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Cannabinoids and novelty investigation: influence of age and duration of exposure

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Abstract

Administration of the synthetic cannabinoid receptor agonist WIN 55,212-2 has been shown to increase indices of noradrenergic activity. Neuroanatomical, neurochemical and behavioral studies have provided evidence supporting a marked impact of cannabinoids on the rat coeruleo-cortical pathway. As activity of this pathway is implicated in setting specific attentional modes, the present study assessed the influence of acute and repeated systemic administration of WIN 55,212-2 on novelty investigation in adolescent and adult male rats by using the hole board behavioral paradigm. Animals were individually acclimated to the hole board for 10-minute sessions over three days, and novel objects were introduced on the fourth day. Novelty-seeking behavior was measured by comparison of the average number of return visits to a hole containing a novel object versus the average number of return visits to an empty hole. While attenuation of novelty preference was observed in adult rats acutely treated with WIN 55,212-2, both acutely-treated adolescent groups retained their preference for novelty. All groups treated with repeated administration of either drug or vehicle demonstrated novelty preference, and no differences were found in the measure of novelty investigation between the groups. Furthermore, this study reproduced findings showing significant differences in locomotor activity that did not coincide with differences in novelty-seeking behavior. These data thus suggest a complex effect of CB1 receptor modulation on novelty preference in the male rat that is modulated by age and treatment.

Keywords

cannabinoids; attention; novelty investigation; adolescence; WIN 55, 212-2; hole board; locus coeruleus; norepinephrine

Introduction

In human and animal behavioral studies, acute cannabinoid administration results in impaired attention, vigilance and cognitive processing [1, 2], and long-term cannabis use

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results in impairment of attention that worsens with increasing years of regular use [3]. Studies examining the effects of cannabinoids on attention [4, 5] have shown that chronic cannabis use affects information processing [6]. Specifically, cannabis users are unable to filter out extraneous information and there is an inability to effectively focus attention and reject irrelevant information [7]. These data suggest a failure to habituate to irrelevant stimuli and may represent a faulty gating mechanism or inefficient information processing strategies [7]. Furthermore, there is a growing body of evidence that suggests various cannabinoid effects in adolescents may lead to long-term susceptibility for cognitive impairments and drug abuse [8].

In rodents, administration of cannabinoid agonists has been shown to induce alterations in various measures of behavior [9–15]. In the open-field test, acute administration of Delta(9)-tetrahydrocannabinol (Δ^9-THC), WIN 55,212-2, or AM-411 in adult rats has been shown to reduce both horizontal and vertical locomotor activity (ambulation and rearing) and administration of Δ^9-THC or AM-411 has been shown to increase circling activity [12–14]. In adolescent rats, acute administration of CP 55,940 decreases ambulation, rearing, and grooming activity in a dose-dependent manner [15], and acute administration of WIN 55,212-2 results in reduced locomotor activity as measured by a decrease in the number of closed-arm entries in the elevated-plus maze (EPM) [10]. Repeated administration of WIN 55,212-2 in adult rats has been found to induce increases in anxiety-like responses in the EPM, although it has not been found to significantly reduce locomotor activity [9].

Administration of cannabinoid agonists has also been shown to increase indices of noradrenergic activity [9, 16]. Intravenous injection of WIN 55,212-2, CP 55,940, or Δ^9-THC in anaesthetized adult rats increases the spontaneous firing rate of locus coeruleus (LC) neurons in a dose-dependent manner [16, 17]. Acute intraperitoneal administration of WIN 55,212-2 stimulates c-Fos expression in noradrenergic neurons of the LC and modulates norepinephrine efflux in the frontal cortex, suggesting a modulatory effect on the coeruleo-cortical pathway [18]. The anxiety-like behavior observed with repeated WIN 55,212-2 administration further correlates with increases in tyrosine hydroxylase (TH) expression in the LC [18] as well as augmented norepinephrine efflux in frontal cortex [9].

One behavioral trait that is affected by modulation of coeruleo-cortical pathway activity is preference for novelty [12]. With convergent evidence that this pathway is altered by CB1 agonist administration, the goal of the present study was to examine effects of the synthetic cannabinoid receptor agonist, WIN 55,212-2, on this behavioral endpoint. We also chose to examine these effects in both adult and adolescent rats as previous evidence has indicated age-dependent behavioral responses to CB1 agonist administration [11]. Finally, we devised a measure of novelty-seeking behavior that was independent of other behavioral measures. This measure was based on a behavioral paradigm that has been shown to reflect alterations in LC activity [19, 20] by probing a rodent's preference for novel objects in a hole board apparatus [21], and allowing for discrimination between general behavioral activation and attention towards novel stimuli [20]. In this manner, the hole board paradigm has been used to distinguish the attentional states of the rat as a behavioral endpoint [19, 20, 22], and performance has been correlated with environmentally and pharmacologically induced fluctuations of LC activity [19, 20, 22, 23]. Thus, we examined the impact of acute and

repeated WIN 55,212-2 administration on the behavioral endpoint of novelty-investigation in adult and adolescent male rats and constructed a measure of novelty investigation that we have shown to be independent of locomotor and exploratory measures.

Materials and Methods

Animals

Forty-six adolescent and thirty-three adult male Sprague-Dawley rats (Harlan Laboratories, Indianapolis, IN) were housed on a 12-hour light-dark cycle with free access to food and water. Adolescent rats were 30 days old upon arrival to the facility and were housed in cohorts of four. Adults weighed 200–224g upon arrival to and were housed in cohorts of three. Cohorts were assigned to either acute or repeated administration groups. All procedures conformed with The Institutional Animal Care and Use Committee at Thomas Jefferson University according to the revised Guide for the Care and Use of Laboratory Animals (1996), The Health Research Extension Act (1985), and the PHS Policy on Humane Care and Use of Laboratory Animals (1986).

Pharmacological Treatment

Previous studies from our laboratory have shown that a systemic dose of 3.0mg/kg WIN 55,212-2 significantly impacts activity of the coeruleo-cortical pathway [9, 18, 24]. To elaborate on these findings, this dose was chosen for the present study.

Acute administration—Twelve adolescent and six adult rats received a single injection of 3.0mg/kg WIN 55,212-2 (Sigma, St. Louis, MO) dissolved in 10% dimethyl sulfoxide (DMSO) and 0.9% saline to a concentration of 3.0mg/ml (administered in a volume of 1.0ml/kg). Twelve adolescent and five adult animals received a single injection of the vehicle (10% DMSO and 0.9% saline) in a volume of 1.0ml/kg. All injections were administered intraperitoneally 30 minutes prior to the behavioral test of novelty investigation.

Repeated administration—Twelve adolescent and twelve adult rats received an injection of 3.0mg/kg WIN 55,212-2 (Sigma, St. Louis, MO) dissolved in 10% DMSO and 0.9% saline to a concentration of 3.0mg/ml (administered in a volume of 1.0ml/kg) once a day for seven days. Ten adolescent and ten adult animals received an injection of the vehicle (10% DMSO and 0.9% saline) in a volume of 1.0ml/kg once a day for seven days. All injections were administered intraperitoneally, with the first injection being administered on the third day of handling, and the last injection being administered on the day of the behavioral test of novelty investigation.

Hole board Apparatus

The hole board consisted of a clear plexiglass box measuring 43.2cm × 43.2cm × 30.5cm with a raised aluminum platform containing 16 holes 3.175cm in diameter, evenly spaced 4.445cm apart (model ENV-515, MED Associates, St. Albans, VT). Sixteen infrared beams located above the hole board measured ambulation along the X-Y axis. Eight infrared beams positioned below the hole board detected the location and number of head dips at each hole.

The entire apparatus was assembled according to the Activity Monitor version 5 manual (MED Associates, St. Albans, VT). The outer sides of the box were then covered with opaque mat board to prevent the interference of external cues. All data was transmitted to a PC via a USB interface, model DIG-729, and processed using Activity Monitor 5 software, Sof-811, version 5.9.725 (MED Associates, St. Albans, VT).

Behavioral Procedure

One day after arrival to the facility, animals were individually handled for five minutes a day for seven days. On the eighth day, each animal was allowed to acclimate to the hole board by free exploration for 10 minutes. The hole board was cleaned using 50% ethanol and allowed to dry prior to each animal's entry. This acclimation session was repeated for each animal once a day for two additional days, resulting in a total of three daily 10 minute acclimation sessions.

On the final (eleventh) day, each animal was allowed to freely explore the hole board just as in the acclimation sessions. However, prior to beginning the test, a novel object was inserted into each of four holes. The location of these four objects in the hole board was consistent for each animal (Figure 1) and included three small toys and a retractable identification clip.

Data Collection

Data was collected on a PC running Sof-811 Open Field Activity version 5.9.725 (MED Associates, St. Albans, VT). Data for each subject included the distance traveled, the average velocity, and the number of head dips at each hole, and a report was automatically generated categorizing the number of initial head dips at holes containing novel objects, the number of initial head dips at empty holes, the number of subsequent head dips at holes containing novel objects, and the number of subsequent head dips at empty holes.

Data Analysis

Data was cataloged in Microsoft Access, transferred to Microsoft Excel, and analyzed in SPSS version 14. To devise a measure of preference for hole condition, an average number of return visits to filled holes and an average number of return visits to empty holes was calculated for each subject and then compared within each group by a paired t-test. A significant difference represented a hole type preference in the animal, and the higher value indicated the type (filled or empty). 2×2 ANOVAs were then performed to detect differences in overall measures of hole investigation, exploration, and ambulation across groups, and t-tests were used for post-hoc analyses.

Results

Measure of Novelty Investigation

To construct a measure of novelty preference that assesses novelty investigation behavior independent of exploratory behavior, an average number of return visits to either type of hole (object-containing or empty) was calculated for each subject. An individual animal may or may not visit a given hole based on its level of exploratory behavior, that is, the animal may visit all sixteen holes within the duration of the ten minute session, or it may visit none

of them. The initial visit to a given hole is thus exploratory in nature. Secondarily, a visited hole may or may not contain an object due to chance. For instance, a subject may visit three object-containing holes and four empty holes during the session, or it may visit a number of combinations as a function of its exploratory nature. Thus, investigation of a given hole occurs upon a return visit to that hole, and this measure accounts for investigation by describing the animal's tendency to return to either type of hole.

Sof-811 Open Field Activity version 5.9.725 classified the head dips for each subject according to whether they were initial visits or return visits as well as object-containing or empty, specified by the program as novel task entries, novel non-task entries, repeat task entries, and repeat non-task entries. This classification was used to calculate the average number of return visits to both types of holes, which was achieved by dividing the repeat task entries by the novel task entries, and the repeat non-task entries by the novel non-task entries. For instance, if a subject's data included 3 novel task entries, 4 novel non-task entries, 6 repeat task entries, and 2 repeat non-task entries, the subject's average number of return visits would be 2 for task (object-containing) and 0.5 for non-task (empty). A significantly greater average number of returns to a task hole than to a non-task hole thus represented the subject's preference for novelty.

Statistical Analysis

To establish the presence of this novelty preference within each group, a paired t-test was performed comparing the average number of return visits to holes containing an object with the average number of return visits to empty holes. A significant difference indicated a tendency to return to one hole type over another, and thus a significant p value showing a higher average number of returns to object -containing (filled) holes indicated a preference for novelty.

Figure 2 depicts the average visits to filled holes and empty holes for each acutely-treated group. Both adolescent cohorts (vehicle- and drug-treated) showed a preference for novelty [vehicle: $t(11)=2.926$, $p=0.014$; 3.0mg/kg WIN 55,212-2: $t(11)=3.471$, $p=0.005$]. Adult rats acutely treated with vehicle also showed a preference for novelty [$t(4)=2.523$, $p=0.065$], however this behavior was attenuated in adult rats acutely treated with 3.0mg/kg WIN 55,212-2 [$t(5)=0.956$, $p=0.383$]. Alternatively, all groups receiving repeated administration of either the vehicle or 3.0mg/kg WIN 55,212-2 showed a preference for novelty (adolescent rats repeatedly treated with vehicle, $t(9)=3.083$, $p=0.013$; adolescent rats repeatedly treated with 3.0mg/kg WIN 55,212-2, $t(11)=2.634$, $p=0.023$; adult rats repeatedly treated with vehicle, $t(9)=3.984$, $p=0.003$; adult rats repeatedly treated with 3.0mg/kg WIN 55,212-2, $t(11)=4.541$, $p=0.001$) as depicted in Figure 3.

Subsequently, 2×2 ANOVAs were performed within the 1-Day and 7-Day groups to assess any overall differences in the average numbers of hole visits during the 10 minute test period. Within the acutely and repeatedly treated groups, age and treatment condition were crossed for either the average number of visits to filled holes or for the average number of visits to empty holes. In groups treated for 1 day, no main effects or interactions were found. That is, age did not impact the average number of return visits to filled holes [$F(1,31)=2.224$, $p>0.05$] or to empty holes [$F(1,31)=0.374$, $p>0.05$], nor did treatment

condition [filled: F(1,31)=0.151, p>0.05; empty: F(1,31)=2.323, p>0.05], and no significant interactions were found [filled holes: F(1,31)=0.344, p>0.05; empty holes: F(1,31)=0.424, p>0.05]. In groups treated for 7 days, neither a main effect nor an interaction was found for the average return visits to filled holes [age: F(1,40)=2.904, p>0.05; treatment: F(1,40)=0.021, p>0.05, age x treatment: F(1,40)=1.612, p>0.05]. For the average number of visits to empty holes, no significant effect was found for treatment condition [F(1,40)=0.011, p>0.05] and no interaction was found [F(1,40)=0.683, p>0.05]. Although a significant effect of age was found [F(1,40)=7.874, p=0.008] interpretation of this finding is limited.

Exploratory Behavior and Locomotor Activity

2×2 ANOVAs were also performed to assess differences in exploratory and locomotor activity measures, crossing age and treatment condition for total number of visits to all holes or total ambulatory distance covered within the test period. Independent t-tests were performed for post-hoc analyses of ANOVA results.

In groups treated for 1 day, neither the age [F(1,31)=0.014, p>0.05] nor treatment [F(1,31)=2.842, p>0.05] impacted the total number of visits to holes, and no interactions were found [F(1,31)=0.134, p>0.05]. Total ambulatory distance was impacted by age [F(1,31)=21.433, p=0.000] and treatment condition [F(1,31)=34.520, p=0.000], and a significant interaction was found [F(1,31)=11.265, p=0.002, Figure 5]. Subsequent t-tests indicated that the adults covered more distance than the adolescents [t(33)=−2.962, p=0.006, equal variances assumed], and vehicle-treated groups covered more distance than drug-treated groups [t(33)=3.504, p=0.001, equal variances assumed].

In groups treated for 7 days, a significant impact of age was found on the total number of hole visits [F(1,40)=6.282, p=0.016, Figure 6], such that adolescents visited more holes overall than adults did [t(42)=2.422, p=0.020, equal variances assumed]. However no effect was found for treatment condition [F(1,40)=0.021, p>0.05], and no interaction was found [F(1,40)=1.458, p>0.05]. There was also no significant impact of age or treatment on the total ambulatory distance covered [age: F(1,40)=0.002, p>0.05; treatment: F(1,40)=0.006, p>0.05; age x treatment: F(1,40)=0.904, p=0.348].

Discussion

The present findings indicate that the CB1 receptor agonist, WIN 55,212-2 (3.0mg/kg) attenuates novelty investigation in the adult male rat when administered acutely, but not after seven days of administration, and that adolescent male rats showed similar novelty-investigation behavior in both control and WIN 55,212-2-treated groups, which did not differ whether the administration was acute or repeated. Furthermore, the novelty-investigation behavior within each group did not correspond with differences in ambulatory or exploratory activity. These data suggest complex behavioral effects of CB1 receptor modulation in the male rat that are modulated by age and duration of treatment.

Multiple behavioral studies have shown dose-dependent effects of cannabinoid agonists in rats [12–15]. Although acute low doses of WIN 55,212-2 have not been shown to alter

motor activity [25], administration of higher doses of WIN 55,212-2 or Δ^9-THC have been shown to reduce both ambulatory (horizontal) and rearing (vertical) activity in adult rats [12, 13]. In adolescent rats, acute administration of CP 55,940 (0.1, 0.2, 0.4, or 0.6 mg/kg) decreased ambulatory and rearing activity in a dose-dependent manner [15], and acute administration of WIN 55,212-2 (2.5mg/kg) was found to decrease the number of closed arm entries in the elevated plus maze [10]. Our results supported these findings from previous studies, showing a significant reduction in ambulatory distance after acute administration of 3.0mg/kg WIN 55,212-2 in both adult and adolescent rats.

A decrease in ambulatory activity might indicate a possible cause for a reduction in the measure of novelty-seeking behavior. However, despite the significant decrease in ambulatory distance observed in both groups treated acutely with WIN 55,212-2, the adolescent group did not lose preference for novelty. Furthermore, it was also shown that the adult animals covered more distance than the adolescents for both drug- and vehicle-treated groups, indicating and even greater likelihood that novelty-seeking behavior and motor activity are differentially impacted by administration of WIN 55,212-2. Additionally, no significant changes in the total number of hole visits were observed, again indicating a distinction between the measures of exploration, motor activity, and novelty-preference.

Indications for heterogeneous impacts of cannabinoid agonist administration have been observed in previous behavioral experiments. In a study by Arevalo et al., dose-dependent effects of CP 55,940 were shown to independently modulate motor, exploratory, and anxiety-like behavior [26]. By utilizing a range of doses, they observed a reduction in exploratory behavior at a dose where changes in motor activity were not yet observed. Arevalo et al. thus suggested that separate circuits are involved in regulation of these behaviors. A similar trend was observed by Romero et al. in a study examining the effects of CP 55,940 on nociception and motor activity in adolescent and adult rats. Their results suggested that these systems were not only separate but that they functionally mature at different rates, such that the responses for nociception are observed at an earlier age than for motor activity [15]. Thus, the results of our present study extend the notion that separate systems are regulating behaviors observed in the hole board and that these systems may follow differential developmental trajectories.

One system that has been implicated in novelty-seeking behavior is the noradrenergic coeruleo-cortical pathway. Specifically, novelty-preference in the hole board has been correlated with environmentally and pharmacologically induced fluctuations of LC activity [23, 27]. Such novelty investigation requires the intact function of the LC-NE system, and an increase in phasic LC activity is required to promote continued investigation of the novel object [19, 23]. The present findings are consistent with other studies showing that vehicle-treated rats exhibit novelty preference [20, 21, 27] and provide further evidence for the efficacy of this paradigm. The attenuation of novelty investigation seen with acute treatment of WIN 55,212-2, in the present study, thus may suggest a shift in coeruleo-cortical activity, and the absence of an observed effect after seven days of administration may suggest a recovery of function.

Evidence for the modulation of coeruleo-cortical activity by CB1 receptor agonists is well-established. Acute administration of WIN 55,212-2 modulates norepinephrine efflux in the frontal cortex and stimulates c-Fos expression in noradrenergic neurons of the LC [18], and acute administration of WIN 55,212-2 (2–4 mg/kg) as well as Δ^9-THC (5–20mg/kg) increased synthesis of norepinephrine in brainstem slices containing LC by upwards of 173% [28]. Repeated cannabinoid administration of WIN 55,212-2 has been shown to induce anxiety-like behavior in the EPM [9] that correlates with increases in tyrosine hydroxylase (TH) expression in the LC [18] and augmented norepinephrine efflux in frontal cortex [9]. Sites of action for CB1 receptor modulation include noradrenergic axon terminals in the frontal cortex [24] as well as noradrenergic cell bodies in the LC [29, 30]. It is thus tempting to speculate that modulation of CB1 receptors localized to presynaptic axon terminals in the cortex or axon terminals in the LC may underlie the alterations observed in novelty-seeking behavior in the present study.

However, the coeruleo-cortical pathway may represent one system impacting modulation of novelty-seeking behavior. The utilization of intraperitoneal drug administration limits interpretation of the effects observed on a specific system, as multiple pathways are impacted by CB1 receptor activation. Thus, local administration of the cannabinoid agonist, for example via intracerbroventricular injection, is required to yield more definitive insight as to the particular involvement of the coeruleo-cortical pathway. Local administration in adolescent cohorts may also be useful to elucidate maturational differences in the activity of this pathway.

Further investigation is also necessary to delineate the absence of effects observed in cohorts receiving repeated drug treatment requires. Because these cohorts did not demonstrate such alterations in novelty preference or exploratory measures, this suggests that underlying alterations of coeruleo-cortical activity are occurring subsequently to repeated cannabinoid administration. One factor that our model did not account for was the duration of the hole visits. It is possible that differences in the amount of time spent investigating novel objects may be detected after seven days of WIN 55,212-2 administration whereas the number of visits themselves may not be affected.

Additionally, the effects of intraperitoneal administration may be confounded by differences in drug metabolism among the experimental groups. In this study, both adult and adolescent animals were tested in the same timeframe subsequent to identical drug administration. If the adolescent cohorts experienced faster metabolism of the drug, this may have resulted in the absence of observed effects. However, our results showing a significant decrease in ambulatory distance in both acutely drug-treated adolescent and adult cohorts suggests an effective impact of the drug during the testing period. Additionally, a similar administration paradigm was used by Pandolfo et al., in which an acute dose of 2.5mg/kg WIN 55,212-2 was used in both adult and adolescent rats 30 minutes prior to testing [10].

But despite possible metabolic differences, adolescent rats show elevated levels of novelty seeking and exploratory behaviors [31, 32], and exhibit differential expression of anxiety-like behaviors [33, 34]. The present findings of an overall trend for higher novelty-preference in adolescent rats within the repeatedly-treated groups is therefore in agreement

with other reports. In the case of CB1 receptor activation, behavioral studies have also showed additional age-related effects. As mentioned previously, Romero et al. found underdeveloped responses to CP 55,940 on nociception and motor activity in adolescent rats [15], and a place-conditioning study by Quinn et al. indicated that repeated administration of Δ^9-THC was less aversive to adolescent rats than to adult rats, however, it resulted in greater memory deficits and hippocampal alterations later on [11]. It is thus possible that an age-related tendency for novelty-seeking might either mask the behavioral impacts of drug administration, or that, as suggested by previous studies, systems modulated by CB1 receptor activation are not functionally mature.

Thus the evidence that initial CB1 activation in adolescents does not show an immediate impact on behavior suggests particular vulnerability to its continued long-term use. Unfortunately, long-lasting cognitive impairments have been correlated with early use and repeated use of cannabinoids during adolescence may thus interfere with the developmental regulation of CB1 receptor function downstream. Additional behavioral assessments are be needed to uncover the long-term effects of WIN 55,212-2 on the coeruleo-cortical pathway in adolescent rats, and studies examining the upregulation of TH, c-Fos, or CB1 receptor proteins in adolescents may further reveal altered regulatory pathways. As mentioned previously, the lack of modulation by CB1 receptor activation on novelty-preference in the adolescent animals suggests a developmental difference in CB1 receptor activity. Thus the ontogeny of CB1 receptor-related effects requires further study.

The convergent results that separate systems are differentially impacted by CB1 agonist administration and that these systems have disparate developmental trajectories has extensive implications for cannabinoid exposure during adolescence. Additional longitudinal studies may yield information about the long-term effects of repeated CB1 receptor activation on attentional mode, and despite the apparent absence of modulatory effects of WIN 55,212-2 on the investigatory behavior of the adolescent cohorts, it is possible that long-term repeated exposure to CB1 agonists will have lasting effects on novelty investigation that are not evident until adulthood. Thus the results of this study provide further indications that separate systems are differentially impacted by activation of CB1 receptors, and that further studies are required to elucidate the full implications of cannabinoid exposure during adolescence.

References

1. Casswell S, Marks D. Cannabis induced impairment of performance of a divided attention task. Nature. 1973; 241(5384):60–1. [PubMed: 4696937]
2. Chait, LD.; Pierri, J. Effects of smoked marijuana on human performance: A critical review. In: Murphy, L.; Bartke, A., editors. Marijuana/Cannabinoids: Neurobilogy and Neurophysiology. CRC Press; Boca Raton, FL: 1992. p. 387-423.
3. Solowij N, et al. Does marijuana use cause long-term cognitive deficits? Jama. 2002; 287(20):2653–4. author reply 2654. [PubMed: 12020296]
4. Hillyard SA, Kutas M. Electrophysiology of cognitive processing. Annu Rev Psychol. 1983; 34:33–61. [PubMed: 6338812]
5. Naatanen R. The role of attention in auditory information processing revealed by event-related brain potentials. Behav Brain Sci. 1990; 13:201–288.

6. Kempel P, et al. Auditory-evoked potentials and selective attention: different ways of information processing in cannabis users and controls. Neuropsychobiology. 2003; 48(2):95–101. [PubMed: 14504418]
7. Solowij N, Michie PT, Fox AM. Differential impairments of selective attention due to frequency and duration of cannabis use. Biol Psychiatry. 1995; 37(10):731–9. [PubMed: 7640328]
8. Schneider M. Puberty as a highly vulnerable developmental period for the consequences of cannabis exposure. Addict Biol. 2008; 13(2):253–63. [PubMed: 18482434]
9. Page ME, et al. Repeated cannabinoid administration increases indices of noradrenergic activity in rats. Pharmacol Biochem Behav. 2007; 86(1):162–8. [PubMed: 17275893]
10. Pandolfo P, et al. Increased sensitivity of adolescent spontaneously hypertensive rats, an animal model of attention deficit hyperactivity disorder, to the locomotor stimulation induced by the cannabinoid receptor agonist WIN 55,212-2. Eur J Pharmacol. 2007; 563(1–3):141–8. [PubMed: 17374533]
11. Quinn HR, et al. Adolescent Rats Find Repeated Delta(9)-THC Less Aversive Than Adult Rats but Display Greater Residual Cognitive Deficits and Changes in Hippocampal Protein Expression Following Exposure. Neuropsychopharmacology. 2008; 33(5):1113–26. [PubMed: 17581536]
12. Jarbe TU, et al. Effects of the CB1R agonist WIN-55,212-2 and the CB1R antagonists SR-141716 and AM-1387: open-field examination in rats. Pharmacol Biochem Behav. 2006; 85(1):243–52. [PubMed: 16996123]
13. Jarbe TU, Andrzejewski ME, DiPatrizio NV. Interactions between the CB1 receptor agonist Delta(9)-THC and the CB1 receptor antagonist SR-141716 in rats. Open-field revisited. Pharmacol Biochem Behav. 2002; 73(4):911–9. [PubMed: 12213538]
14. Jarbe TU, et al. (−)-Adamantyl-delta8-tetrahydrocannabinol (AM-411), a selective cannabinoid CB1 receptor agonist: effects on open-field behaviors and antagonism by SR-141716 in rats. Behav Pharmacol. 2004; 15(7):517–21. [PubMed: 15472574]
15. Romero EM, et al. Antinociceptive, behavioural and neuroendocrine effects of CP 55,940 in young rats. Brain Res Dev Brain Res. 2002; 136(2):85–92. [PubMed: 12101025]
16. Mendiguren A, Pineda J. Systemic effect of cannabinoids on the spontaneous firing rate of locus coeruleus neurons in rats. Eur J Pharmacol. 2006; 534(1–3):83–8. [PubMed: 16483566]
17. Muntoni AL, et al. Cannabinoids modulate spontaneous neuronal activity and evoked inhibition of locus coeruleus noradrenergic neurons. Eur J Neurosci. 2006; 23(9):2385–94. [PubMed: 16706846]
18. Oropeza VC, Page ME, Van Bockstaele EJ. Systemic administration of WIN 55,212-2 increases norepinephrine release in the rat frontal cortex. Brain Res. 2005; 1046(1–2):45–54. [PubMed: 15927549]
19. Sara SJ, Dyon-Laurent C, Herve A. Novelty seeking behavior in the rat is dependent upon the integrity of the noradrenergic system. Brain Res Cogn Brain Res. 1995; 2(3):181–7. [PubMed: 7580400]
20. Mansour AA, et al. Novel objects in a holeboard probe the role of the locus coeruleus in curiosity: support for two modes of attention in the rat. Behav Neurosci. 2003; 117(3):621–31. [PubMed: 12802890]
21. File SE, Wardill AG. Validity of head-dipping as a measure of exploration in a modified hole-board. Psychopharmacologia. 1975; 44(1):53–9. [PubMed: 1197580]
22. Devauges V, Sara SJ. Activation of the noradrenergic system facilitates an attentional shift in the rat. Behav Brain Res. 1990; 39(1):19–28. [PubMed: 2167690]
23. Kitchigina V, et al. Novelty-elicited, noradrenaline-dependent enhancement of excitability in the dentate gyrus. Eur J Neurosci. 1997; 9(1):41–7. [PubMed: 9042567]
24. Oropeza VC, Mackie K, Van Bockstaele EJ. Cannabinoid receptors are localized to noradrenergic axon terminals in the rat frontal cortex. Brain Res. 2007; 1127(1):36–44. [PubMed: 17113043]
25. Drews E, Schneider M, Koch M. Effects of the cannabinoid receptor agonist WIN 55,212-2 on operant behavior and locomotor activity in rats. Pharmacol Biochem Behav. 2005; 80(1):145–50. [PubMed: 15652390]

26. Arevalo C, de Miguel R, Hernandez-Tristan R. Cannabinoid effects on anxiety-related behaviours and hypothalamic neurotransmitters. Pharmacol Biochem Behav. 2001; 70(1):123–31. [PubMed: 11566149]

27. Devauges V, Sara SJ. Memory retrieval enhancement by locus coeruleus stimulation: evidence for mediation by beta-receptors. Behav Brain Res. 1991; 43(1):93–7. [PubMed: 1650233]

28. Moranta D, Esteban S, Garcia-Sevilla JA. Differential effects of acute cannabinoid drug treatment, mediated by CB1 receptors, on the in vivo activity of tyrosine and tryptophan hydroxylase in the rat brain. Naunyn Schmiedebergs Arch Pharmacol. 2004; 369(5):516–24. Epub 2004 Apr 3. [PubMed: 15064921]

29. Scavone, JL.; Mackie, K.; Van Bockstaele, EJ. Neuroscience Meeting Planner. San Diego, CA: Society for Neuroscience; 2007. Distribution and trafficking of the cannabinoid receptor CB1 in the rat noradrenergic locus coeruleus. Online., 2007. Program No. 44.10

30. Herkenham M, et al. Neuronal localization of cannabinoid receptors and second messengers in mutant mouse cerebellum. Brain Res. 1991; 552(2):301–10. [PubMed: 1913192]

31. Stansfield KH, Kirstein CL. Effects of novelty on behavior in the adolescent and adult rat. Dev Psychobiol. 2006; 48(1):10–5. [PubMed: 16381024]

32. Douglas LA, Varlinskaya EI, Spear LP. Novel-object place conditioning in adolescent and adult male and female rats: effects of social isolation. Physiol Behav. 2003; 80(2–3):317–25. [PubMed: 14637231]

33. Doremus TL, Varlinskaya EI, Spear LP. Age-related differences in elevated plus maze behavior between adolescent and adult rats. Ann N Y Acad Sci. 2004; 1021:427–30. [PubMed: 15251922]

34. Slawecki CJ. Comparison of anxiety-like behavior in adolescent and adult Sprague-Dawley rats. Behav Neurosci. 2005; 119(6):1477–83. [PubMed: 16420152]

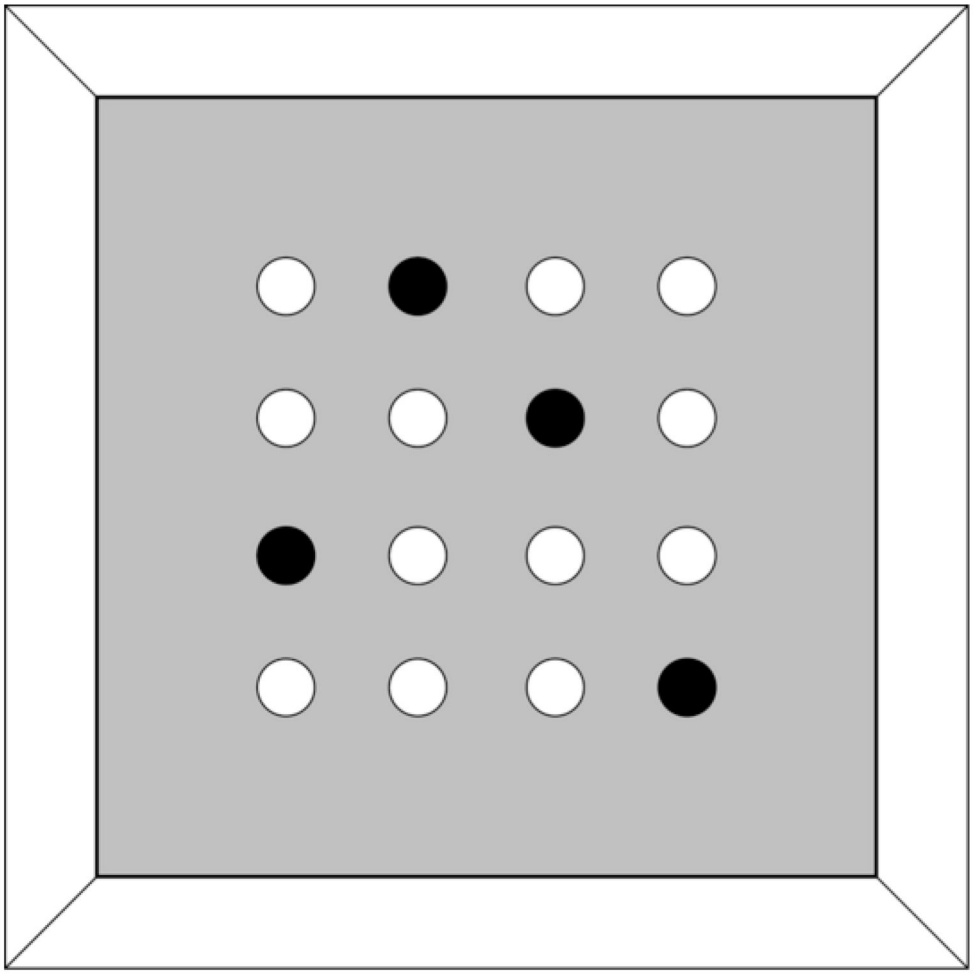

Figure 1.
Schematic of hole board apparatus (to scale). Black holes indicate placement of objects and white holes were empty. Infrared beams were located above and below the floor to detect locomotor activity and hole visits respectively.

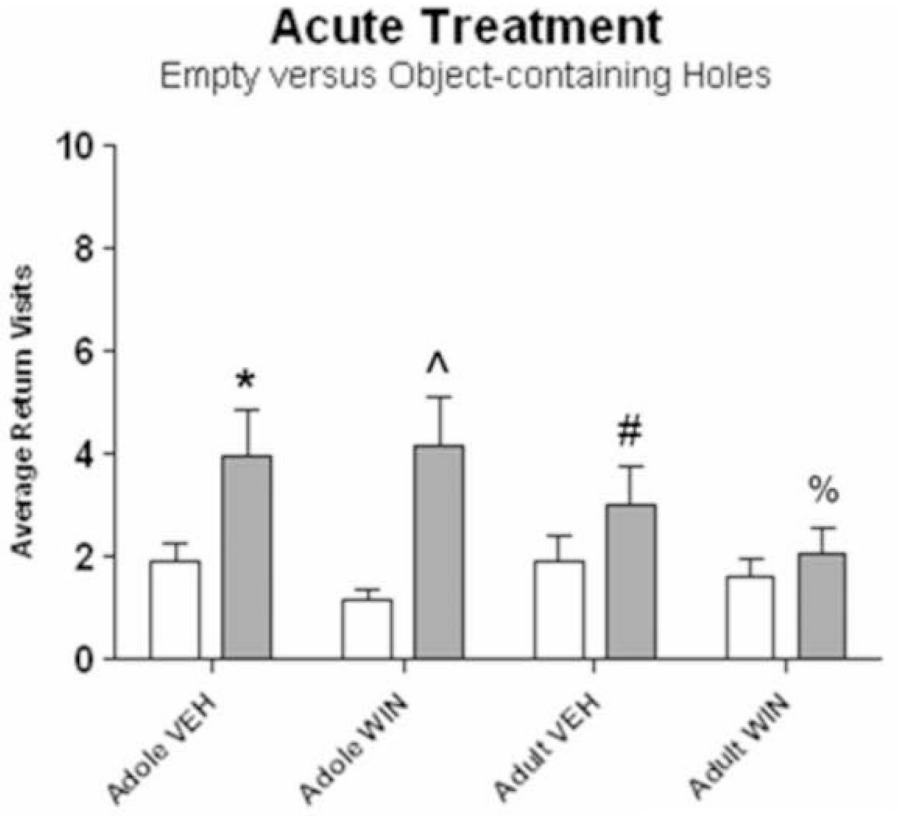

Figure 2.

Average number of return visits to an empty hole (white bars) versus a hole containing an object (grey bars) during the 10 min test in the hole board for groups receiving acute administration of WIN 55,212-2 (3.0 mg/kg) or the vehicle. A significantly greater average number of returns to a hole containing an object indicated a preference for novelty and intact novelty investigation behavior. This can be seen in all groups except the WIN 55,212-2-treated adults. *p=0.014, ^p=0.005, #p=0.065, %p=0.383. The drug did not affect the total number of visits to holes (p>0.05).

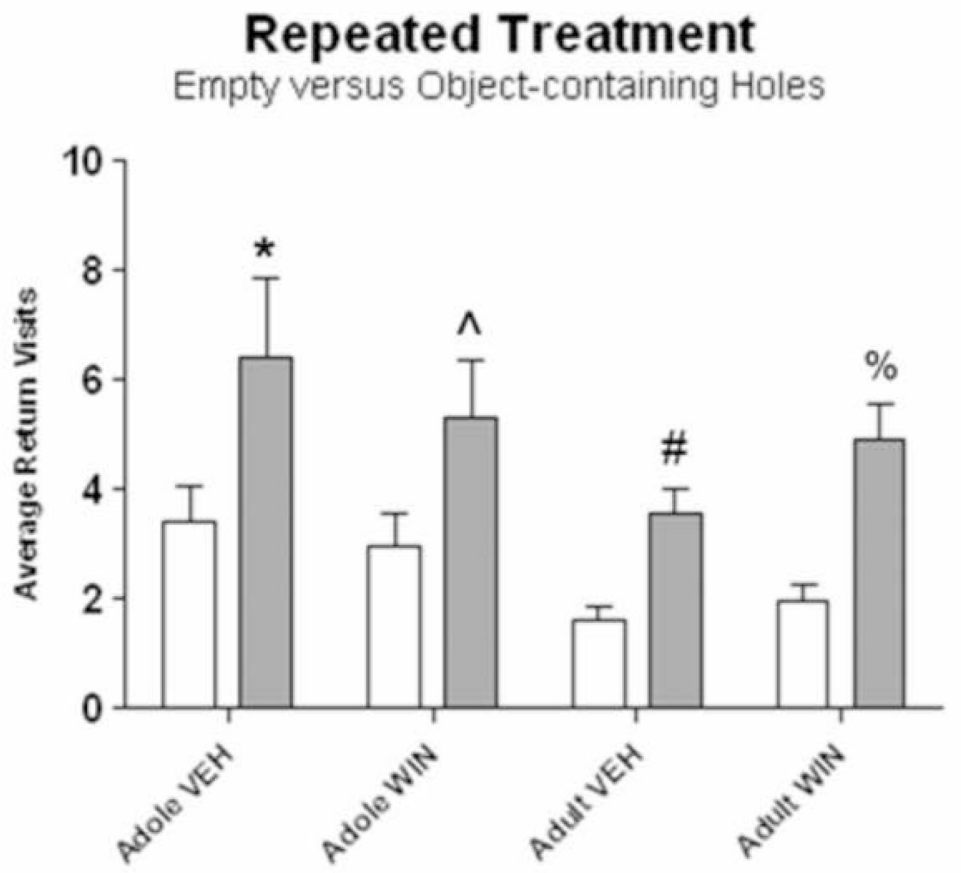

Figure 3.

Average number of return visits to an empty hole (white bars) versus a hole containing an object (grey bars) during the 10 min test in the hole board for groups receiving repeated administration of WIN 55,212-2 (3.0 mg/kg) or the vehicle. A significantly greater average number of returns to a hole containing an object indicated a preference for novelty and intact novelty investigation behavior. This can be seen in all groups. *p=0.013, ^p=0.023, #p=0.003, %p=0.001. The drug did not affect the total number of visits to holes (p>0.05).

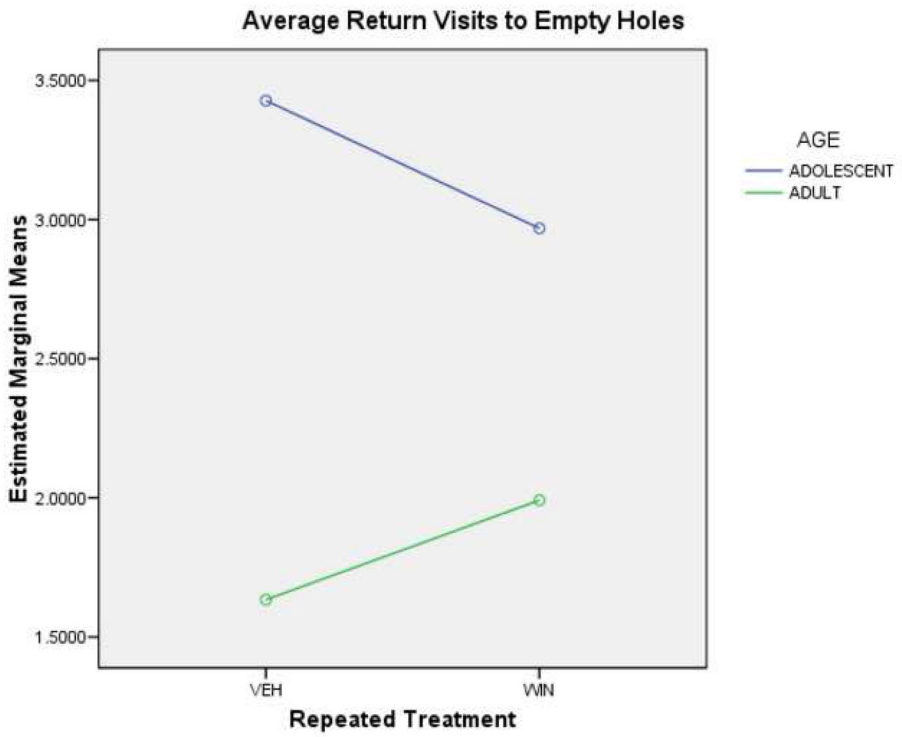

Figure 4.

For repeatedly-treated groups, 2×2 ANOVA crossing age and treatment for average return visits to empty holes. The average number of visits to empty holes was significantly impacted by age was found [$F(1,40)=7.874$, $p=0.008$]

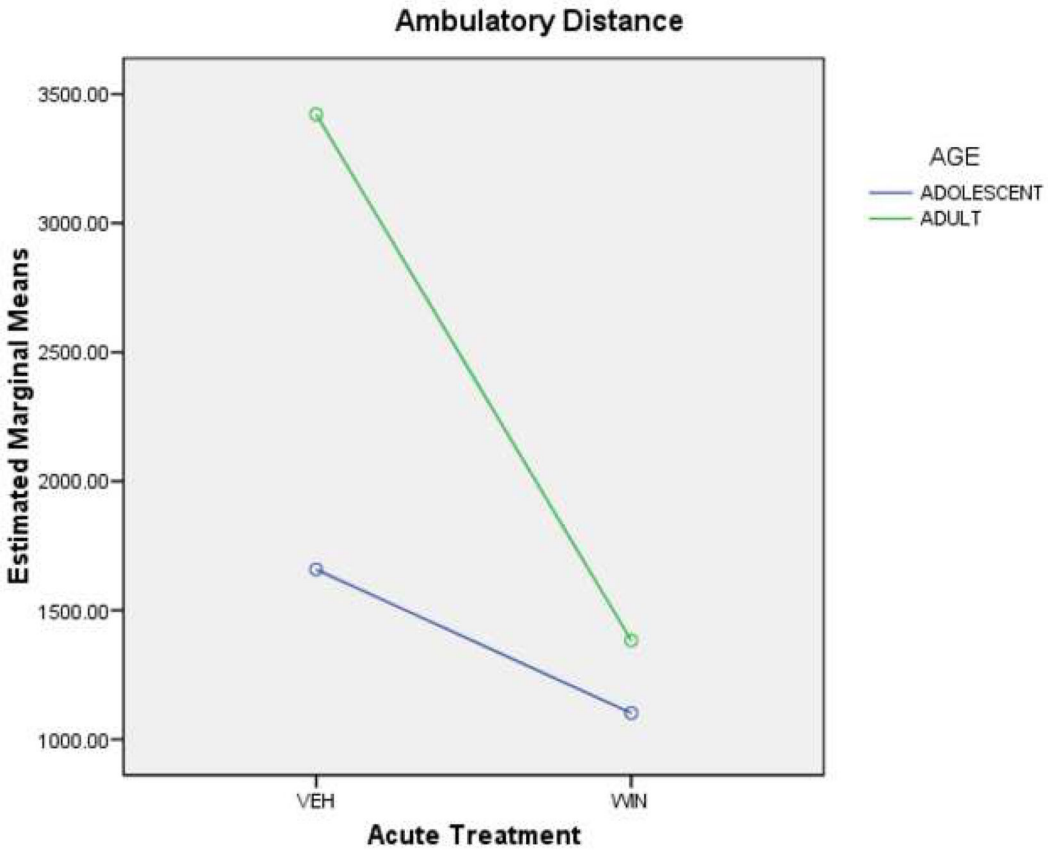

Figure 5.

For acutely-treated groups, 2×2 ANOVA crossing age and treatment for total ambulatory distance. Total ambulatory distance was impacted by age [$F(1,31)=21.433$, $p=0.000$] and treatment condition [$F(1,31)=34.520$, $p=0.000$], and a significant interaction was found [$F(1,31)=11.265$, $p=0.002$, Figure 5]. Subsequent t-tests indicated that the adults covered more distance than the adolescents [$t(33)=-2.962$, $p=0.006$, equal variances assumed], and vehicle-treated groups covered more distance than drug-treated groups [$t(33)=3.504$, $p=0.001$, equal variances assumed].

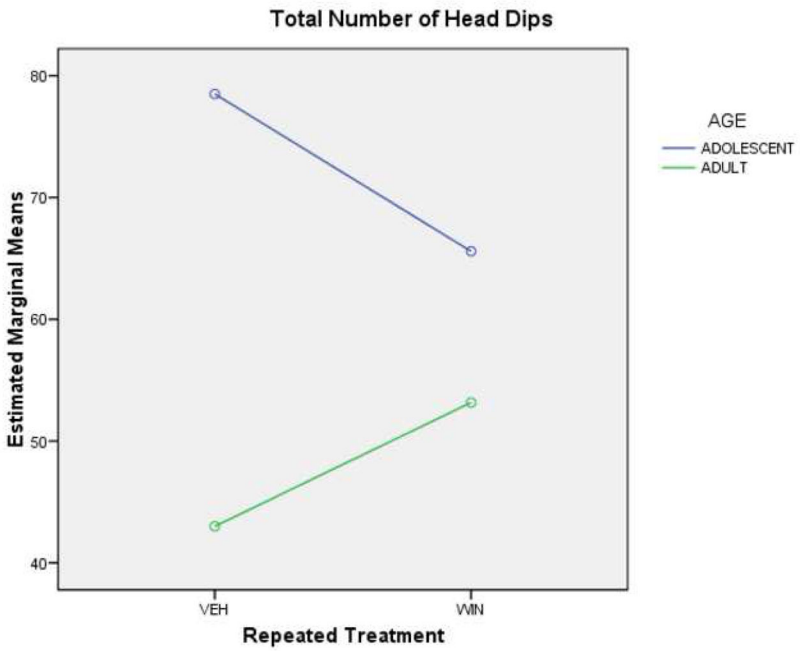

Figure 6.

For repeatedly-treated groups, 2×2 ANOVA crossing age and treatment for total number of visits to all holes. The total number of hole visits was significantly impacted by age [$F(1,40)=6.282$, $p=0.016$, Figure 6], such that adolescents visited more holes overall than adults did [$t(42)=2.422$, $p=0.020$, equal variances assumed].