0.2 mM EGTA, 50 mM Tris-HCl, pH 7.4). Protein concentration was determined by the Bradford protocol (Bradford, 1976). Before assay, membranes (2–8 μg of protein) were preincubated for 25 min with adenosine deaminase (3 mU mL^{-1}) at 30°C in assay buffer. Concentration-effect curves were generated by incubating the appropriate amount of membrane protein (4–8 μg) in assay buffer B (assay buffer A plus 1.25 g L^{-1} BSA) with CP55,940 (0.01–10 μM) in the presence of 30 μM GDP and 0.1 nM [^{35}S]GTPγS in 0.5 mL volume at 30°C for 2 h. Basal binding was measured in the absence of agonist, and nonspecific binding was measured in the presence of 20 μM unlabelled guanosine 5′-3-O-(thio)triphosphate. The reaction was terminated by vacuum filtration though Whatman GF/B glass fiber filters, followed by two washes with 4°C Tris buffer (50 mM Tris-HCl, pH 7.4). Bound radioactivity was determined by liquid scintillation spectrophotometry at 95% efficiency after 10 h extraction in econosafe 1 scintillation fluid.

[^{3}H]SR141716A binding

Membranes were prepared as described above. Saturation analysis was performed by incubating 12 μg membrane protein with 0.2–3 nM [^{3}H]SR141716A (CB_1 receptor antagonist) in assay buffer A + BSA (0.5 g^{-1}) in the presence or absence of 5 μM unlabelled SR141716A (to determine non-specific and total binding, respectively) for 90 min at 30°C. The reaction was terminated by vacuum filtration through Whatman GF/B glass fiber filter that was pre-soaked in Tris buffer containing 5 g L^{-1} BSA (Tris–BSA), followed by three washes with 4 °C Tris–BSA. Bound radioactivity was determined by liquid scintillation spectrophotometry at 45% efficiency after extraction in ScintiSafe Econo 1 scintillation fluid.

Data analysis

Data are reported as mean (±SEM) of at least six experiments, each performed in triplicate. Non-specific binding was subtracted from each sample. Net stimulated [^{35}S]GTPγS binding was defined as agonist-stimulated minus basal [^{35}S]GTPγS binding, and percent stimulation was defined as (net-stimulated/basal [^{35}S]GTPγS binding) × 100%. Non-linear iterative regression analyses of agonist concentration–effect and saturation binding curves were performed with Prism 4.0 (GraphPad Software, Inc., La Jolla, CA). Separate age × sex ANOVAs were used to analyse E_{max} values, mean percent desensitization and mean percent down-regulation for each region. Separate age × sex × treatment ANOVAs were used to analyse B_{max} values for each region. Significant interactions revealed by ANOVAs were further analysed by Tukey *post hoc* tests ($\alpha = 0.05$) to specify the nature of the differences.

Materials

SR141716A and CP55,940 were provided by the Drug Supply Program of the National Institute on Drug Abuse (Rockville, MD, USA). All compounds were dissolved in ethanol. Guanosine 5′-3-*O*-(thio)triphosphate was purchased from Roche Diagnostics (Indianapolis, IN, USA). [^{35}S]GTPγS (1150–1250 Ci/mmol) was purchased from PerkinElmer Life and Analytical Sciences (Waltham, MA, USA). [^{3}H]-SR141716A (44.0 Ci/mmol) was purchased from GE Healthcare (Chalfont St. Giles, UK). WIN 55,212-2, GDP, bovine serum albumin (BSA) and adenosine deaminase were purchased from Sigma-Aldrich (St. Louis, MO, USA). Scintillation fluid (Econosafe 1) was purchased from Thermo Fisher Scientific (Waltham, MA, USA).

Results

This study compared the effects of chronic THC or vehicle treatment for 9.5 days on CB_1 receptor density and G-protein activity in adolescent and

Table 1

Maximal CP55,940-stimulated G-protein activation (E_{max}) in membranes from various brain regions in vehicle-treated adolescent and adult rats of both sexes

Brain region	Female adolescents	Female adults	Male adolescents	Male adults
Prefrontal cortex [$F(1,20) = 1.5$, $P = 0.2$]	185 (24)	146 (14)	131 (12)	134 (15)
Striatum [$F(1,20) = 2.5$, $P = 0.1$]	183 (19)	148 (16)	158 (12)	180 (23)
Hypothalamus [$F(1,20) = 0.3$, $P = 0.6$]	154 (13)	123 (17)	146 (9)	128 (7)
PAG [$F(1,20) = 0.002$, $P = 0.96$]	148 (10)	130 (11)	153 (12)	134 (10)
Hippocampus [$F(1,20) = 0.1$, $P = 0.7$]	167 (22)	114 (31)	198 (16)	159 (18)
Ventral midbrain [$F(1,20) = 2.2$, $P = 0.1$]	139 (17)	154 (19)	204 (22)	161 (19)
Cerebellum [$F(1,20) = 1.9$, $P = 0.2$]	264 (10)	261 (14)	242 (11)	269 (10)

E_{max} values for vehicle-treated rats of each age and sex are expressed as mean percent CP55,940-stimulated [^{35}S]GTPγS binding (SEM). For all regions, E_{max} values were not significantly different across age and sex. (*F* values for the age–sex interactions are presented in brackets in the leftmost column underneath the name of the region.)
PAG = periaqueductal gray.

adult male and female rats. Dosing began on PN 29 or >PN 60 for adolescent and adult rats, respectively, and brains were collected 24 h after the last injection. G-protein activation was assessed by measuring CP55,940-stimulated [^{35}S]GTPγS binding whereas CB_1 receptor density was measured using [^{3}H]SR141716A saturation binding. Tissue preparations from various brain regions were used to determine regional specificity of any treatment, age and sex effects. Basal [^{35}S]GTPγS binding did not significantly differ across age and sex in any region following THC treatment (data not shown).

Table 1 shows the results of CP55,940-stimulated [^{35}S]GTPγS binding assays on membranes prepared from various brain regions. The extent of G-protein activation (E_{max}) in vehicle-treated rats varied quite widely across brain region within rats of the same age and sex (e.g. 114% stimulation and 261% stimulation in hippocampus and cerebellum, respectively, of adult female rats). This regional variation in the magnitude of G-protein activation in vehicle-treated rats is in agreement with previous reports (Sim-Selley and Childers, 2002). Differences across groups in the same region also occurred (e.g. 167% stimulation and 114% stimulation in hippocampus for adolescent and adult females, respectively), but tended to be of lesser magnitude and none were statistically significant when analysed by region with age × sex factorial ANOVAs (*F* values presented in Table 1). When compared with vehicle treatment in corresponding membranes from rats of the same age and sex, THC reduced G-protein activation (i.e. lower E_{max} values) in all brain regions, but did not alter EC_{50} values (concentration of CP55 940 required to produce 50% of maximal stimulation), suggesting that THC-induced desensitization of CB_1 receptors occurred throughout the brain in both adolescents and adults. Figure 1 shows concentration-response curves for CP55,940-stimulated [^{35}S]GTPγS binding for two brain regions (hippocampus and cerebellum) in vehicle- or THC-treated rats of each sex and age.

In order to standardize THC-induced desensitization data across regions, desensitization in THC-treated rats was expressed as a percent of the maximal stimulation (E_{max}) of membrane homogenates from vehicle-treated rats separately for each region (Figure 2). Although regional differences in the magnitude of desensitization were evident, age and sex differences were also prominent. A consistent finding across most assessed brain regions [except the cerebellum: $F(1,20) = 0.45$, $P > 0.05$] was that tissues from female adolescents exhibited significantly greater desensitization as compared with one or more of the other groups. Significant age × sex differences in the magnitude of desensitization were observed in the prefrontal cortex [$F(1,20) = 9.8$, $P = 0.005$], striatum [$F(1,20) = 5.6$, $P = 0.028$], PAG [$F(1,20) = 45.9$, $P < 0.001$], hippocampus [$F(1,20) = 28.9$, $P < 0.001$], and ventral midbrain [$F(1,20) = 7.7$, $P = 0.012$] (Figure 2A, B, D, E and F, respectively). Tukey *post hoc* tests on the interaction revealed that significantly greater desensitization occurred in female adolescent rats than in female adult rats in the prefrontal cortex, PAG, hippocampus, and ventral midbrain. Age differences were also observed for males in three of the four areas (prefrontal cortex, PAG and hippocampus), but in the opposite direction. Lesser desensitization was seen in male adolescent rats than in male adult rats. In addition to these age differences, significant sex differences occurred in each of these four regions and in the striatum, with

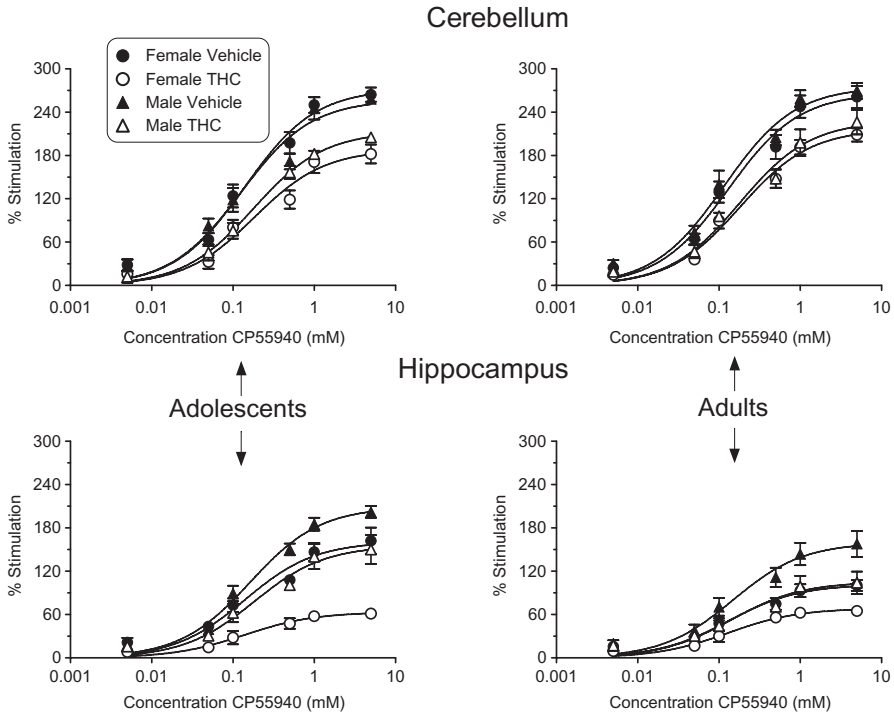

Figure 1

Concentration effect curves for CP55,940-stimulated [^{35}S]GTPγS binding in tissues from cerebellum (top panels) and hippocampus (bottom panels) of adolescent and adult rats (left and right panels, respectively) of both sexes that had received vehicle or THC treatment. E_{max} values for vehicle-treated rats are presented in Table 1.

desensitization being greater in female adolescent rats than in male adolescent rats. Interestingly, the only sex difference in adult rats was in the PAG where male adult rats exhibited greater desensitization than did female adult rats, an effect that was opposite to that seen in adolescent rats. However, the magnitude of this difference was small (albeit statistically significant). In the hypothalamus, the age–sex interaction was not significant; however, analysis of significant main effects of age [$F(1,20) = 43.8$, $P < 0.001$] and sex [$F(1,20) = 15.9$, $P < 0.001$] revealed that adolescents showed greater overall desensitization than adults and that females showed greater overall desensitization than males, respectively (Figure 2C). These findings are consistent with those obtained in other regions as described above. Finally, desensitization in the cerebellum did not significantly differ across age or sex (Figure 2G).

In order to determine the degree to which the observed desensitization was related to CB_1 receptor down-regulation, analysis of [^{3}H]SR141716A saturation curves was conducted on membrane homogenates derived from selected brain regions (prefrontal cortex, striatum, hypothalamus and ventral midbrain) [Table 2]. These regions were selected because they corresponded with regions most likely to be involved in mediation of pharmacological effects of THC that we previously measured in rats that had received treatments identical to those used here (Wiley *et al.*, 2007). These effects included catalepsy (ventral midbrain/striatum), hypothermia (hypothalamus) and locomotor activity (multiple regions). In each of these regions, vehicle-treated adolescent rats of both sexes had significantly higher densities of CB_1 receptors than did vehicle-treated adult rats (Table 2). THC treatment produced significant decreases in B_{max} across all regions in membranes derived from the brains of THC-treated adolescent and adult rats of both sexes (Table 2), although no change in the K_D value was seen as a result of THC treatment (data not shown). These results show that residual THC was not present in the membranes because the K_D value of [^{3}H]SR141716A was unchanged. These findings suggest that repeated treatment with THC caused a decrease in the total density of CB_1 receptors in these brain regions, as has been reported previously (Breivogel *et al.*, 1999). Unlike the data obtained for [^{35}S]GTPγS binding, however, the degree of THC-induced down-regulation (B_{max} of THC-treated rats expressed as a percentage change from the B_{max} of vehicle-treated rats of corresponding age and sex) did not significantly differ across sex or age (Table 2). These results suggest that the group

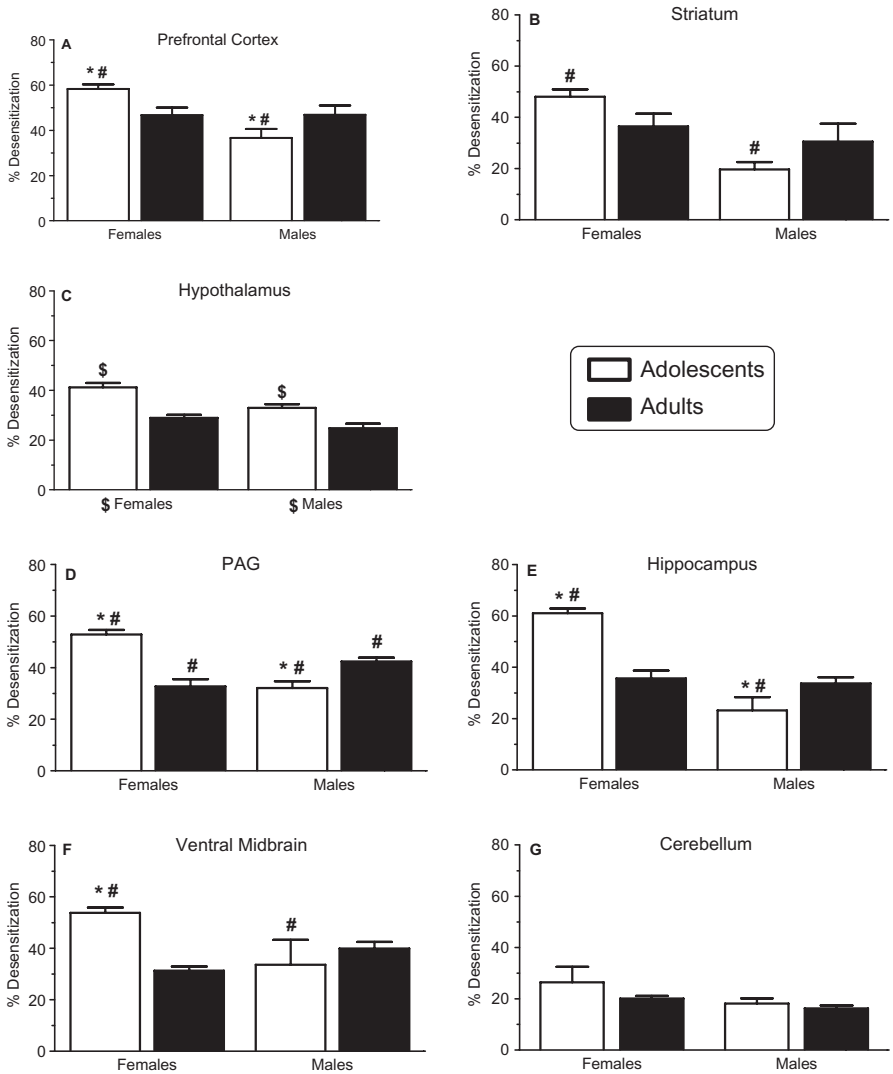

Figure 2

Desensitization, defined as decrease in maximal stimulation (E_{max}) of CP55,940-stimulated [^{35}S]GTPγS binding in THC-treated tissue. Data are expressed as a percent decrease in the E_{max} value, compared with vehicle-treated tissue from rats of the corresponding age and sex, in membrane homogenates derived from THC-treated female and male adolescent and adult Long-Evans rats treated twice daily with 10 mg kg^{-1} THC for 9.5 days. (A–G) Desensitization in the prefrontal cortex, striatum, hypothalamus, periaqueductal gray (PAG), hippocampus, ventral midbrain and cerebellum, respectively. Values represent mean (±SEM) percent desensitization for each group. * Significant ($P < 0.05$) age difference for adolescents versus adults of the same sex (based on age–sex interaction). # Significant ($P < 0.05$) sex difference within a single age group (based on age–sex interaction). $ Significant ($P < 0.05$) main effect for age or sex without an accompanying significant interaction.

differences observed in the percent CB_1 desensitization were not entirely mediated by CB_1 receptor down-regulation. Because there were no significant differences in the percent CB_1 down-regulation between different sex/age groups in these four regions, [^{3}H]SR141716A binding assays with tissue from other brain regions were not conducted.

Discussion

The primary goal of this study was to evaluate CB_1 receptor adaptation following repeated THC treatment in adolescent versus adult male and female rats. Administration of THC produced CB_1 receptor desensitization and down-regulation in all four groups, consistent with previous studies conducted in adult male rodents (Breivogel *et al.*, 1999; Sim-Selley, 2003), but the relative level of desensitization varied by group. Overall, female adolescent rats were more sensitive to THC-induced desensitization. While these differences reached significance in only certain regions when compared with male adolescent or to female adult rats, the overall trend is evident across several brain regions. Interestingly, adolescent male rats were less sensitive to

Table 2

[^{3}H]SR141716A B_{max} values and % down-regulation in membranes from various brain regions in vehicle- and THC-treated adolescent and adult rats of both sexes

Brain region	Female adolescents			Female adults			Male adolescents			Male adults		
	Veh	THC	% DR	Veh	THC	% DR	Veh	THC	% DR	Veh	THC	% DR
Prefrontal Cortex	4.5	3.4	23%	2.3	1.7	23%	4.8	3.8	20%	2.4	1.8	26%
	(0.15)	(0.18)	(5.8)	(0.13)	(0.09)	(6.5)	(0.11)	(0.14)	(3.2)	(0.09)	(0.17)	(5.0)
Striatum	3.4	2.8	17%	1.8	1.5	16%	4.1	3.4	18%	2.0	1.6	20%
	(0.12)	(0.06)	(3.8)	(0.05)	(0.03)	(3.8)	(0.08)	(0.24)	(4.6)	(0.04)	(0.07)	(4.5)
Hypothalamus	5.5	2.9	47%	3.8	1.9	50%	5.4	3.0	44%	2.7	1.5	45%
	(0.12)	(0.06)	(1.4)	(0.10)	(0.08)	(2.2)	(0.15)	(0.06)	(1.5)	(0.09)	(0.06)	(3.8)
Ventral midbrain	4.1	2.9	30%	2.9	2.1	29%	5.2	3.7	28%	3.7	2.6	29%
	(0.08)	(0.08)	(2.5)	(0.04)	(0.05)	(2.3)	(0.19)	(0.15)	(3.6)	(0.14)	(0.09)	(2.9)

Each value in columns labelled 'Veh' and 'THC' represents mean B_{max} value (SEM), expressed in pmol per mg of membrane protein. % DR = % down-regulation = B_{max} value of THC-treated rats expressed as a percent of maximal CB_1 receptor binding (B_{max}) of vehicle-treated rats of corresponding age and sex. Age × sex × treatment ANOVAs followed by Tukey *post hoc* tests revealed that B_{max} values for adolescents were significantly higher than those for adults, regardless of treatment, with age-treatment interaction terms (and associated probability levels) for each region as follows: [prefrontal cortex $F(1,40) = 5.5$, $P = 0.02$], [striatum $F(1,40) = 4.5$, $P = 0.04$], [hypothalamus $F(1,40) = 9.4$, $P = 0.004$], [ventral midbrain $F(1,40) = 6.0$, $P = 0.02$]. In addition, the main effects for treatment were significant, indicating that THC treatment decreased B_{max} values in each region. In contrast, percent down-regulation for each region did not differ across age and sex.

THC-induced desensitization than were male adult rats in three brain regions (prefrontal cortex, PAG and hippocampus), albeit the magnitude of difference was much less than for females. Subsequent receptor binding experiments revealed that the greater desensitization seen in female adolescents and the decreased desensitization in male adolescents did not result from differences in down-regulation of CB_1 receptors in the regions examined.

A second major finding was that vehicle-treated female and male adolescent rats had higher expression of CB_1 receptors than adults in several brain regions examined, as is consistent with CB_1 receptor pruning that occurs over the course of adolescence (Rodriguez de Foseca *et al.*, 1993). Interestingly, this difference did not reliably translate into greater receptor-mediated G-protein activation in vehicle-treated rats, suggesting that signalling may be less efficient in the brains of adolescent rats. In contrast, neither CB_1 receptor number nor their activation as determined using agonist-stimulated [^{35}S]GTPγS binding differed between male and female adult rats. This result was somewhat surprising, given reports that female rats are more sensitive to the anti-nociceptive, memory and motor effects of THC (Tseng and Craft, 2001; Wiley, 2003; Cha *et al.*, 2007), and suggest that *in vivo* differences seen acutely might relate more to pharmacokinetic than pharmacodynamic factors (see also Tseng *et al.*, 2004), although possible sex differences in downstream signalling mechanisms cannot be excluded (see Auger, 2003; Zhang *et al.*, 2003). To date, age differences in pharmacokinetics of THC between adolescent and adult rats have not been reported for either sex.

With the exception of the PAG, sex differences in CB_1 receptor desensitization following THC treatment were only present in adolescents, where females exhibited greater desensitization than males. In the PAG, male adults showed greater desensitization than female adults, an effect that was opposite to that seen in the PAG in adolescent males and females. To our knowledge, one other study has examined desensitization of CB_1 receptors throughout the brain immediately following THC treatment in adolescence (Rubino *et al.*, 2008). Despite some methodological differences (Sprague-Dawley rats, different THC regimen, autoradiographic analysis) the same overall conclusion was reached: CB_1 receptor desensitization was greater in female compared with male adolescent rats. In fact, in the previous report, desensitization was found in only four regions in male rats, despite the presence of regionally widespread down-regulation. Surprisingly, the age- and sex-dependent differences in the magnitude of CB_1 receptor desensitization were not reflected by *in vivo* studies from our laboratory in which rats received THC according to the regimen in this study (Wiley *et al.*, 2007) and then were assessed in a tetrad of tests in which psychoactive cannabinoids produce characteristic effects (reduction in spontaneous activity, anti-nociception, hypothermia and catalepsy; Martin *et al.*, 1991). In part, this disparity between *in vivo* and *in vitro* data

might be related to a floor effect because maximal tolerance (i.e. resulting in a small or absent effect) was observed in most of the *in vivo* assays. Further, the *in vivo* assessment did not include dependent variables that are likely to be mediated by some of the brain areas in which desensitization was observed to differ between adolescent and adult rats or between sexes in adolescent rats (e.g. cognitive tasks involving hippocampal functioning). Moreover, several factors that affect *in vivo*, but not *in vitro*, measures might have contributed to the discrepancy between the *in vivo* and *in vitro* effects of repeated dosing with THC. First, G-protein activation represents a relatively early signalling event. As many other downstream pathways affect *in vivo* responses, compensatory adaptation could occur in any of these signalling mechanisms. Additionally, *in vivo* activity results from the interaction of several receptors and anatomical regions, so that concomitant adaptations in non-cannabinoid receptor systems could also affect results.

The finding that female adolescent rats were more sensitive to CB_1 receptor desensitization suggests that their endocannabinoid system might differ somewhat from the other groups examined, but the mechanism(s) underlying enhanced THC-induced desensitization in female adolescents has not yet been identified. Ellgren *et al*. (2008) systematically evaluated endocannabinoid levels and CB_1 receptors in the striatum and prefrontal cortex of vehicle- and THC-treated male rats at early (PN 29), mid (PN 38) and late (PN 50) adolescence. Results in vehicle-treated rats revealed that the levels of the endocannabinoids anandamide and 2-arachidonyl glycerol (2-AG), as well as the density of CB_1 receptors, varied over the adolescent period, with different patterns emerging in different regions. For example, anandamide levels peaked in the nucleus accumbens at mid-adolescence, whereas 2-AG decreased in this region from early to mid-adolescence. In contrast, endocannabinoids were stable in the caudate-putamen over the period examined. CB_1 receptor density increased in the nucleus accumbens shell over adolescence, but decreased in the nucleus accumbens core and in the prefrontal cortex. Intermittent THC administration (1.5 mg kg^{-1} given every 3 days) did not alter CB_1 receptor expression, probably because the dose was rather low (Ellgren *et al*., 2007). The effect of THC on the endocannabinoids was complex, but the overall pattern was a perturbation in the normal ratio of anandamide to 2-AG in the nucleus accumbens (significant at mid adolescence) and prefrontal cortex (significant in late adolescence). The functional significance of this ratio has been shown in the adult striatum, where anandamide attenuated the electrophysiological effects of 2-AG on GABA neurons via modulation of its synthesis by diacylglycerol lipase (Maccarrone *et al*., 2008). These findings suggest that the immediate effects of THC treatment, such as desensitization and tolerance, could vary throughout adolescence due to its disruption of this ratio as well as to its direct effects on receptor expression and activation (as shown here). Moreover, the present study suggests that effects might be more pronounced in females, especially during certain developmental windows.

Studies in which the FAAH inhibitor URB597 was administered during adolescence provide further evidence that perturbation of anandamide during adolescence can have long lasting consequences (Marco *et al*., 2009). Examination of brains from adult male rats that had received URB597 in adolescence revealed region-specific changes in CB_1 receptors that included reduced receptor expression in the caudate-putamen, nucleus accumbens, ventral tegmental area (VTA) and hippocampus, with no change in most other regions examined. The effects of THC might be further exacerbated by differences in THC pharmacokinetics based on age and/or sex. Following an acute injection of THC, previous research has shown that higher peak brain levels of THC and its major active metabolite, 11-hydroxy-THC, are observed in adult female rats than in males (Tseng *et al*., 2004); however, this study did not measure the effects of THC after repeated administration, as was performed for the desensitization assessment reported here.

In conclusion, the present study showed age- and sex-dependent differences in the magnitude of CB_1 receptor desensitization following repeated treatment with THC. Adolescent female rodents consistently showed the greatest degree of desensitization across all regions, suggesting that teenagers, especially females, might be particularly sensitive to the effects of repeated marijuana use on the brain endocannabinoid system, although whether this enhanced desensitization reflects increased vulnerability to disruption or protective adaptation is still uncertain. Nevertheless, if CB_1 receptor desensitization and down-regulation occur in teenagers, as they do in rats, the consequences of chronic marijuana use during this critical window of synaptic maturation could have profound consequences in later life. In light of data from previous studies, increased susceptibility to desensitization in female adolescents might result from interactions between THC (and its metabolites) and endocannabinoids, but this area has not been well defined in either adolescent or adult models. Clearly further research is needed in this area, specifically to examine the mechanisms of differential age- and sex-dependent

sensitivity to THC-induced CB_1 receptor desensitization and to determine the temporal profile of repeated adolescent THC exposure on CB_1 receptor function immediately following treatment and in adulthood.

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Conflict of interest

None.

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