Published in final edited form as:

Neurosci Lett. 2011 January 10; 487(3): 411–414. doi:10.1016/j.neulet.2010.10.067.

The synthetic cannabinoid WIN 55212-2 differentially modulates thigmotaxis but not spatial learning in adolescent and adult animals

Shawn K Acheson, PhD^{1,3}, Nicole LT Moore, PhD², Cynthia M Kuhn, PhD², Wilkie A Wilson, PhD^{2,3}, and H Scott Swartzwelder, PhD^{1,3}

¹ Department of Psychiatry and Behavioral Sciences, Duke University Medical Center, Durham, NC

² Department of Pharmacology and Cancer Biology, Duke University Medical Center, Durham, NC

³ Neurobiology Research Laboratory, Durham Veterans Affairs Medical Center, Durham, NC

Abstract

Unlike Δ^9 -THC, the synthetic compound WIN 55212-2 (WIN) is a full agonist of endogenous cannabinoid receptors. Previous work has shown Δ^9 -THC to affect adolescent and adult animals differently on numerous behavioral measures of spatial memory, anxiety, and locomotor activity. However, far less is known about the developmental and neurobehavioral effects of WIN. To address this, we assessed the effect of WIN (1mg/kg) on spatial learning in adolescent and adult rats using the Morris water maze. While all animals demonstrated decreased swim distance across days, WIN affected adolescents and adults differently. It improved performance in adolescents and resulted in a nearly significant performance decrement in adults. However, these effects were significantly related to thigmotaxis, which declined across days in the water maze testing protocol. WIN reduced thigmotaxis on days 1 and 2 (but not days 3 – 5) only in adolescents. The effect of age, treatment, and the age $\times$ treatment interaction was eliminated after controlling for thigmotaxis. These results indicate that WIN affects thigmotaxis rather than spatial reference memory. More importantly, these findings indicate a dissociation between the developmental effects of THC and the synthetic CB1 receptor agonist, WIN 55212-2. We suggest that the role of thigmotaxis be carefully evaluated in future neurodevelopmental studies of spatial learning, especially those investigating the endocannabinoid system.

Keywords

Adolescence; Cannabinoid; WIN 55212-2; thigmotaxis

Corresponding Author: Shawn K. Acheson, Ph.D., Rm. 25 Bldg. 16, Durham VAMC (151), 508 Fulton Street Durham, NC 27705, Telephone: 919-286-0411, x6093, Fax: 919-286-0411, x7432, shawn.acheson@duke.edu.

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.

INTRODUCTION

Δ^9 -THC has been found to possess psychoactive characteristics including euphoria and altered perception [2] that make its use appealing. It also possesses medicinal properties [3] including antinociception [8,16,31], appetite stimulation ([6], and antiemesis [4]. While these characteristics have helped to promote the use of marijuana for thousands of years [1], Δ^9 -THC also possesses characteristics that pose health risks. These include immunosuppression [15], increased risk of psychosis in some individuals [17], as well as impairments in learning and memory. The latter is a cognitive domain of great importance, especially among adolescents engaged in the academic and intellectual pursuits associated with formal education.

Although Δ^9 -THC induced impairments in learning and memory are well described in human research [30], the most extensive work has involved animal models [9,12]. Δ^9 -THC and related cannabinoid agonists have been shown to impair numerous hippocampally dependent forms of learning including operant tasks [20,40], spatial working memory [24,25,27,45], and spatial reference memory [14,32,43,44]. These effects are thought to be mediated by CB1 receptors in the dorsal hippocampus [46]. However, since THC is not a specific agonist for CB1 receptors, and many cannabinoid agents under development for potential clinical use are specific for CB1 receptor activation, it is critical to determine if there are differences in the neurobehavioral effects of THC and other, more specific agents.

Exposure to THC or related CB1 agonists may have significant adverse effects on development. For example, chronic treatment with the full CB1 agonist WIN 55212-2 (WIN) during adolescence was found to produce long-term impairments in sensory motor gating, object/social recognition, performance in a progressive ratio operant task, social behavior, social play and self-grooming [36,37]. Such impairments were not observed in adults. Our laboratory has recently demonstrated numerous neurobehavioral differences in the effects of Δ^9 -THC on adolescent and adult animals. These neurodevelopmental effects include greater spatial and non-spatial learning impairment among adolescent male animals (PD30-32) than adult male animals (PD65-70) [11]. These effects are further complicated by sex with adolescent females showing impairment consistently across days while adolescent males differed from age matched controls only on days 2 and 3. Adult females were impaired by the same dose (5mg/kg) on days 1 and 2 of training and there were no differences across days for adult males (all compared to age matched vehicle controls; [10]). There is also evidence for a neurodevelopmental effect on anxiogenesis, conditioned aversion, and locomotor performance. More specifically, Δ^9 -THC has been found to be more anxiogenic and aversive in adult animals (relative to adolescents) and to reduce locomotor activity more in adults than in adolescents [38].

As Δ^9 -THC has been shown to produce impairments in hippocampally dependent spatial learning [14,32,43,44], it seems logical to attribute the age differences in THC-induced learning impairments to a similar hippocampally mediated process. However, age differences in both the neurobehavioral effects [38] and the non-spatial deficits induced by Δ^9 -THC [11] suggest an alternative interpretation. It is possible that these non-cognitive processes might mediate the effect of Δ^9 -THC we observed in the spatial learning task reported above, and it is not clear whether similar effects would be observed with specific CB1 receptor agonists.

To address this issue we evaluated the effect of WIN 55212-2 (WIN) on spatial learning in adolescent and adult animals. WIN is a synthetic compound that acts as a full agonist at the CB1 receptor. By comparison, Δ^9 -THC is only a partial agonist and therefore may be contributing to these non-cognitive processes in ways that are not yet understood. The

purpose of this study was to evaluate the effect of a full CB1 receptor agonist on spatial learning in adolescent and adult animals.

METHODS

Subjects

Male Sprague-Dawley rats (Charles River Laboratories, Raleigh, NC) were used in this study. Adolescent animals (n=11) were PD30 and adult animals (n=11) were PD65. They were given 2 days to acclimate to our AAALAC accredited vivarium before the study was initiated. Animals were maintained on a 12 hour light-dark cycle and had *ad libitum* access to food and water. All procedures were reviewed and approved by the Animal Care and Use Committees at the Durham VA Medical Center and Duke University Medical Center prior to implementation. Two WIN treated animals (1 adult and 1 adolescent) and 1 adult vehicle animals were eliminated from the analysis due to erratic behavior. This reduced within group variability but did not alter the direction of results.

Drug Treatment

The WIN55212-2 (Sigma-Aldrich) solution was prepared using a vehicle consisting of ethanol (95%), emulphor, and normal saline (1:1:18) for a final concentration of 1mg/ml. Half the animals were dosed i.p. with 1mg/kg WIN and the other half received isovolumetric vehicle 30 minutes prior to behavioral testing on each day.

Water Maze

Training The water maze tank was constructed of white fiberglass and measured 1.6m wide and 0.75m deep. The tank was filled with water (~22°C) until the white plastic platform (9" diameter) was submerged 2cm below the water line. The platform location remained constant throughout the study. The thigmotaxis zone was a concentric area consisting of the outermost 23% of the maze field. The inside perimeter of this thigmotaxis zone was 18cm from the outer perimeter. The non-thigmotaxis zone was the remainder of the maze field. These zones were used to calculate the distance animals swam in thigmotaxis and non-thigmotaxis.

Any-maze software (Stoelting, Wood Dale, IL) and a ceiling mounted digital camera were used to record and digitize the swim path of each animal on each trial. The room was equipped with numerous distal extra-maze cues to facilitate spatial learning. Each animal was given 4 trials per day for 5 consecutive days. Animals were placed into the tank facing the wall at each of the cardinal locations (N, S, E, W—randomly chosen). Each trial lasted 60s or until the animal located the platform. Animals were given 15s on the platform and then allowed to rest for 30s in a warm dry towel before the next trial. Trials were run consecutively for each animal on each day.

Statistical Analyses

Distance traveled from the starting point to trial end and average swim speed across each trial served as primary dependent measures. Distance traveled within the thigmotaxis and non-thigmotaxis zones was also recorded. Mean daily measures were subjected to repeated measures analyses of variance (RM-ANOVA) using age and dose as between subjects variables and day as a repeated measure. Reductions in distance traveled across days were used as an indication of learning. Mauchley's Test was used to evaluate the sphericity of within-subjects effects and when necessary, Greenhouse-Geisser was applied to adjust the degrees of freedom. All analyses were performed using SPSS v18 (Chicago, IL).

RESULTS

All animals learned to locate the hidden platform ($F(4,60)=62.89$, $p\leq 0.009$; Figure 1). Although learning did not differ as a function of Age or Treatment, there were differences between adolescents and adults ($F(1,15)=15.46$, $p=0.001$) and this effect differed as a function of treatment ($F(1,15)=11.2$, $p=0.004$). Subsequent analyses revealed that adolescents learned the platform location ($F(4,32)=36.18$, $p\leq 0.009$) and, vehicle treated adolescents swam farther to reach the hidden platform than WIN treated adolescents ($F(1,8)=7.99$, $p=0.02$). Adult animals also learned to locate the hidden platform effectively ($F(4,28)=27.98$, $p\leq 0.009$). However, unlike adolescents, adult animals treated with WIN swam farther to find the hidden platform ($F(1,7)=5.52$, $p=0.05$).

In reviewing group performance in the water maze, we observed that the greatest apparent differences occurred during the first 2 days (Figure 1). Qualitative observation of the swim paths suggested the presence of thigmotaxis early in the training process. We assessed thigmotaxis by measuring that portion of the swim path that occurred within the thigmotaxis zone (see above). We found that the distance swam in thigmotaxis declined across days ($F(4,60)=74.52$, $p\leq 0.009$) and that this decline occurred as a function of Age and Treatment ($F(4,60)=4.35$, $p=0.01$; Figure 2). Analyzing adolescent and adult data separately, thigmotaxis was found to decline across days in both adolescents ($F(4,32)=55.83$, $p\leq 0.009$) and adults ($F(4,28)=22.49$, $p\leq 0.009$). However, in adolescents, that decline was treatment dependent ($F(4,32)=4.3$, $p=0.007$). Further analyses revealed that WIN treatment resulted in less thigmotaxis on day 1 ($F(1,8)=6.31$, $p=0.04$) and day 2 ($F(1,8)=12.57$, $p=0.008$) among adolescents. There was no treatment effect or treatment $\times$ day interaction among adults ($p\geq 0.05$). These findings are nearly identical to those reported for total distance. In addition, these age dependent effects on thigmotaxis were still present even after controlling for overall path length using the ratio of thigmotactic distance : total distance.

To assess the effect of age and treatment on learning across days in the absence of thigmotaxis, we performed the original analysis (Day $\times$ Age $\times$ Treatment RM-ANOVA) using just the distance traveled in the non-thigmotactic zone. Results reveal a significant decline in swim distance across days ($F(4,60)=13.32$, $p\leq 0.009$), but no effect of age, treatment, or any interactions ($p\geq 0.05$). Swim speed declined across days ($F(4,60)=3.62$, $p=0.01$) and on average, adolescents swam faster than adults ($F(1,15)=5.63$, $p=0.03$). Collapsing across day and treatment, adolescents swam less than 15% faster than adults (0.21 vs. 0.18, respectively).

DISCUSSION

Our results indicate that although animals of both ages learned to find the hidden platform, adult performance was consistently better than adolescents and this age difference was dependent upon treatment condition. That is, WIN treated adolescents performed better across days than age-matched controls while WIN treated adults performed worse than age-matched controls. It is important to note the distinction we make between performance and learning. These findings indicate the presence of an age by treatment interaction collapsing across days. There was no differential improvement between treatment groups across days (no interaction with day). That is, the rate at which the animals' performance improved across days was not related to treatment group. More importantly, these effects were entirely accounted for by thigmotaxis. Thus, as overall performance improved across training days (Fig. 1), thigmotaxis diminished across days (Fig. 2). When distance swam in thigmotaxis was removed from the analysis or controlled for statistically, age and treatment effects were eliminated.

Thigmotaxis refers to an animal's propensity to move along the edge of its environment. This behavior is frequently attributed to anxiety or fear and is considered a phylogenetically adaptive response [13,19,23]. In the laboratory, thigmotaxis can be observed as wall-hugging in several behavioral tasks including the open field test, the elevated plus maze and the Morris water maze. The validity of thigmotaxis as an indicator of anxiety or fear is demonstrated via its reduction by anxiolytic agents [42]; its association with changes in corticosteroid levels or the systemic administration of corticosteroids [5,22,39]; its decline following pre-training and environmental enrichment [21]; and its correlation with elevated plus maze performance [22].

In light of the current understanding of thigmotaxic behavior, we conclude that the animals in the present study habituated to the stress of the novel task over time (significant reduction in thigmotaxis across days); that adolescent animals were initially more stressed than adults (adolescents are more thigmotaxic on days 1 and 2 than adults); and that WIN (1mg/kg) moderated that stress response in adolescents but not adults (WIN reduced thigmotaxis in adolescents but not adults on days 1 and 2). This is not to suggest that spatial learning did not occur in this study. The distance traveled in the non-thigmotaxic zone declined significantly over days but was not affected by age, treatment, or the interaction of age $\times$ treatment.

The difference in performance between adolescent and adult controls may seem inconsistent with previous work from our laboratory, but similar differences between adolescent and adult controls have been observed in previous experiments from our lab [10,11]. However, that distinction was not observed consistently across experiments and when it was, it never exceeded 1 – 2 meters in swim distance on Day 1. In the present study, there was a 4 – 5 meter difference in swim distance (in favor of adults) on Day 1. One notable difference between our earlier work with THC and the present study was the period of acclimation prior to the start of the experiment. In our earlier THC studies ([10,11]), animals were allowed to adjust to their new surroundings for 4 – 5 days after arrival in our vivarium. In the present study, animals were allowed only 2 days of acclimation. Thus, the adult-adolescent difference in initial performance that we observed could be related to the stress associated with adjusting to the novel environment. Regardless of why these adolescents were more thigmotaxic, it is important to note that WIN reversed this effect.

While it is not clear why our adolescent controls were more thigmotaxic than the adult controls, we contend that the effect of WIN was to modulate this stress response differentially in adolescents and adults. We have previously shown that THC was more anxiogenic in adults than adolescents [38], which may seem inconsistent with our explanation of the current findings. However, this earlier work did not assess the effect of THC on stress induction by external forces. Those experiments evaluated whether THC was more or less anxiogenic compared to vehicle control, and whether that effect varied as a function of age. Moreover, there is increasing evidence that the effect of cannabinoid agonists is dose dependent with low doses being anxiolytic and high doses being potentially anxiogenic [26,28,33,34]. This dose-response effect may further distinguish the current findings from previous work published from our lab.

The relationship between the endocannabinoid system and stress or anxiety is far from clear. THC has clear anxiogenic properties in humans [41] and there is evidence that THC and other CB1 receptor agonists are anxiogenic in animals [35]. However, there is also evidence that WIN may be anxiolytic or reduce the effect of stress. For example, WIN has been shown to dose-dependently increase the amount of time spent in the open arms of the elevated plus maze and was found to act synergistically with diazepam [26]. Low doses of WIN and Rimonabant (a CB1 receptor antagonist) have also been shown to reverse the

anxiogenic effects of acute doses of amphetamine and nicotine and alternatively, reduce the anxiolytic effect of subchronic doses of amphetamine and nicotine [7]. In this context it is noteworthy that WIN did not powerfully disrupt learning in this study. This result was unexpected, given the effects of THC on learning, and underscores the potentially important differences in efficacy between full and partial CB1 receptor agonists.

As others have hypothesized, the key to understanding the relationship between stress/anxiety and the endocannabinoid system may not lie in the anxiolytic or anxiogenic properties of various CB1 receptor ligands, but in the ability of the endocannabinoid system to modulate reactivity of affective or HPA processes [18]. Further evidence comes from findings that CB1 receptor agonists such WIN and CP55940 reverse the corticosteroid elevations associated with acute stress [18,29]. If THC operates in a similar fashion, it may work to blunt affective responsivity more potently in adolescents than adults. Although additional work will be required to confirm these hypotheses and delineate the underlying mechanisms, these findings may help to explain why marijuana use is more prevalent among adolescents than adults. It should also be recognized that stress related thigmotaxis could produce a substantial effect on water maze performance. We suggest that the role of thigmotaxis be carefully evaluated in future neurodevelopmental studies of spatial learning, especially those investigating the endocannabinoid system.

Highlights

- WIN 55212-2 (1mg/kg) was found to affect adolescents and adults differently.
- Thigmotaxis was reduced on days 1 and 2 in adolescent but not adult animals.
- WIN 55212-2 affects thigmotaxis rather than spatial reference memory.
- The neurodevelopmental effect of WIN differs from those previously observed for Δ^9 -THC.

Acknowledgments

This work was supported by the National Institute of Drug Abuse (DA019346 to HSS), and VA research career scientist and Merit Review awards to HSS and WAW.

References

1. Abel, EL. A Comprehensive Guide to the Cannabis Literature. Greenwood Press; Westport, CN: 1979.
2. Adams IB, Martin BR. Cannabis: pharmacology and toxicology in animals and humans. *Addiction* 1996;91:1585–1614. [PubMed: 8972919]
3. Aggarwal SK, Carter GT, Sullivan MD, ZumBrunnen C, Morrill R, Mayer JD. Medicinal use of cannabis in the United States: historical perspectives, current trends, and future directions. *J Opioid Manag* 2009;5:153–168. [PubMed: 19662925]
4. Bagshaw SM, Hagen NA. Medical efficacy of cannabinoids and marijuana: a comprehensive review of the literature. *J Palliat Care* 2002;18:111–122. [PubMed: 12164099]
5. Beiko J, Lander R, Hampson E, Boon F, Cain DP. Contribution of sex differences in the acute stress response to sex differences in water maze performance in the rat. *Behav Brain Res* 2004;151:239–253. [PubMed: 15084440]
6. Berry EM, Mechoulam R. Tetrahydrocannabinol and endocannabinoids in feeding and appetite. *Pharmacol Ther* 2002;95:185–190. [PubMed: 12182965]
7. Biala G, Kruk M, Budzynska B. Effects of the cannabinoid receptor ligands on anxiety-related effects of d-amphetamine and nicotine in the mouse elevated plus maze test. *J Physiol Pharmacol* 2009;60:113–122. [PubMed: 19617654]

8. Burns TL, Ineck JR. Cannabinoid analgesia as a potential new therapeutic option in the treatment of chronic pain. *Ann Pharmacother* 2006;40:251–260. [PubMed: 16449552]
9. Castellano C, Rossi-Arnaud C, Cestari V, Costanzi M. Cannabinoids and memory: animal studies. *Curr Drug Targets CNS Neurol Disord* 2003;2:389–402. [PubMed: 14683467]
10. Cha YM, Jones KH, Kuhn CM, Wilson WA, Swartzwelder HS. Sex differences in the effects of delta9-tetrahydrocannabinol on spatial learning in adolescent and adult rats. *Behav Pharmacol* 2007;18:563–569. [PubMed: 17762524]
11. Cha YM, White AM, Kuhn CM, Wilson WA, Swartzwelder HS. Differential effects of delta9-THC on learning in adolescent and adult rats. *Pharmacology, biochemistry, and behavior* 2006;83:448–455.
12. Chaperon F, Thiebot MH. Behavioral effects of cannabinoid agents in animals. *Crit Rev Neurobiol* 1999;13:243–281. [PubMed: 10803637]
13. Choleris E, Thomas AW, Kavaliers M, Prato FS. A detailed ethological analysis of the mouse open field test: effects of diazepam, chlórdiazepoxide and an extremely low frequency pulsed magnetic field. *Neurosci Biobehav Rev* 2001;25:235–260. [PubMed: 11378179]
14. Da S, Takahashi RN. SR 141716A prevents delta 9-tetrahydrocannabinol-induced spatial learning deficit in a Morris-type water maze in mice. *Prog Neuropsychopharmacol Biol Psychiatry* 2002;26:321–325. [PubMed: 11817511]
15. Eisenstein TK, Meissler JJ, Wilson Q, Gaughan JP, Adler MW. Anandamide and Delta9-tetrahydrocannabinol directly inhibit cells of the immune system via CB2 receptors. *J Neuroimmunol* 2007;189:17–22. [PubMed: 17640739]
16. Farquhar-Smith WP, Rice AS. A novel neuroimmune mechanism in cannabinoid-mediated attenuation of nerve growth factor-induced hyperalgesia. *Anesthesiology* 2003;99:1391–1401. [PubMed: 14639155]
17. Fernandez-Espejo E, Viveros MP, Nunez L, Ellenbroek BA, Rodriguez de Fonseca F. Role of cannabis and endocannabinoids in the genesis of schizophrenia. *Psychopharmacology* 2009;206:531–549. [PubMed: 19629449]
18. Ganon-Elazar E, Akirav I. Cannabinoid Receptor Activation in the Basolateral Amygdala Blocks the Effects of Stress on the Conditioning and Extinction of Inhibitory Avoidance. *J Neurosci* 2009;29:11078–11088. [PubMed: 19741114]
19. Grossen NE, Kelley MJ. Species-specific behavior and acquisition of avoidance behavior in rats. *J Comp Physiol Psychol* 1972;81:307–310. [PubMed: 5084446]
20. Hampson RE, Deadwyler SA. Cannabinoids reveal the necessity of hippocampal neural encoding for short-term memory in rats. *J Neurosci* 2000;20:8932–8942. [PubMed: 11102504]
21. Harris AP, D'Eath RB, Healy SD. Environmental enrichment enhances spatial cognition in rats by reducing thigmotaxis (wall hugging) during testing. *Anim Behav* 2009;77:1459–1464.
22. Herrero AI, Sandi C, Venero C. Individual differences in anxiety trait are related to spatial learning abilities and hippocampal expression of mineralocorticoid receptors. *Neurobiol Learn Mem* 2006;86:150–159. [PubMed: 16580234]
23. Kavaliers M, Choleris E. Antipredator responses and defensive behavior: ecological and ethological approaches for the neurosciences. *Neurosci Biobehav Rev* 2001;25:577–586. [PubMed: 11801283]
24. Lichtman AH, Martin BR. Delta 9-tetrahydrocannabinol impairs spatial memory through a cannabinoid receptor mechanism. *Psychopharmacology* 1996;126:125–131. [PubMed: 8856831]
25. Mishima K, Egashira N, Hirose N, Fujii M, Matsumoto Y, Iwasaki K, Fujiwara M. Characteristics of learning and memory impairment induced by delta9-tetrahydrocannabinol in rats. *Jpn J Pharmacol* 2001;87:297–308. [PubMed: 11829149]
26. Naderi N, Haghpars A, Saber-Tehrani A, Rezaii N, Alizadeh AM, Khani A, Motamedi F. Interaction between cannabinoid compounds and diazepam on anxiety-like behaviour of mice. *Pharmacology Biochemistry and Behavior* 2008;89:64–75.
27. Nakamura EM, da Silva EA, Concilio GV, Wilkinson DA, Masur J. Reversible effects of acute and long-term administration of delta-9-tetrahydrocannabinol (THC) on memory in the rat. *Drug Alcohol Depend* 1991;28:167–175. [PubMed: 1657563]

28. Patel S, Hillard CJ. Pharmacological evaluation of cannabinoid receptor ligands in a mouse model of anxiety: further evidence for an anxiolytic role for endogenous cannabinoid signaling. *J Pharmacol Exp Ther* 2006;318:304–311. [PubMed: 16569753]
29. Patel S, Roelke CT, Rademacher DJ, Cullinan WE, Hillard CJ. Endocannabinoid Signaling Negatively Modulates Stress-Induced Activation of the Hypothalamic-Pituitary-Adrenal Axis. *Endocrinology* 2004;145:5431–5438. [PubMed: 15331569]
30. Ranganathan M, D'Souza D. The acute effects of cannabinoids on memory in humans: a review. *Psychopharmacology (Berl)* 2006;188:425–444. [PubMed: 17019571]
31. Rice AS, Farquhar-Smith WP, Nagy I. Endocannabinoids and pain: spinal and peripheral analgesia in inflammation and neuropathy. *Prostaglandins Leukot Essent Fatty Acids* 2002;66:243–256. [PubMed: 12052040]
32. Robinson L, Goonawardena AV, Pertwee RG, Hampson RE, Riedel G. The synthetic cannabinoid HU210 induces spatial memory deficits and suppresses hippocampal firing rate in rats. *Br J Pharmacol* 2007;151:688–700. [PubMed: 17502849]
33. Rubino T, Sala M, Vigano D, Braida D, Castiglioni C, Limonta V, Guidali C, Realini N, Parolaro D. Cellular Mechanisms Underlying the Anxiolytic Effect of Low Doses of Peripheral [Delta]9-Tetrahydrocannabinol in Rats. *Neuropsychopharmacology* 2007;32:2036–2045. [PubMed: 17287821]
34. Rubino T, Vigano D, Realini N, Guidali C, Braida D, Capurro V, Castiglioni C, Cherubino F, Romualdi P, Candeletti S, Sala M, Parolaro D. Chronic Delta (9)-Tetrahydrocannabinol During Adolescence Provokes Sex-Dependent Changes in the Emotional Profile in Adult Rats: Behavioral and Biochemical Correlates. *Neuropsychopharmacology*. 2008
35. Scherma M, Medalie J, Fratta W, Vadivel SK, Makriyannis A, Piomelli D, Mikics E, Haller J, Yasar S, Tanda G, Goldberg SR. The endogenous cannabinoid anandamide has effects on motivation and anxiety that are revealed by fatty acid amide hydrolase (FAAH) inhibition. *Neuropharmacology* 2008;54:129–140. [PubMed: 17904589]
36. Schneider M, Koch M. Chronic pubertal, but not adult chronic cannabinoid treatment impairs sensorimotor gating, recognition memory, and the performance in a progressive ratio task in adult rats. *Neuropsychopharmacology* 2003;28:1760–1769. [PubMed: 12888772]
37. Schneider M, Schomig E, Leweke FM. Acute and chronic cannabinoid treatment differentially affects recognition memory and social behavior in pubertal and adult rats. *Addiction Biology* 2008;13:345–357. [PubMed: 18782382]
38. Schramm-Sapota NL, Cha YM, Chaudhry S, Wilson WA, Swartzwelder HS, Kuhn CM. Differential anxiogenic, aversive, and locomotor effects of THC in adolescent and adult rats. *Psychopharmacology* 2007;191:867–877. [PubMed: 17211649]
39. Snihur AWK, Hampson E, Cain DP. Estradiol and corticosterone independently impair spatial navigation in the Morris water maze in adult female rats. *Behav Brain Res* 2008;187:56–66. [PubMed: 17913254]
40. Suenaga T, Kaku M, Ichitani Y. Effects of intrahippocampal cannabinoid receptor agonist and antagonist on radial maze and T-maze delayed alternation performance in rats. *Pharmacology, biochemistry, and behavior* 2008;91:91–96.
41. Thomas H. A community survey of adverse effects of cannabis use. *Drug and Alcohol Dependence* 1996;42:201–207. [PubMed: 8912803]
42. Treit D, Fundytus M. Thigmotaxis as a test for anxiolytic activity in rats. *Pharmacology, biochemistry, and behavior* 1989;31:959–962.
43. Varvel SA, Anum E, Niyuhire F, Wise LE, Lichtman AH. Delta(9)-THC-induced cognitive deficits in mice are reversed by the GABA(A) antagonist bicuculline. *Psychopharmacology* 2005;178:317–327. [PubMed: 15322725]
44. Varvel SA, Hamm RJ, Martin BR, Lichtman AH. Differential effects of delta 9-THC on spatial reference and working memory in mice. *Psychopharmacology* 2001;157:142–150. [PubMed: 11594438]
45. Wegener N, Kuhnert S, Thuns A, Roese R, Koch M. Effects of acute systemic and intra-cerebral stimulation of cannabinoid receptors on sensorimotor gating, locomotion and spatial memory in rats. *Psychopharmacology* 2008;198:375–385. [PubMed: 18446326]

46. Wise LE, Thorpe AJ, Lichtman AH. Hippocampal CB(1) receptors mediate the memory impairing effects of Delta(9)-tetrahydrocannabinol. *Neuropsychopharmacology* 2009;34:2072–2080. [PubMed: 19322169]

Figure 1.

Mean daily path length between the starting location and the hidden platform. WIN treated animals are identified by open markers and adolescents are identified by square markers. All animals learned across days. Collapsing across days, adolescents swam farther to reach the platform than adults. Adolescent controls swam the farthest. Data are presented in meters $\pm$ SEM. $\square$ indicates significantly different from adolescent WIN.

Figure 2.

Mean daily path length within the outer most region (thigmotaxis). Group markers are identical to those in Figure 1. Thigmotactic distance declined across days for all animals but did so in an age by treatment dependent fashion. Adolescent controls swam farther in thigmotaxis on Day 1 & 2 than any other group. Data are presented in meters $\pm$ SEM. □ indicates significantly different from adolescent WIN.