Published in final edited form as:

Neurotoxicol Teratol. 2008 ; 30(3): 213–219. doi:10.1016/j.ntt.2007.12.007.

PERINATAL DELTA-9-TETRAHYDROCANNABINOL EXPOSURE DISRUPTS SOCIAL AND OPEN FIELD BEHAVIOR IN ADULT MALE RATS

R.J. Newsom and S.J. Kelly

Department of Psychology, University of South Carolina, Columbia, SC 29208

Abstract

Marijuana is the most frequently used illegal drug among women of reproductive age, but little is known about the consequences of using marijuana during pregnancy. Delta-9-tetrahydrocannabinol (Δ^9 -THC), one of the active chemicals in marijuana, has been shown to cross the placental barrier easily. In this study, pregnant Long Evans rats were assigned to one of three treatment groups (Δ^9 -THC-exposed, vehicle control, and non-treated control) on day 1 of gestation. Drug exposure consisted of 2 mg/kg of natural Δ^9 -THC, administered twice daily by subcutaneous (s.c.) injection, from gestational day 1 through 22. Pups continued to receive drug exposure via s.c. injection from postnatal day 2 through 10. Male rats from each group were tested starting on postnatal day 90 in a battery of tests which included open field activity, active social interaction, and the forced-swim test. There were no significant differences in weight gained by dams or weight of offspring when compared to controls. Δ^9 -THC-exposed rats showed decreased time in the inner part of the open field and an increase in investigation time in the test of social interaction compared to both control groups. There were no differences among groups in the forced-swim test. Perinatal Δ^9 -THC exposure may result in increased susceptibility to anxious behavior and alter social functioning in adult offspring.

Keywords

THC; marijuana; prenatal; perinatal; social behavior; anxiety; forced swim test

1. Introduction

Marijuana is a complex compound containing numerous active ingredients. One of the most prevalent psychoactive ingredients of marijuana is delta-9-tetrahydrocannabinol (Δ^9 -THC) which acts at cannabinoid (CB) receptors [35]. Two subtypes of cannabinoid receptors (CB₁ and CB₂) have been identified. The CB₁ receptors are found predominantly in the brain and mediate the subjective effects of marijuana [1]. The CB₂ receptors are found predominantly in the spleen and immune system but have recently been shown to be on microglia, astrocytes and some neuronal populations [15,33,50]. Importantly, developmental studies of rodents [6]

Corresponding author: Sandra J. Kelly, Department of Psychology, University of South Carolina, Columbia, SC 29208, Tel: 803-777-7610, Fax: 803-777-9558, Email: sandra-kelly@sc.edu.

Conflict of Interest

There were no conflicts of interest for either author.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

and humans [34] suggest CB₁ receptors are present and active starting in early prenatal periods, with receptor density gradually but substantially increasing throughout development until adult levels are reached. CB receptor binding has been detected as early as gestational day (GD) 11 in rodent brains [7,27] and week 14 of gestation in human brains [8,34]. Endogenous cannabinoid activity at both CB₁ and CB₂ receptors has been proposed to have functions specific to development of the nervous system [14,15,27]. In particular, activation of CB₁ receptors has been suggested to be involved in cell survival and differentiation [for a review, see 14,27] and the CB₂ receptor has been proposed to be involved in cell death and neuroprotection [for a review, see 15].

Given the involvement of the endogenous cannabinoid system in development of the nervous system, it is not surprising that a variety of behavioral and cognitive abnormalities have been reported in children of women who self-reported marijuana use during pregnancy [12,22,23, 26,45]. While changes in attention and cognitive function have been widely reported [19,42, 45], socioemotional changes after developmental exposure to marijuana have been less frequently investigated. Nevertheless, increased fearfulness and shorter length of play have been reported in children after prenatal exposure to marijuana [12]. Marijuana-exposed children from the Maternal Health Practices and Child Development Project were reported to show impulsivity, hyperactivity, increased delinquency and increased externalizing problems [23] and an increase in prevalence childhood depressive and anxiety symptoms at ten years of age [26].

In order to control for the confounding factors in human studies of prenatal marijuana exposure, animal models have been used and are critical for isolating the effects of prenatal marijuana use from other confounds (for reviews, see [14,27,40]). As in the human literature, the research on animals has focused on cognitive deficits but there is some research on socioemotional changes. Exposure to hashish during the perinatal period has been shown to alter behavior in a social preference test in adult male but not adult female rats [39]. There is also evidence that perinatal exposure to Δ^9 -THC results in a sexually dimorphic change in the hypothalamic-pituitary-adrenal axis, with females showing an up-regulation and males showing a down-regulation of this system after exposure to Δ^9 -THC during development [46]. CB₁ receptor knockout mice show suppression of the endocrine and behavioral response to repeated stresses [18]. There are also reports of increased exploratory behavior in a plus-maze paradigm [46] and in the defensive withdrawal test [47] in Δ^9 -THC -exposed rats, suggesting less anxiety. Behavior in an open field is influenced by some, but not all, aspects of anxiety [44] with a decrease in locomotion, particularly in the inner portion of the open field, often interpreted as an increase in anxiety [44]. The findings on the effects of gestational exposure to cannabinoids on locomotor activity in rodents are mixed and may be both age- and sex-dependent. In adult rats, perinatal exposure to Δ^9 -THC has been shown to result in either decreased activity at long intervals (with stronger effects in males) [38] or increased activity in females [41,46]. Perinatal exposure to hashish has been shown to have no significant effects on activity in adult rats [39] whereas prenatal exposure to anandamide, the endogenous ligand for the CB₁ receptor, results in decreased activity in both sexes [17].

Rodent models of marijuana exposure during development have been such that the exposure period either has been limited to gestation in the rat [e.g., 29,37] or has been through the dam's milk during the early postnatal period [e.g., 39,41]. The prenatal period in the rat does not include the period equivalent to the third trimester in humans [5]. The third trimester in humans or the postnatal period in the rat, which is equivalent to the third trimester, is a critical period for teratogenic effects on the brain [11]. While Δ^9 -THC crosses into the milk of a lactating animal, the amount that actually gets into the offspring is less than 1% of the dose [10,30] (compared to almost complete transfer across the placental barrier [2,4]) and is unlikely to mimic third trimester exposure in humans. Furthermore, the exposure of the lactating female

rat to cannabinoids disrupts its maternal behavior and milk release which will impact pup development [9] in a manner different than a direct teratogenic effect of cannabinoid exposure on the offspring.

This study uses a pre- and early postnatal exposure period which closely mimics the period of central nervous system development that occurs during the three trimesters of human pregnancy [5]. Importantly, the drug administration during the postnatal period in the rat was directly to the pup to ensure that exposure of the pup to a moderate dose of Δ^9 -THC and that maternal behavior was not directly impacted. In this study, drug administration is standardized in two 2 mg/kg subcutaneous injections daily of natural Δ^9 -THC which mimics estimates of low to moderate daily exposure to this compound in humans and is comparable to the dose used in previous studies [41,46]. The oral route of administration was avoided due to significant first pass metabolism and inconsistent absorption as well as the creation of new cannabinoids during digestion [35]. The daily dosage was divided into two injections to mimic the pattern of heavy human users of marijuana who report smoking multiple joints throughout the day [3], in a manner that would cause neuronal exposure to an elevated level of cannabinoids for a significant portion of every day.

Importantly, this study examines socioemotional behavior in the adult animal to determine any long-lasting or emerging effects due to Δ^9 -THC exposure across the period equivalent to all three trimesters in the human. Measures of socioemotional behavior included open field behavior, social interactions, and the behavioral despair test. Open field behavior is sensitive to both changes in overall activity and anxiety levels [44], with the activity in the inner field being particularly sensitive to anxiety [44]. Measurement of social interactions [36] can reflect changes in both anxiety and social behavior. The forced swim test has been used as an assay for antidepressant effects of drugs and is often considered a model of depression [43]. Given that adolescents exposed to marijuana during the prenatal period show high rates of depression [26], a test sensitive to anti-depressants was considered to be an important addition to the battery of tests to assess the socioemotional effects of perinatal administration of Δ^9 -THC in adult rats.

2. Methods

2.1. Subjects

Twenty-four female Long Evans rats were obtained from Harlan and bred in the laboratory after a 1 week period of adaptation. Breeding consisted of overnight housing of virgin females and proven stud males in breeder cages (4–5 females and 1 male in each cage). Pregnancy was determined the following morning by vaginal swabs that were smeared on culture slides and examined under microscope for the presence of sperm. Dams were randomly assigned to one of three groups: Δ^9 -THC-exposed (Δ^9 -THC), Non-treated control (NC), and Vehicle-injected control (IC). Each group consisted of 8 dams. Rats were housed in a colony with temperature and humidity controls, and were under a 12/12 light cycle. Food consumption was not limited. Pups were paw-marked for identification on PD 10 and weaned on PD 21 and housed in same-sex groups of 2–3. One male rat was randomly selected from each litter for testing resulting in a total of 8 rats per group. The selected offspring were tested in all behavioral tests. The pregnant dams were weighed daily from GD 1 to GD22, and offspring were weighed daily from PD 2 to PD 10, and on PD 21, 30, 60, and 90.

2.2. Drug Treatment

Drug treatment began on day 1 of gestation and consisted of 2 mg/kg injections of natural Δ^9 -THC (NIDA), administered twice daily. Δ^9 -THC was administered via subcutaneous injection in a vehicle of ethanol, Tween 80, and 0.9% saline, in a ratio of 1:1:18. Drug exposure

continued from gestational day (GD) 1 to GD 22, and PD 2 to PD 10. IC rats received twice daily vehicle injections to control for the effects of the stress from drug administration and the vehicle. NC rats received no treatment. The injection volume for both the Δ^9 -THC and the vehicle was 1.06 ml/kg.

2.4. Behavioral Testing

Behavioral testing began on PD 90, when rats are considered fully developed adults [48]. The order of testing was as follows: observation of activity in an open field, observation of active social interactions, and behavior in the forced swim test.

2.4.1. Open Field Activity—Testing was as described in [25]. Each rat was habituated to a square box ($40.6 \times 40.6 \times 40.6$ cm) with high walls for three periods of 10 minutes daily for three days. The box was electronically divided into an inner square (10.2×10.2 cm), and an outer area surrounding the inner square. Testing consisted of one ten-minute period in which movement was measured via a camera mounted above the open field and the Ethovision automated tracking program (Noldus). The box was wiped with alcohol before each testing session to minimize the scent of other rats. Measurements included distance and time spent in the inner and outer sections.

2.4.2. Active Social Interaction—Active social interactions were assessed as described in [31,36]. After three daily ten minute habituation periods to a clear housing box, test rats were placed with an unfamiliar, non-experimental rat of the same size and sex. A new, clean housing box was used for each session. Each rat was videotaped for a 15 minute period. The tapes were scored for incidence of sniffing, dominant mounts, social grooming, chasing, pinning, biting, boxing, and avoidance. For each videotaped session, behaviors were scored every 5 seconds.

2.4.3 Forced Swim Test—The forced swim test was conducted as described in [43]. Each rat was placed in a tank of room temperature water for a habituation period of 15 minutes in order to induce a state of helplessness. Approximately 24 hours later, each rat was again placed in the water tank for a 5 minute period. The latency to immobility (judged as passively floating with movement only to keep one's head above the water) was measured.

3. Results

3.1 Physical Data

Dams were weighed daily from gestational day (GD) 1 until GD 22. Analysis of body weights using a repeated measures analysis of variance (ANOVA) indicated no significant effect of group or interaction of group and gestational day. There was a main effect of gestational day ($F(21,252) = 105.2$, $p < 0.001$) indicating that all dams gained weight over pregnancy (see Table 1).

Length of gestation did not differ between groups; all dams gave birth on gestational day 23 and there was no mortality among the pups. The mean weights of the experimental rats were analyzed over PD 2 through PD 10 and on PD 21, 30, 60, and 90. A repeated measures ANOVA indicated no significant differences among groups or interaction of group with postnatal day. There was a main effect of postnatal day ($F(12,252) = 3,338.3$, $p < 0.001$) indicating that all pups gained weight across postnatal day (see Table 1).

3.2. Behavioral Data

3.2.1. Open Field Activity—An ANOVA on time spent in the inner section showed a significant main effect of group [$F(2,21) = 3.7$, $p < 0.05$] and LSD *post hoc* tests showed that the Δ^9 -THC rats spend less time in the inner section compared to NC and IC control rats (p's

< 0.05) (see Figure 1). An ANOVA on distance traveled in the inner section did not show a main effect but did show a trend towards a main effect of group [$F(2,21) = 3.5$, $p = 0.051$]. LSD *post hoc* tests showed that the Δ^9 -THC rats traveled less distance in the inner section compared to NC and IC controls (p 's < 0.05) (see Table 2). Analyses of distance traveled or time spent in the outer section did not reveal a significant difference or a statistical trend towards a significant difference (p 's > 0.10) among groups (see Table 2).

In order to further explore these effects, a time ratio was constructed where the time spent in the inner section was divided by the sum of the time spent in the inner and outer sections of the open field. A distance ratio was constructed in a similar manner. These data are shown in Table 2. Planned comparisons revealed that the Δ^9 -THC rats had significantly smaller time and distance ratios compared to the control rats (p 's < 0.05), suggesting that the effects on activity were specific to the inner section.

3.2.2 Active Social Interaction—To assess social interaction, investigation (sniffing) time was analyzed by an ANOVA. There was a main effect of group ($F(2,21) = 4.2$, $p < 0.05$); the Δ^9 -THC rats exhibited an increase in sniffing compared to both NC and IC rats (Tukey's *post-hoc* tests, p 's < 0.05) (See Figure 2). There were no incidences of social grooming, biting, or chasing. The incidence of dominant mounts, pinning, boxing, and avoidance were shown by only a few animals within each group and were too low to analyze (data shown in Table 3).

3.2.3. Forced Swim Test— Δ^9 -THC rats did not differ significantly from NC and IC rats in latency to immobility in a five minute session 24 hours after inducing a state of helplessness. The average latencies to immobility and SEMs in seconds were 219.2 ± 23.3 , 217.4 ± 11.6 and 223.8 ± 12.8 in the NC, IC and THC groups, respectively.

4. Discussion

The results of this study suggest that perinatal exposure to Δ^9 -THC causes alterations in the central nervous system that result in disruptions of social and emotional behavior in adult male Long Evans rats. The presence of these behavioral differences was observed on PD 90 and later, implying that they are long-lasting changes. Physical measurements of growth and length of gestation support the consensus of the majority of the existing literature that perinatal Δ^9 -THC exposure does not alter weight gained by dams or offspring, and does not shorten the length of gestation [20,21,37]. The few reports of shortened gestation period and reduced weight of human offspring due to prenatal marijuana use may be due to confounds such as additional drug use or environmental factors.

The behavioral results indicate specific effects on anxiety-like behavior and social interaction. In the test of open field activity, Δ^9 -THC-exposed rats spent significantly less time in the center area of the maze compared control rats. Distance traveled in the inner section of the field showed a trend towards significant effect by group. This type of behavior has been interpreted as anxious or fearful [25]. Ratios of time spent and distance traveled in the inner section divided by time and distance (respectively) in both the inner and outer sections suggest the decrease in behavior seen in Δ^9 -THC rats in the open field was specific to the inner section and not an overall disruption of psychomotor activity as reported elsewhere [38]. These results suggest increased susceptibility to anxiety-like behaviors in adulthood in the Δ^9 -THC -exposed rats.

The measurement of active social interactions revealed significant differences in investigative behavior (seen as sniffing in rats). Δ^9 -THC rats displayed a substantial increase in sniffing compared to controls. The increase in active social interactions could be interpreted as a decrease in anxiety [16] although this interpretation is inconsistent with our findings on open field activity described above. However, given the multi-faceted nature of anxiety, it is not

unusual for different measures of anxiety to be inconsistent with each other [16,44]. Another possibility is that the increased investigative behavior observed in this study is a precursor to aggression induced by the anxiety of exposure to a novel animal. This interpretation would be consistent with clinical reports of prenatal marijuana exposure correlating with behavioral disruptions such as shorter length of play, increased fearfulness, hyperactivity and increased delinquency and externalizing problems [12,23]. However, Δ^9 -THC rats did not show increases in other aggressive behaviors (pinning, biting, chasing, or boxing) suggesting the effect of perinatal Δ^9 -THC on social interactions may be subtle. The specific nature of the change in social interactions induced by exposure to Δ^9 -THC during development could be further analyzed with more direct tests of aggression, play and sexual behaviors.

The results from the forced swim test suggest that perinatal Δ^9 -THC exposure is not correlated with increased susceptibility to depression or learned helplessness. The forced swim test was chosen for this study as a model of depression, since it has been shown to be sensitive to a variety of anti-depressants [32,43]. A growing body of recent literature has suggested a relationship of endogenous cannabinoid activity to depressive and anxiety behaviors [28,49, 51] and an increase in depressive and anxiety symptoms has been reported at 10 years of age [26] in a sample of children exposed to marijuana during gestation. It is possible that environmental stress and/or higher amounts of exposure to Δ^9 -THC are necessary to induce changes in behavior in the forced swim test. Another possibility is that the increased susceptibility to depression is corrected by adulthood, and therefore was not detected in our observations of adult rats.

The neural changes that mediate the effects of exposure to Δ^9 -THC during the perinatal period remain to be elucidated. Δ^9 -THC acts on both CB₁ and CB₂ receptors and both of these receptors are involved in development of the nervous system. Actions on the CB₁ receptors are likely to have complex effects on cell number, neuronal differentiation, and synaptic connections whereas actions on CB₂ receptors may have more specific effects on cell number [15,27]. Repeated cannabinoid exposure in adults is associated with down regulation or desensitization of CB₁ receptors [24], and is reversible in fully developed brains; it is plausible that this may not be the case in the developing brain. Furthermore, given the interactions of the cannabinoid system with other systems that modulate development of the nervous system [27], it is likely that exposure to Δ^9 -THC during development may not only alter the endogenous cannabinoid system but other systems as well.

There are a number of limitations to this study. The first is the use of a single dose of Δ^9 -THC, since it is likely that the effects of perinatal exposure to cannabinoids are dependent upon dose. However, this study did use a dose that was within a moderate range [41,46] and thus, the effects might reflect those that are typical. Another limitation is the testing of behavior at one time point. The ninety day time point was chosen because it is well past adolescence in a rat and considered to be a period where behavior is stable until aging effects occur [48]. Nevertheless, it might be revealing to study the time course of Δ^9 -THC effects across development both with respect to neural bases and behavioral mechanisms. It is also possible that the effects of exposure to Δ^9 -THC during early development on adult rats might either be ameliorated or exacerbated by the aging process or exposure to environmental stressors or enrichment. Examination of whether the Δ^9 -THC effects in rats can be ameliorated by various interventions would be an interesting avenue of research with important clinical implications.

Care must be taken when generalizing the current findings to other populations or measures. This study only examined behavior in males and the findings may differ in females. Many of the behavioral tests for rodents have been validated in males only. The performance in tests by females, particularly in tests of anxiety, does not necessarily reflect the same aspects of behavior as that in males [13]. This may explain some of the observed sexually dimorphic

effects of perinatal Δ^9 -THC exposure [38,40]. It is also important to note that this study only used two measures of anxiety in rats, namely open field behavior and the forced swim test. As already mentioned, anxiety is a multifaceted concept with multiple ways to measure it and each of those measures has various strengths and weaknesses (see [16,44] for reviews). It will be important to use other measures of anxiety, such as the elevated plus maze and potentiated startle test, to assess the extent to which the current findings can be generalized.

The results of this study are consistent with previous findings that the brain is altered by exposure to Δ^9 -THC during development and this alteration does not fully recover [14,27]. Importantly, these findings are based on a rat model where the moderate exposure occurs throughout the period equivalent to all three human trimesters [5] and maternal behavior is not directly altered by the Δ^9 -THC exposure. Increased anxiety, as measured by decreased locomotion in the inner portion of an open field, and increased social investigation were found to be altered in adults that had been exposed to Δ^9 -THC during development, suggesting long-lasting changes in socioemotional behavior. Given that the cannabinoid system is involved in anxiety in a dose- and context-dependent manner [51], a permanent change in this system would impact anxiety and possibly social behaviors in adult rats. Clearly, more research needs to be conducted, particularly examining the impact of development exposure to Δ^9 -THC on cannabinoid receptors and endogenous cannabinoid activity and on other measures of anxiety and social behaviors. Long-term changes in socioemotional behaviors after exposure to cannabinoids during development have direct implications for behavioral and pharmacological treatments.

Acknowledgements

This work was funded by NIAAA grant 11566 to S.J.K and a fellowship from the University of South Carolina Honors College to R.J.N. The delta-9-tetrahydrocannabinol was supplied without cost by NIDA. These agencies had no involvement in the conduct or the publication of the study.

References

1. Alicia T, Appel JB. Increasing the selectivity of the discriminative stimulus effects of delta-9-tetrahydrocannabinol: complete substitution with methanandamide. *Pharmacol Biochem Behav* 2004;79:431–437. [PubMed: 15582014]
2. Ash RH, Smith CG. Effects of delta 9-THC, the principal psychoactive component of marijuana, during pregnancy in the rhesus monkey. *J Reprod Med* 1986;31:1071–1081. [PubMed: 3025441]
3. Ashton CH. Pharmacology and effects of cannabis: a brief review. *Br J Psychiatry* 2001;178:101–106. [PubMed: 11157422]
4. Bailey JR, Cunny HC, Paule MG, Slikker W. Fetal disposition of delta 9-tetrahydrocannabinol (THC) during later pregnancy in the rhesus monkey. *Toxicol Appl Pharmacol* 1987;90:315–321. [PubMed: 2820086]
5. Bayer SA, Altman J, Russo RJ, Zhang X. Timetables in the human brain based on experimentally determined patterns in the rat. *Neurotoxicology* 1993;14:83–144. [PubMed: 8361683]
6. Belue RC, Howlett AC, Westlake TM, Hutchings DE. The ontogeny of cannabinoid receptors in the brain of postnatal and aging rats. *Neurotoxicol Teratol* 1995;17:25–30. [PubMed: 7708016]
7. Berrendero F, Sepe N, Ramos JA, Di Marzo V, Fernandez-Ruiz JJ. Analysis of cannabinoid receptor binding and mRNA expression and endogenous cannabinoid contents in the developing rat brain during late gestational and early postnatal period. *Synapse* 1999;33:181–191. [PubMed: 10420166]
8. Biegon A, Kerman IA. Autoradiographic study of pre- and postnatal distribution of cannabinoid receptors in human brain. *Neuroimage* 2001;14:1463–1468. [PubMed: 11707102]
9. Bromley BL, Rabii J, Gordon JH, Zimmerman E. Delta-9-tetrahydrocannabinol inhibition of suckling-induced prolactin release in the lacting rat. *Endocr Res Commun* 1978;5:271–278. [PubMed: 752632]

10. Chao FC, Green DE, Forrest IS, Kaplan JN, Winship-Ball A, Braude M. The passage of 14C- Δ -9-tetrahydrocannabinol into the milk of lactating squirrel monkeys. *Res Comm Chem Path Pharmacol* 1976;15:303–317.
11. Cronise K, Marino MD, Tran TD, Kelly SJ. Critical periods for the effects of alcohol exposure on learning in rats. *Behav Neurosci* 2001;115:138–145. [PubMed: 11256437]
12. Faden VB, Graubard BI. Maternal substance abuse during pregnancy and developmental outcomes at age three. *J Subst Abuse* 2000;12:329–340. [PubMed: 11452837]
13. Fernandes C, González MI, Wilson CA, File SE. Factor analysis shows that female rat behaviour is characterized primarily by activity, male rats are driven by sex and anxiety. *Pharmacol Biochem Beh* 1999;64:731–738.
14. Fernández-Ruiz J, Berrendero F, Hernández ML, Ramos JA. The endogenous cannabinoid system and brain development. *TINS* 2000;23:14–20. [PubMed: 10631784]
15. Fernández-Ruiz J, Romero J, Velasco G, Tolón RM, Ramos JA, Guzmán M. Cannabinoid CB₂ receptor: a new target for controlling neural cell survival. *TIPS* 2006;28:39–45. [PubMed: 17141334]
16. File, SE. Animal models of different anxiety states. In: Biggio, G.; Sanna, E.; Costa, E., editors. *GABA_A Receptors and Anxiety: From Neurobiology to Treatment, Advances in Biochemical Psychopharmacology*. New York: Raven Press; 1995. p. 93-113.
17. Fride E, Mechoulam R. Ontogenetic development of the response to anandamide and Δ^9 -tetrahydrocannabinol in mice. *Dev Brain Res* 1996;95:131–134. [PubMed: 8873985]
18. Fride E, Suris R, Weidenfeld J, Mechoulam R. Differential response to acute and repeated stress in cannabinoid CB₁ receptor knockout newborn and adult mice. *Behav Pharmacol* 2005;16:431–440. [PubMed: 16148448]
19. Fried PA. Adolescents prenatally exposed to marijuana: examination of facets of complex behaviors and comparisons with the influence of in utero cigarettes. *J Clin Pharmacol* 2002;42:97S–102S. [PubMed: 12412842]
20. Fried PA. Conceptual issues in behavioral teratology and their application in determining long-term sequelae of prenatal marihuana exposure. *J Child Psyc Psychiatry* 2002;43:81–102.
21. Fried PA, Smith AM. A literature review of the consequences of prenatal marihuana exposure: an emerging theme of a deficiency in aspects of executive function. *Neurotoxicol Teratol* 2001;23:1–11. [PubMed: 11274871]
22. Fried PA, Watkinson B, Gray R. Differential effects on cognitive functioning in 13- to 16-year olds prenatally exposed to cigarettes and marihuana. *Neurotoxicol Teratol* 2003;25:427–436.
23. Goldschmidt L, Richardson GA, Cornelius MD, Day NL. Prenatal marijuana and alcohol exposure and academic achievement at age 10. *Neurotoxicol Teratol* 2004;26:521–532. [PubMed: 15203174]
24. Gonzalez S, Cebeira M, Fernandez-Ruiz J. Cannabinoid tolerance and dependence: a review of studies in laboratory animals. *Pharmacol Biochem Behav* 2005;81:300–318. [PubMed: 15919107]
25. Gonzalez S, Fernandez-Ruiz J, Di Marzo V, Hernandez M, Arevalo C, Nicanor C, Cascio MG, Ambrosio E, Ramos JA. Behavioral and molecular changes elicited by acute administration of SR141716 to delta9-tetrahydrocannabinol-tolerant rats: an experimental model of cannabinoid abstinence. *Drug Alcohol Depend* 2004;74:159–170. [PubMed: 15099659]
26. Gray KA, Day NL, Leech S, Richardson GA. Prenatal marijuana exposure: Effect on child depressive symptoms at ten years of age. *Neurotoxicol Teratol* 2005;27:439–448. [PubMed: 15869861]
27. Harkany T, Guzmán M, Galve-Roperh I, Berghuis B, Devi LA, Mackie K. The emerging functions of the endocannabinoid signaling during CNS development. *TIPS* 2007;28:83–92. [PubMed: 17222464]
28. Hill MN, Gorzalka BB. Pharmacological enhancement of cannabinoid CB₁ receptor activity elicits an antidepressant-like response in the rat forced swim test. *Neuropsychopharmacology* 2005;15:593–599.
29. Hutchings DE, Gamagaris Z, Miller N, Fico TA. The effects of prenatal exposure to delta-9-tetrahydrocannabinol on the rest-activity cycle of the preweanling rat. *Neurotoxicol Teratol* 1989;11:353–356. [PubMed: 2552274]
30. Jakubovič A, Hattori T, Geer PL. Radioactivity in suckled rats after giving 14C-tetrahydrocannabinol to the mother. *Eur J Pharmacol* 1973;22:221–223. [PubMed: 4736671]

31. Kelly SJ, Dillingham RR. Sexually dimorphic effects of perinatal alcohol exposure on social interactions and amygdala DNA and DOPAC concentrations. *Neurotoxicol Teratol* 1994;16:377–384. [PubMed: 7526147]
32. Kulkarni SK, Dhir A. Effect of various classes of antidepressants in behavioral paradigms of despair. *Prog Neuro-Psychopharmacol Biol Psychiatry* 2007;31:1248–1254.
33. Maresz K, Carrier EJ, Ponomarev ED, Hillard CJ, Dittel BN. Modulation of the cannabinoid CB₂ receptor in microglial cells in inflammatory stimuli. *J Neurochem* 2005;95:437–445. [PubMed: 16086683]
34. Mato S, Del Olmo E, Pazos A. Ontogenetic development of cannabinoid receptor expression and signal transduction functionality in the human brain. *Eur J Neurosci* 2003;17:1747–1754. [PubMed: 12752773]
35. McKim, WA. *An Introduction to Behavioral Pharmacology*. New Jersey: Pearson/Prentice Hall; 2007. Drugs and Behavior; p. 313–335.
36. Meaney MJ, Stewart J. A descriptive study of social development in the rat (*Rattus norvegicus*). *Animal Behavior* 1981;29:34–45.
37. Mereu G, Fa M, Ferraro L, Cagiano R, Atonelli T, Tattoli M, Ghiglieri V, Tanganelli S, Gessa GL, Cuomo V. Prenatal exposure to a cannabinoid agonist produces memory deficits linked to dysfunction in hippocampal long-term potentiation and glutamate release. *Proc Natl Acad Sci U S A* 2003;100:4915–4920. [PubMed: 12679519]
38. Moreno M, Trigo JM, Escuredo L, Rodríguez de Fonseca F, Navarro M. Perinatal exposure to Δ^9 -tetrahydrocannabinol increases presynaptic dopamine D2 receptor sensitivity: a behavioral study in rats. *Pharmacol Biochem Behav* 2003;75:565–575. [PubMed: 12895674]
39. Navarro M, de Miguel R, Rodríguez de Fonseca F, Ramos JA, Fernández-Ruiz JJ. Perinatal cannabinoid exposure modifies the sociosexual approach behavior and the mesolimbic dopaminergic activity in adult male rats. *Behav Brain Res* 1996;75:91–98. [PubMed: 8800663]
40. Navarro M, Rubio P, Rodríguez de Fonseca F. Behavioral consequences of maternal exposure to natural cannabinoids in rats. *Psychopharmacol* 1995;122:1–14.
41. Navarro M, Rubio P, Rodríguez de Fonseca F. Sex-dimorphic psychomotor activation after perinatal exposure to (–)-delta-9-tetrahydrocannabinol. An ontogenic study in Wistar rats. *Psychopharmacol* 1994;116:414–422.
42. Noland JS, Singer LT, Short EJ, Minnes S, Arendt RE, Kirchner HL, Bearer C. Prenatal drug exposure and selective attention in preschoolers. *Neurotoxicol Teratol* 2005;27:429–438. [PubMed: 15939203]
43. Porsolt RD, Anton G, Blavet N, Jalfre M. Behavioural despair in rats: a new model sensitive to antidepressant treatments. *Eur J Pharmacol* 1978;47:379–391. [PubMed: 204499]
44. Prut L, Belzung C. The open field as a paradigm to measure the effects of drugs on anxiety-like behaviors: a review. *Eur J Pharmacol* 2003;463:3–33. [PubMed: 12600700]
45. Richardson GA, Ryan C, Willford J, Day NL, Goldschmidt L. Prenatal alcohol and marijuana exposure: Effects on neuropsychological outcomes at 10 years. *Neurotoxicol Teratol* 2002;24:309–320. [PubMed: 12009486]
46. Rubio P, Rodríguez de Fonseca F, Munoz RM, Ariznavarreta C, Martín-Calderón JL, Navarro M. Long-term behavioral effects of perinatal exposure to delta 9-tetrahydrocannabinol in rats: possible role of pituitary-adrenal axis. *Life Sci* 1995;56:2169–2176. [PubMed: 7776846]
47. Rubio P, Rodríguez de Fonseca F, Martín-Calderón JL, Del Arco I, Bartolomé S, Villanúa MA, Navarro M. Maternal exposure to low doses of Δ^9 -tetrahydrocannabinol facilitates morphine-induced place conditioning in adult male offspring. *Pharmacol Biochem Behav* 1998;61:229–238. [PubMed: 9768557]
48. Spear LP. Adolescent brain development and animal models. *Ann NY Acad Sci* 2004;1021:23–26. [PubMed: 15251870]
49. Valverde O, Karsak M, Zimmer A. Analysis of the endocannabinoid system by using CB1 cannabinoid receptor knockout mice. *Handbook Exp Pharmacol* 2005;168:117–145.
50. Van Sickle MD, Duncan M, Kingsley PJ, Mouihate A, Urbani P, Macki K, Stella N, Markkriyannis A, Piomelli D, Davison JS, Marnett LJ, Di Marzo V, Pittman QJ, Patel KD, Sharkey KA.

Identification and functional characterization of cannabinoid CB2 receptors. *Science* 2005;310:329–332. [PubMed: 16224028]

51. Viveros MP, Marco EM, File SE. Endocannabinoid system and stress and anxiety responses. *Pharmacol Biochem Beh* 2005;81:331–342.

Figure 1. Open Field Activity: Time spent in inner section

THC-exposed rats spent significantly less time in the inner area compared to NC and IC controls. Error bars represent SEMs.

Figure 2. Active Social Interaction

THC-exposed rats exhibited significantly more investigative sniffing compared to NC and IC controls. Error bars represent SEMs.

Table 1

Mean body weights (in g) and standard error of the means (SEMs) for dams across gestational days (GD) and offspring across postnatal days (PD)

Groups	GD 5	GD 10	GD 21
Dams in NC group	258.1 ± 2.6	273.1 ± 4.1	360.5 ± 12.1
Dams in IC group	259.8 ± 7.6	275.6 ± 7.6	342.3 ± 11.8
Dams in THC group	253.5 ± 3.4	263.3 ± 4.9	333.2 ± 5.7
	PD 2	PD 10	PD 90
Pups in NC group	7.3 ± 0.2	19.8 ± 1.1	415.8 ± 15.0
Pups in IC group	7.1 ± 0.2	19.7 ± 0.8	385.8 ± 13.7
Pups in THC group	6.9 ± 0.2	19.9 ± 0.6	379.3 ± 7.6

Table 2 Activity measures (means $\pm$ SEMs) in the open field

Groups	Distance in inner section (cm)	Time in outer section (sec)	Distance in outer section (cm)	Time ratio	Distance ratio
NC	537.9 $\pm$ 78.2	259.2 $\pm$ 7.2	3030.3 $\pm$ 175.5	0.15 $\pm$ 0.02	0.15 $\pm$ 0.02
IC	519.1 $\pm$ 87.5	247.0 $\pm$ 10.4	3089.6 $\pm$ 299.3	0.18 $\pm$ 0.03	0.15 $\pm$ 0.03
THC	287.1 $\pm$ 56.6	270.3 $\pm$ 7.7	2722.7 $\pm$ 185.4	0.09 $\pm$ 0.02	0.09 $\pm$ 0.02

Table 3

Incidence of behaviors during active social interactions (mean and SEMs)

Groups	Dominant mounts	Pinning	Boxing	Avoidance
NC	0.25 ± 0.25	1.88 ± 1.04	0	0.50 ± 0.50
IC	0	0.25 ± 0.25	0.25 ± 0.25	0.62 ± 0.32
THC	0	0.38 ± 0.26	0	0