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Lack of behavioral sensitization after repeated exposure to THC in mice and comparison to methamphetamine

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Abstract

Rationale—Recent evidence has provided support for the incentive-sensitization model of addiction, where repeated stimulation of neural reward circuits leads to a long-lasting sensitization of mesolimbic dopaminergic activity. This phenomenon has been demonstrated with many drugs of abuse, most often by measuring progressively increased activating effects of drugs on locomotor activity, thought to reflect an underlying neural sensitization. Whether cannabinoids, and in particular Δ^9-tetrahydrocannabinol (THC), produce similar effects in this model is somewhat controversial, with mixed evidence in the literature.

Objectives—These experiments were conducted to determine whether behavioral sensitization could be established in mice after repeated exposure to THC. Sensitization to repeated methamphetamine treatment was used as a positive control.

Methods—The effects of acute and repeated intermittent (every 3–4 days) treatment with THC or methamphetamine on locomotor activity were determined in Institute of Cancer Research (ICR) mice. Additional experiments with THC employed a dosing regimen that increased the number of injections, controlled for behavioral tolerance, examined different aspects of behavior, and used a different species (Sprague—Dawley rats).

Results—Both methamphetamine and THC acutely increased activity. A robust dose-dependent sensitization was observed after intermittent treatment with methamphetamine but not with THC. Additionally, no evidence for behavioral sensitization to the effects of THC was found with any of the various protocols.

Conclusion—These data suggest that repeated THC treatment is less likely to produce behavioral sensitization than are other drugs of abuse. It appears that this phenomenon may only occur under very particular conditions, which raises doubts about its relevance to chronic cannabis users.

Keywords

THC; Methamphetamine; Behavioral sensitization; Locomotor activity; Addiction

Assessment of the addictive potential of cannabis is an important issue faced by researchers and may have implications for public policy as well as the development of cannabinoid-based therapeutics. While the addictive nature of cannabis has long been controversial, recent epidemiological studies have estimated that approximately 4% of cannabis users develop a DSM-defined cannabis dependence syndrome (Anthony et al. 1994; Chen et al. 2005), and a substantial number of cannabis users seek substance abuse treatment (e.g., SAMHSA 2002). Furthermore, a growing consensus has recognized that chronic cannabis use can sometimes

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lead to physical dependence, defined as the emergence of withdrawal effects during abstinence, most often including cannabis craving, anxious or depressed mood, cognitive impairments, restlessness and irritability, sleep disturbances, appetite or weight changes, and gastrointestinal problems (e.g., Budney et al. 2003; Haney et al. 1999; Jones et al. 1976; Kouri and Pope 2000). While development of strategies to alleviate withdrawal symptoms are likely to provide some benefit to cannabis users seeking treatment (e.g., Haney et al. 2004), it seems clear that negative reinforcement (i.e., avoidance of withdrawal effects) is an insufficient explanation for the complex and varied patterns of compulsive use exhibited by some addicts. Recently, a biologically oriented view of compulsive drug use has been proposed that can account for many characteristics of addiction, referred to as the "incentive-sensitization model" (Robinson and Berridge 1993, 2000). The thrust of this approach is that addictive substances share an ability to produce long-lasting changes in neural systems responsible for reward and incentive motivation (i.e., the mesolimbic dopamine system), causing them to become hypersensitive to drugs and drug-related stimuli. This model is supported at the neurobiological level, as repeated treatments with known addictive drugs have been shown to produce a "neural sensitization" of the mesolimbic dopamine system—characterized by increases in dopamine release, increased sensitivity of dopamine receptors, and even morphological changes that work together to increase the responsiveness of the system (Robinson and Berridge 1993, 2000).

At the behavioral level, increases in mesolimbic dopamine transmission have long been associated with increased spontaneous locomotor activity in rodents (e.g., Costall et al. 1977; Jackson et al. 1975; Pijnenburg et al. 1975), and this effect can become progressively greater with repeated administration of drugs that enhance dopamine transmission (Robinson and Becker 1986; Segal et al. 1981; Stewart and Badiani 1993). This behavioral sensitization has been proposed to be related to the neural sensitization of the mesolimbic dopamine system and thus could be used to model the progressive sensitization of incentive-salience attribution (i.e., addiction). This progressive increase in locomotor-activating effects has been observed after repeated treatment with a variety of substances such as cocaine, amphetamine, methylphenidate, morphine, phencyclidine, 3,4-methylenedioxy-*N*-methylamphetamine (MDMA), nicotine, and ethanol, suggesting a common mechanism shared by many drugs of abuse (e.g., Fish et al. 2002; Hirabayashi and Alam 1981; Kalivas et al. 1998; Kalivas and Stewart 1991; Kita et al. 1992; Xu and Domino 1994). Additional characteristics of this phenomenon that may relate to addiction in humans include the observations that such a sensitized state can last for months to years, often becomes more pronounced after a period of drug abstinence, and is heavily influenced by contextual stimuli (reviewed in Robinson and Berridge 1993, 2000). While there is as yet little direct evidence of sensitization in human addicts, the progressive nature of amphetamine and cocaine-induced psychosis (e.g., Angrist 1994) isconsistentwiththismodel, and several studies have demonstrated sensitization to effects of amphetamine in human subjects (reviewed in Sax and Strakowski 2001).

Therefore, can repeated treatment with tetrahydrocanabinol (THC) produce a behavioral sensitization similar to that observed with other drugs? Despite early controversies, acute administration of THC has been shown to act on brain reward systems in a manner that resembles other common drugs of abuse (for reviews, see Gardner 2002; Tanda and Goldberg 2003). For example, THC has been shown to stimulate mesolimbic dopamine activity (Chen et al. 1990; Tanda et al. 1997), is self-administered in laboratory animals (Braida et al. 2004; Justinova et al. 2003; Tanda et al. 2000), lowers thresholds for intracranial self-stimulation (Gardner et al. 1989), and can be used to establish a conditioned place preference (Braida et al. 2004; Lepore etal. 1995; Valjent and Maldonado 2000). Similarly, while the locomotor depression seen after high doses of THC has been better characterized, low to moderate doses of THC have long been known to produce increases in locomotor activity (e.g., Davis et al. 1972; Sanudo-Pena et al. 2000). However, many of these effects are less robust than those observed with other drugs of abuse and seem to be highly dependent on dose and experimental

conditions. For example, THC has been shown to produce conditioned place aversions just as often as place preferences (Mallet and Beninger 1998; Parker and Gillies 1995). Furthermore, the effects of repeated stimulation of CB_1 receptors on reward systems are still poorly understood. The first study to explicitly look for cannabinoid-mediated behavioral sensitization found no effects of repeated administration of the potent CB_1 agonist CP 55,940 (Arnold et al. 1998). In contrast, two other groups have reported evidence of behavioral sensitization in rats (Cadoni et al. 2001; Rubino et al. 2001). The present experiments were designed to determine whether behavioral sensitization to the loco-motor-increasing effects of THC could be demonstrated in mice. Development of such a model would be useful for characterizing the mechanisms responsible for its development and expression, allowing the use of transgenic mouse lines, and could serve as a platform for developing pharmacological strategies to prevent or reverse it. The first goal was to establish optimal parameters by characterizing the behavioral sensitization produced by a positive control, methamphetamine (METH). The second goal was to determine whether repeated THC exposure induces behavioral sensitization.

Materials and methods

Subjects

Male Institute of Cancer Research (ICR) mice weighing 22–30 g (Harlan Laboratories, Dublin, VA, USA) and Sprague—Dawley rats (Harlan Laboratories) weighing 280–300 g were housed in plastic cages with stainless steel wire tops in groups of five (mice) or two (rats) in a temperature-controlled vivarium on a 12-h light/dark cycle. Food and water were available ad libitum. All animal protocols were approved by the Virginia Commonwealth University Institutional Animal Care and Use Committee (VCU IACUC), and followed the Principles of Laboratory Animal Care.

Drugs

Δ^9-THC and METH HCl were provided by the National Institute on Drug Abuse (Bethesda, MD, USA). Δ^9-THC was dissolved in a 1:1 mixture of absolute ethanol and alkamuls-620 (Rhone-Poulenc, Princeton, NJ, USA) and diluted with saline to a final ratio of 1:1:18 (ethanol/alkamuls/saline). METH was dissolved in saline. All drug injections were given via the i.p. route of administration with an injection volume of 0.1 ml/kg.

Behavioral assessment

Automatic assessment of locomotor activity was conducted in plastic containers (16×32 cm) located in a dark cabinet with a small fan generating white noise. A series of eight photocell beams on the horizontal plane of each cage were monitored using a Digiscan Animal Activity Monitor (Med Associates, St. Albans, VT), and activity was recorded as the total number of beam interruptions per 5-min bins. For some experiments, plastic cages (16×32 for mice, 20×40 cm for rats) were placed on the countertop, and behavior was recorded with a videocamera. Tapes were later scored by an observer blinded to the treatment condition who recorded the amount of time spent in the following categories of exploratory behavior (locomotion, rearing, investigative sniffing), grooming (licking), stereotyped behavior (gaping, writhing, head bobbing/swaying/shaking, and other odd repetitive behaviors), and stillness/inactivity. For one experiment, antinociception was assessed with the radiant heat tail-flick test (D'Amour and Smith 1941). Control withdrawal latencies were obtained before drugs were administered, and tests were conducted 30 min after injections, immediately after the observation period. All animals were allowed to acclimate to the laboratory environment for at least 1 h before being administered drug and assessed. In all cases, subjects were placed in activity cages immediately after drug administration.

Procedure

Experiment 1—The first set of experiments was designed to characterize the behavioral sensitization produced by METH in ICR mice as a positive control. The acute effects of METH on spontaneous locomotor activity were determined by treating separate groups of mice with METH (0.25, 0.5, 1, or 2 mg/kg) or vehicle (veh) and recording locomotor activity (beam breaks) for 30 min. Based on these acute results, new groups of mice were treated seven times (once every 3–4 days) with either veh, 0.5, or 1 mg/kg METH, and locomotor activity was assessed for 60 min after each treatment.

Experiment 2—The second set of experiments evaluated THC under conditions similar to those described above. The acute effects of THC on locomotor activity were determined by treating separate groups of mice with veh, 1, 3, or 10 mg/kg THC and recording activity (beam breaks) for 60 min. The possibility of behavioral sensitization developing to the locomotor-activating effects of THC was assessed by treating mice seven times (once every 3–4 days) with either veh or 10 mg/kg THC and recording activity for 60 min immediately after each treatment.

Experiment 3—Next, a series of protocol variations were employed to determine whether behavioral sensitization to THC could be observed under altered conditions. First, the effects of a more rigorous dosing regimen were evaluated by treating mice twice daily with either 10 mg/kg THC or vehicle and assessing activity for 60 min after each morning treatment. Second, possible effects of the context in which the drug was administered (i.e., behavioral tolerance) were evaluated by treating mice in their home cages twice daily for 6 1/2 days with vehicle or 10 mg/kg THC, and 21 days later, the dose—response of THC was determined in both repeated treatment groups by acute administration of THC (1, 3, or 10 mg/kg) or vehicle and assessing locomotor activity for 60 min. This particular repeated dosing regimen was selected based on previous demonstrations that show it leads to pronounced tolerance to several behavioral effects of THC, including the decreases in activity seen at higher doses (e.g., Bass and Martin 2000). To determine whether behavioral sensitization may occur to specific types of behaviors (i.e., stereotypies) rather than gross locomotor activity under this protocol, separate groups of mice were treated with 10 mg/kg THC or vehicle twice daily for 6 1/2 days in their home cages and tested 14 days later. After an acclimation period and a baseline assessment of nociception, mice were placed in plastic cages on the countertop and videotaped for 30 min. Behavior was later scored and categorized as described above. After 30 min, mice were tested for antinociception (tailflick).

Experiment 4—Finally, an experiment was conducted in Sprague—Dawley rats using a protocol similar to the one in which sensitization to the effects of THC had been previously reported (Rubino et al. 2001). Separate groups of rats were treated in their home cages twice daily for 5 days with an escalating regimen of THC (5 mg/kg on day 1, 10 mg/kg on day 2, 20 mg/kg on day 3, and 40 mg/kg on days 4 and 5) or vehicle. Twenty days later, rats from both groups received either 5 mg/kg THC or vehicle, and their behavior was videotaped for 60 min and manually scored as described above.

Results

Experiment 1—methamphetamine produces behavioral sensitization

As expected, acute administration of METH produced significant increases in spontaneous locomotor activity, $F(4, 43)=5.0$, $p<0.01$ (Fig. 1a). Overall, activity was higher in mice treated with 0.5, 1, and 2 mg/kg METH when compared to the vehicle group. When treated and evaluated every 3–4 days, sensitization readily developed to this locomotor-activating effect (Fig. 1b), as evidenced by a significant interaction between dose and test session, $F(12, 192)$

=4.5, $p<0.001$. Subsequent one-way analyses of variance (ANOVAs) conducted for each treatment level revealed significant session effects for the 0.5 mg/kg METH group, F (6, 66) =2.7, $p<0.05$, and for the 1.0 mg/kg METH group, F (6, 54)=9.7, $p<0.001$, but not for the veh group.

Experiment 2—intermittent THC treatment fails to produce behavioral sensitization

Acute THC also produced a dose-dependent increase in locomotor activity, $F(3, 20)=3.1$, $p<0.05$ (Fig. 2a), with mice treated with 10 mg/kg THC being more active than vehicle-treated mice, $p<0.05$. When evaluated with the same intermittent treatment protocol described above for METH, no evidence of behavioral sensitization to the activating effects of 10 mg/kg THC was observed (Fig. 2b). Significant main effects of THC treatment were observed, $F(1, 108) =31.0$, $p<0.0001$, where THC increased activity on sessions 1, 3, 4, 6, and 7 ($p<0.05$).

Experiment 3—evaluation of THC under several procedural variations

Three distinct additional protocols were used to determine whether ICR mice could develop sensitization to THC after repeated dosing. First, the development of short-term (acute) sensitization was assessed by treating mice twice per day with 10 mg/kg THC or vehicle for 5 days. Immediately after AM injections, mice were placed in an automated activity monitor and assessed for 1 h. As shown in Fig. 3a, there was a significant effect of session ($F[4, 80]=10.5$, $p<0.0001$), where mice in both groups displayed a decrease in activity across days. There was a clear trend towards tolerance, not sensitization, to the locomotor increasing effects of THC as t tests revealed significant effects of THC on days 1 and 3 ($p<0.05$), but not days 4 and 5, although the main effect of THC treatment across all days just failed to reach significance ($p=0.06$).

Next, the development of a prolonged sensitization was assessed by treating a different set of ICR mice twice per day with 10 mg/kg THC or vehicle for 6 1/2 days. Twenty-one days later, mice were acutely challenged with veh, 1, 3, or 10 mg/kg THC and assessed in the automated activity monitors for 1 h. As shown in Fig. 3b, acute treatment with THC increased activity ($F[3, 32]=3.2$, $p<0.05$), although there were no effects of THC pretreatment.

To characterize more fully the behavioral responses to acute THC treatment after repeated dosing, new groups of mice were treated twice per day with 10 mg/kg THC or vehicle for 6 1/2 days. Fourteen days later, mice were treated with vehicle or THC (1 or 10 mg/kg) and placed in plastic cages. Behavior was videotaped for 30 min and scored later by an observer blinded to the drug condition. Behaviors were grouped into three main categories—exploratory activity (locomotion, sniffing, rearing), non-exploratory activity (grooming), and inactivity. As represented in Fig. 4a, there was a significant interaction between acute THC and repeated treatment group on time spent in exploratory activity ($F[2, 44]=3.7$, $p<0.05$), where activity was significantly increased by 10 mg/kg THC in the repeated vehicle group ($F[2, 20]=10.4$, $p<0.001$) but not the repeated THC treatment group. As shown in Fig. 4b, acute THC robustly reduced time spent grooming ($F[2, 44]=19.0$, $p<0.0001$), irrespective of repeated treatment group. The tail-flick antinociception test performed at the end of the observation period revealed a small but significant effect of THC ($F[2, 44]=9.1$, $p<0.001$), demonstrating that 10 mg/kg THC is a threshold dose for antinociception in ICR mice when administered i.p.

Experiment 4—assessment of THC sensitization in Sprague—Dawley rats

Finally, we evaluated a rat model that has been previously used to demonstrate sensitization to THC. As shown in Fig. 5a, exploratory activity decreased across the hour session for all groups ($F[3, 84]=51.1$, $p<0.0001$). However, no effects of acute or repeated THC treatment were observed. As shown in Fig. 5b, acute THC robustly reduced time spent grooming in both repeated treatment groups ($F[1, 84]=39.0$, $p<0.0001$). There was also a significant effect of

time, where grooming behaviors decreased during the hour (F[3, 84]=6.9, p<0.001). Finally, acute THC-attenuated stereotyped behaviors (Fig. 5c; F[1, 84]=9.8, p<0.01), while there were no differences between repeated treatment groups. An interaction between acute THC and time (F[3, 84]=5.3, p<0.01) reflected how stereotypies decreased across time in the rats treated acutely with veh (F[3, 45]=9.3, p<0.0001), but not in those rats treated acutely with THC, which started with fewer stereotypies and remained stable across the hour.

Discussion

Understanding the degree to which drugs of abuse from different classes share common neural mechanisms is a vital part of investigating addiction processes and potentially even non-drug-related compulsive behaviors. However, whether or not THC produces long-term effects on brain reward group circuits similar to those seen with many other drugs of abuse remains controversial. While the results of the present experiments demonstrate that THC can produce acute activating effects on locomotor activity that resemble those seen after METH administration, they provide no evidence that repeated administration of THC leads to a behavioral sensitization similar to that observed with METH.

A potential conceptual difficulty encountered in attempting to demonstrate behavioral sensitization to THC is the fact that moderate to high doses of cannabinoid agonists are well known to depress motor activity, an effect often associated with the basal ganglia (e.g., Chaperon and Thiebot 1999; Shi et al. 2005). It is possible that acute depressant effects of THC could mask the expression of any behavioral sensitization, even if an underlying neural sensitization in the nucleus accumbens was occurring. However, in addition to depressant effects observed with relatively high doses of THC, it has been shown that low to moderate doses can sometimes produce motor-activating effects. For example, one early study showed 5 mg/kg THC increased spontaneous activity of rats, while higher doses reduced activity (Davis et al. 1972). Interestingly, tolerance developed to both of these effects upon daily administration. Another study reported triphasic effects of THC on activity of Sprague—Dawley rats—decreases at very low doses (0.2 mg/kg), increases at moderate doses (2 mg/kg), and then decreases at higher doses, which corresponded to the emergence of catalepsy (Sanudo-Pena et al. 2000). Similarly, we have shown a biphasic effect on locomotor activity of C57 mice under “normal tetrad conditions” (i.p. 5–15 min), with increases observed at 10 mg/kg, and decreases at 30 mg/kg (Varvel et al. 2005). For the present experiments, we addressed this issue by determining a dose of THC and set of experimental parameters that reliably demonstrated the activating effects of THC with a series of pilot studies (data not shown). We found that 10 mg/kg THC administered i.p. to ICR mice produced reliable increases in activity (consistently by around 25–50%) when the mice were placed immediately into the observation chambers and assessed for 60 min.

The primary finding of these experiments was that behavioral sensitization did not develop in mice treated repeatedly with THC, even under the intermittent conditions in which sensitization readily developed to METH’s behavioral-activating effects. This effect of METH has been well characterized in mice, including in the ICR strain used in the present experiments (e.g., Kuribara and Tadokoro 1989). To the extent that this behavioral sensitization reflects the development of an underlying neural sensitization of brain reward systems, we would predict based on this data that repeated THC administration does not produce comparable effects on reward systems that repeated METH does, although aspects of their acute effects appear similar.

However, given the enormous differences between the mechanisms mediating the effects of THC and METH, including the nature of their effects on mesolimbic dopamine (i.e., direct vs indirect stimulation of dopamine release), it is possible that the conditions necessary to develop

or express such a sensitization are different and that the observed differences in ability to induce sensitization are quantitative rather than qualitative in nature. Thus, we evaluated mice under several different protocols designed to systematically address several potential factors. While prior work with METH and other abused drugs has shown that sensitization tends to be more pronounced when drugs are administered intermittently (Hirabayashi and Alam 1981), it is possible that more frequent treatments are required for sensitization to develop to THC. Indeed, the two published reports of THC sensitization both administered THC twice per day with an escalating dose regimen, reaching high doses (Cadoni et al. 2001; Rubino et al. 2001). Thus, we investigated the effects of twice daily treatment with 10 mg/kg THC, observing activity after each AM treatment. We did not see any sensitization; in fact, tolerance developed to THC's activating effects by the fourth day of treatment. Another issue we addressed was the possibility that a behavioral tolerance had developed as a result of repeated pairings between THC's effects and the testing environment. However, mice treated in their home cages throughout the development phase also showed no sensitization when tested 21 days later in a novel context. We also investigated whether our failure to observe sensitization was due to our reliance on automatic measures of gross locomotor activity, which may have missed subtle differences in other types of behavior. While most of the experiments discussed above used locomotor activity as the primary dependent measure, other dopamine-influenced behaviors such as stereotypies have also been shown to develop sensitization to repeated treatment with psycho-stimulants and other drugs of abuse. In fact, both prior reports of THC sensitization showed a greater effect on stereotypies than increases in locomotor activity (Cadoni et al. 2001; Rubino et al. 2001). However, visual analysis of mice treated under identical conditions also found no incidence of increased stereotypies nor increased locomotion. In fact, no evidence of stereotypical behaviors as defined above was noted in any mice, although they were detected in rats. Interestingly, acute THC dose-dependently reduced grooming behavior in both treatment groups.

The experiments discussed above failed to demonstrate behavioral sensitization to repeated THC exposures in ICR mice. However, it is possible that species or strain differences render ICR mice insensitive to this effect. As previously mentioned, the existing literature regarding whether behavioral sensitization develops to cannabinoids in rats is mixed. The first report to directly address this issue was Arnold et al. (1998), who found no evidence of sensitization to intermittent treatments with several doses of the potent cannabinoid agonist CP 55, 940. Similarly, a recent study reported no sensitization to repeated THC treatment (Kolb et al. 2006). However, two groups have reported intriguing evidence of behavioral sensitization in rats (Cadoni et al. 2001; Rubino et al. 2001), which was associated with an upregulation of CB_1 receptors in the cerebellum (Rubino et al. 2003). Both groups investigated the effects of twice daily treatments of escalating doses of THC, assessed 15 or 20 days later. However, neither study included important control groups (acute vehicle), leaving open the possibility that residual tolerance to locomotor-depressing effects accounted for the differences they reported. We failed to replicate these effects when using a protocol very similar to the one used by Rubino et al. (2001). One potentially important difference between our study and the Rubino report is that their rats were young while ours were fully mature (125–150 g vs 275–300 g). It is possible that younger rats may be particularly susceptible to these adaptive changes. However, Ellgren et al. (2004) found that repeated treatments with THC or WIN 55, 212 to adolescent rats did not cause them to be more susceptible to locomotor or dopamine-increasing effects of subsequent amphetamine treatment. In addition, an ongoing study looking at adolescent rats using a different protocol has found no evidence of sensitization to THC (Jenny Wiley, personal communication). Another potentially important parameter is the route of administration. Cadoni et al. (2001) used intravenous administration of THC to assess sensitization, which may produce increases in mesolimbic dopamine more effectively than i.p. administration. Thus, it appears that behavioral sensitization to THC is a difficult phenomenon

to demonstrate and may depend on a number of factors such as species, route of drug administration, and other subtle aspects of experimental protocol.

However, it should be emphasized that failure to demonstrate sensitization to locomotor effects of cannabinoid agonists does not rule out the possibility that long-term adaptive changes may occur in brain reward systems in response to repeated drug treatments. While there have been to date no reports of THC (or other cannabinoid agonists) producing the kind of neural sensitization reported for other drugs of abuse (i.e., sensitized dopamine release, accelerated firing rates, or increased receptor sensitivity, etc.), a recent provocative paper reported that a relatively mild THC-dosing regimen (which did not produce behavioral sensitization) increased dendritic branching and dendritic length in the shell of the nucleus accumbens measured 31 days later, an effect similar to that seen in amphetamine-sensitized rats (Kolb et al. 2006). The relevance of this effect for behavior has not been established, but it does demonstrate that some long-term adaptive changes in reward circuitry can occur after repeated THC treatments.

In conclusion, these experiments show that mice appear resistant to behavioral sensitization of the locomotor-activating effects of THC. Even after systematic variation of several experimental parameters such as the frequency of THC treatments, exposure to the test apparatus during treatments, and manual vs automatic recording of activity, we failed to detect any sensitization. Furthermore, an experiment in rats using a published protocol also failed to demonstrate sensitization. It appears that this phenomenon may only occur under very particular conditions, raising doubts about its relevance to chronic cannabis users. At the very least, it can be concluded that the phenomenon appears much less robust after repeated cannabinoid exposure compared to psychostimulants or opiates. Given the difficulty encountered in this study and others in showing behavioral sensitization, direct evaluation of neural sensitization of mesolimbic dopamine systems after repeated THC exposures with biochemical or electrophysiological techniques may be required to adequately address this issue.

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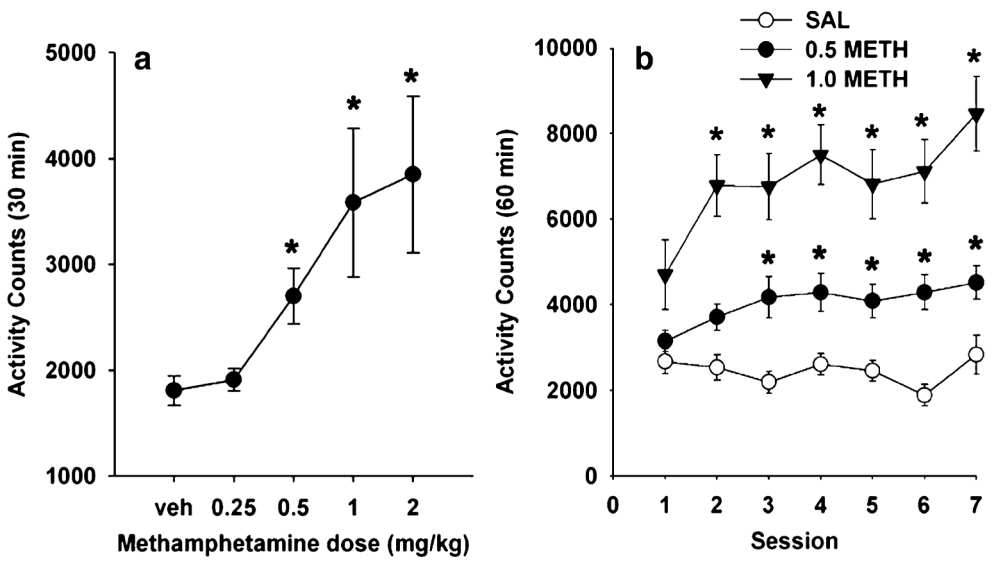

Fig. 1.

Methamphetamine acutely increased locomotor activity (**a**), and sensitization developed to this effect when mice were treated and tested once every 3–4 days (**b**). Values reflect mean±SEM. *Asterisks* indicate significant differences from veh (**a**) or from the first test (**b**), $p<0.05$. N=8–14 per group (**a**), N=10–12 per group (**b**)

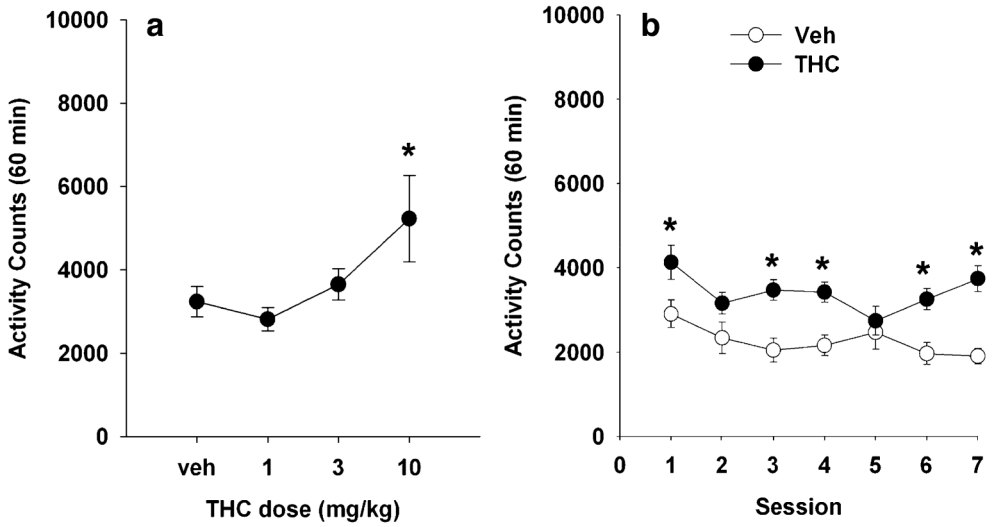

Fig. 2.

THC acutely increased locomotor activity (**a**), and intermittent repeated treatments of 10 mg/kg THC (once every 3–4 days) did not lead to sensitization of this effect (**b**). Values reflect mean±SEM. *Asterisks* indicate significant differences from veh (**a**) and differences between THC and veh on a given day (**b**). *N*=6 per group (**a**), *N*=10 per group (**b**)

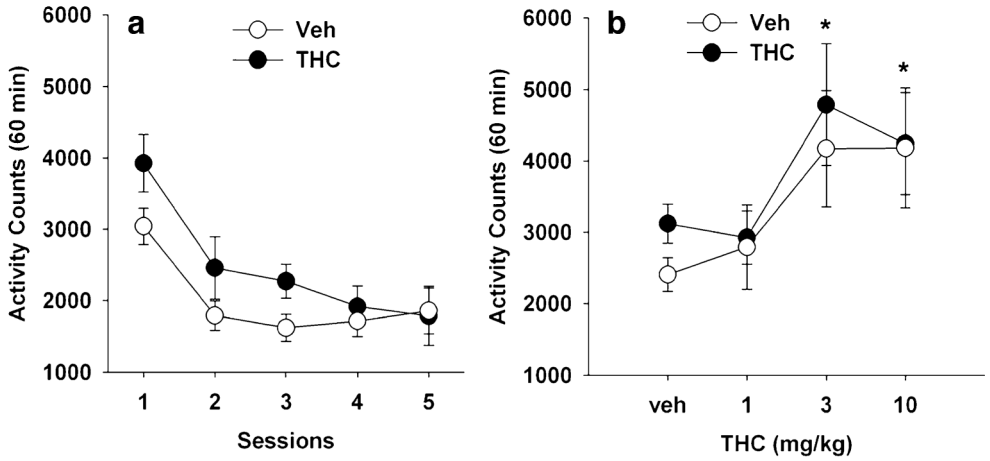

Fig. 3.

No evidence of sensitization to the effects of THC. Mice were treated twice daily with 10 mg/kg THC or veh and assessed for one hour after each morning injection (**a**). Another group of mice was treated with the same regimen for 6 1/2 days in their home cages. Twenty-one days after treatments ended mice were treated acutely with THC, and activity was assessed for 1 hour (**b**). *Asterisks* indicate significant differences from veh when the two pretreatment groups were combined, $p<0.05$. Values reflect mean±SEM. $N=10–12$ per group (**a**), $N=5–6$ per group (**b**)

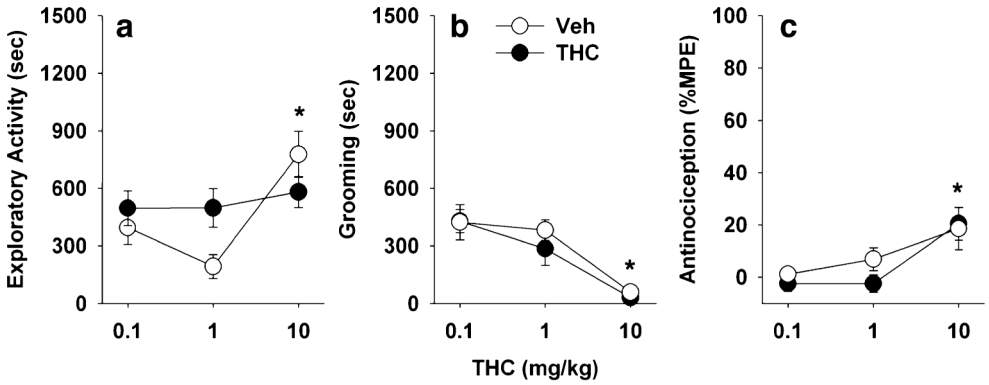

Fig. 4.

Mice were treated once per day with veh or 10 mg/kg THC in their home cages for 5 days. Fourteen days after their last treatment, mice were treated acutely with THC, and behavior was scored for 30 min, followed by the tail-flick antinociception test. THC acutely increased exploratory activity in the veh-treated but not THC-treated (**a**) and reduced grooming behaviors in both groups (**b**). A small antinociceptive effect of 10 mg/kg THC was also observed in both groups (**c**). Values reflect mean±SEM. *Asterisks* indicate significant differences from veh in the veh-treated group (**a**) or differences from veh with both groups combined (**b** and **c**), *N*=7–9 per group

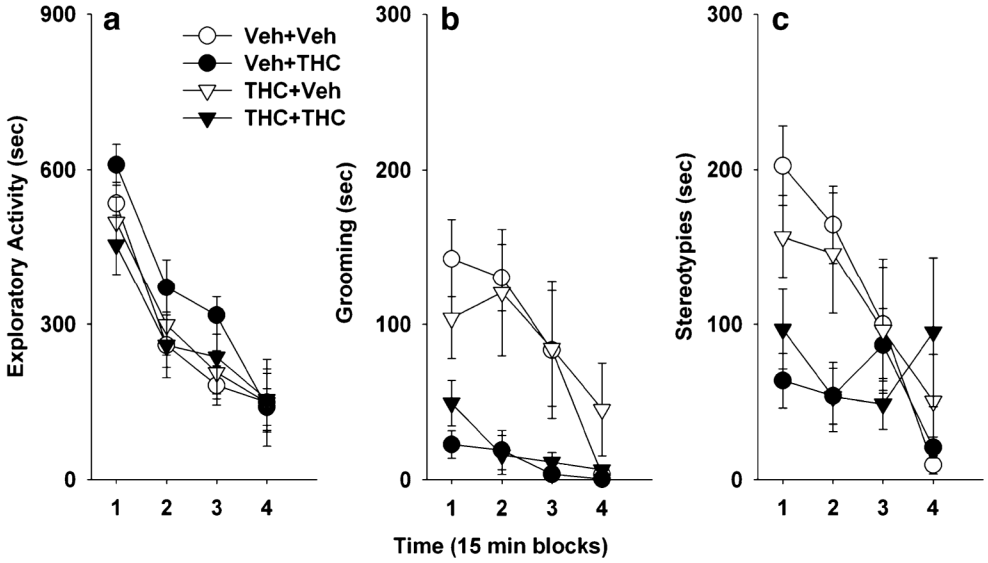

Fig. 5.

No sensitization in Sprague—Dawley rats. Rats were treated in their home cages twice daily with an escalating regimen of THC (5, 10, 20, 40, and 40 mg/kg) or vehicle for 5 days. Twenty days later, rats from both groups received either veh or 5 mg/kg THC, and their behavior was scored for 60 min. The total time spent in exploration (**a**), grooming (**b**), and exhibiting stereotyped behaviors (**c**) are presented as group means±SEM. *N*=8 per group