Research report

Chronic WIN55,212-2 elicits sustained and conditioned increases in intracranial self-stimulation thresholds in the rat

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1. Introduction

Dependence-producing substances with a high abuse potential that produce euphoric states in humans are believed to affect reinforcement and reward mechanisms by actions on certain areas and pathways of the brain, which are collectively known as brain reward systems. In most cases, these drugs also yield reinforcing and rewarding effects in experimental animals, as assessed by relevant animal models, such as self-administration, conditioned place preference and intracranial self-stimulation (ICSS) [29].

Although marijuana is considered to be one of the oldest and most widely used and abused drugs, our knowledge and understanding of the manner it acts in the brain to exert its reinforcing/rewarding effects are far from complete. Animal studies using several behavioral paradigms and under various experimental conditions have shown that Δ\textsubscript{9}-tetrahydrocannabinol (Δ\textsubscript{9}-THC) and other synthetic cannabinoid agonists can induce both appetitive and aversive effects (for reviews, see [7,9,10,14,15,21,31,33]).

Intracranial self-stimulation (ICSS) has been suggested to be a valid approach for studying the rewarding/reinforcing properties of various drugs of abuse [38]. Indeed, drugs with reinforcing properties in animals and addictive potential in humans tend to facilitate the reinforcing effects of brain stimulation in rats, i.e., to lower thresholds for rewarding brain stimulation (ICSS thresholds), shifting to the left the function that relates response strength to stimulation strength. On the other hand, drugs that have a negative impact on reward and reinforcement of behavior typically increase ICSS thresholds shifting the function to the right [4,38].

Interestingly, there are contradictory reports on the effects of cannabinoids on ICSS. Gardner and colleagues found that Δ\textsubscript{9}-THC decreases thresholds for ICSS primarily in Lewis rats [8,13], whereas other studies have found no effect or increased thresholds for ICSS following acute administration of Δ\textsubscript{9}-THC or synthetic cannabinoid agonists in other rat strains [1,2,12,32,34–36]. These discrepancies, which are likely due to methodological differences, remain to be resolved.

A possible explanation for the failure to find a clear reward enhancing effect of cannabinoids in the ICSS model is that the first exposure(s) to the cannabinoid may produce severe dysphoric actions, which may mask the rewarding effects of the drug.

Interestingly, the effects of chronic cannabinoid administration on ICSS have not been examined. Such a study would allow us to track the cannabinoid effects over time, and this information might provide insight into the mechanisms that contribute to the abuse potential of cannabis preparations.
2. Methods

2.1. Animals and surgery

Male Sprague–Dawley rats (n = 21) weighting 300–350 g at the time of surgery were used. Before surgery they were housed in groups of three and maintained on a 12 h light–12 h dark cycle with free access to food and water. The animals were anaesthetized with intramuscular (i.m.) injection of ketamine hydrochloride (100 mg/kg) and xylazine (10 mg/kg). Atropine sulphate (0.6 mg/kg, i.m.) was injected to reduce bronchial secretion. The animals were implanted with a monopolar stimulation electrode aimed at the medial forebrain bundle (MFB) at the level of lateral hypothalamus (2.56 mm posterior to bregma, 1.8 mm lateral from midsagittal suture, and 8.6 below the outer flat skull), according to Paxinos and Watson [23]. The electrodes were constructed from 0.25 mm stainless-steel wire insulated with Epoxyline except for the conically shaped tip. The anode was an uninsulated stainless-steel wire connected to an amphenol pin. Five miniature skull screws, the electrode and the anode were secured to the skull with acrylic dental cement. Following implantation and for the entire duration of the experiments, the animals were housed individually.

Experiments were conducted in accordance with “Principles of laboratory animal care” (NIH publication No. 86-23, revised 1985).

2.2. Apparatus and procedures for self-stimulation

After one-week recovery, the rats were tested for self-stimulation in an operant chamber made of transparent Plexiglas (25 cm wide, 25 cm deep and 30 cm high). Each chamber was equipped with a stainless-steel poke device (lever) 4 cm wide and protruded 2 cm from the left side at a height of 4 cm from the bottom. Each bar-press triggered a constant current stimulator (Med Associates, St. Albans, VT) that delivered a 0.4-s train of rectangular cathodal pulses of constant duration (0.1 ms) and intensity (250 μA) and variable frequency (15–100 Hz, i.e., 6–40 number of pulses/0.4 s). The pulse frequency, i.e., the number of pulses within a train, was progressively increased up to 40 per stimulation train until the subject showed vigorous self-stimulation. During the acquisition phase the animals were trained to self-stimulate using four alternating series of ascending and descending pulse frequencies. The pulse frequency was varied by steps of approximately 0.1 log units. For each trial, there was an initial “priming” phase during which the animals received three trains of stimulation at the frequency which was available for the specific trial. A rate–frequency determination session lasted about 45 min. One rate–frequency curve was established daily, for 10–14 days, depending on the period when the self-stimulation indices (i.e., curve-shift and threshold measure) were stable. The stimulation parameters, intracranial self-stimulation sessions and data collection were controlled by a computer. For a schematic representation of acquisition and training schedules for ICSS, see Fig. 1.

Since cannabinoids seem to affect performance capacity in a dose-dependent manner, the use of a reward selective measure, like the curve-shift, was requisite. In this method, plotting the responses of the animals against the various pulse frequencies yields a sigmoidal rate–frequency curve as shown in Fig. 2. Shifts in the lateral position of the curve provide a selective measure of stimulation-produced reward, as elegantly demonstrated by Edmonds and Gallistel [6], while vertical shifts provide information on motor/performance capacity. Furthermore, this method offers quantitative scaling of drug-induced changes in reward [3] that is useful when comparing the effects of different drugs. In other words, the rate–frequency method appears to have reward selectivity that is required in psychopharmacological research [17,19].

2.3. Drugs and drug administration

WIN55,212-2 mesylate (Tocris, Westwoods Bus. Park Ellisville, U.S.A.) was dissolved in 5% dimethyl sulphoxide (DMSO) and 5% cremophor in 0.9% NaCl and injected intraperitoneally (i.p.) at a volume of 3 ml/kg of body weight.

In previous studies, we had found that a single injection of WIN55,212-2 at the 3 mg/kg, but not at the 1 mg/kg, dose raised ICSS thresholds [34]. For this reason, we
conducted a pilot study under the present experimental conditions that includes 5 days of ICSS testing with all the animals receiving vehicle and found that even the 1 mg/kg dose administered on the 6th day raised ICSS thresholds. Adaptation to the experimental conditions, such as that most likely occurring with animals receiving vehicle for 5 days in the present setting, has been shown to affect the behavioral responses to the cannabinoid receptor agonists Δ2-THC and WIN55,212-2. For example, Pollissidis et al. [24] showed that low doses of these compounds increased locomotion only under habituated conditions, whereas high doses decreased locomotion only under non-habituated conditions. To this end, we speculate that the 5 days of vehicle administration, ICSS testing and further habituation to the experimental procedures rendered the animals more susceptible to the anhedonic effects of WIN55,212-2. Based on the present finding, we continued the study, using the 1 mg/kg dose as the highest dose for the chronic regimen, given that we sought to investigate if changes in ICSS thresholds occurred in either direction after chronic administration.

2.4. Experimental procedure

Rats were trained in the ICSS procedure until stable thresholds were achieved. The criterion for stability was met when the frequency thresholds did not vary by more than 0.1 log units. Rats were then tested daily for 5 consecutive days in order to evaluate the baseline threshold. Immediately after the first ICSS session (preinjection) the animals were removed from the ICSS chamber and injected with vehicle. Rats were returned to the ICSS chamber and thresholds were assessed 10 min later (postinjection threshold). The mean of the first 4 days of vehicle administration (pre- and post-injection) was considered as baseline, while the response on the 5th day was considered as control (B5). After these 5 days, animals were injected with vehicle [n = 5] or WIN55,212-2 [0.1 [n = 5], 0.3 [n = 5] or 1 mg/kg [n = 6]), returned to the ICSS chamber and thresholds were assessed 10 min later. During this phase, pre- and post-injection thresholds were assessed for 20 consecutive days (testing phase). Thereafter, WIN55,212-2 was substituted with vehicle and thresholds were assessed for 5 more days (washout phase). For a schematic representation of the experimental procedures, see Fig. 1.

2.5. Data analysis and statistics

The analysis was performed on two aspects of data obtained from the rate–frequency curve: the intracranial self-stimulation (ICSS) threshold and the maximum rate of responding or asymptote. [18]. Threshold and asymptote values at B5, testing and washout phases were expressed as percentage of baseline values. Data were initially analyzed using two-way analysis of variance (ANOVA) with repeated measures followed whenever appropriate by one-way ANOVAs or one-way ANOVAs with repeated measures and correlated t-test using Bonferroni’s adjustment for multiple comparisons.

2.6. Histology

At the end of the experiment, the animals were given a lethal dose of sodium pentothal. The location of the terminal stimulation site was then marked according to the following procedure: a direct anodal current of 0.1 mA and 15 s duration was passed through the electrode tip. The animals were perfused intracardially with 10% formalin for 3 days, and 2 days in a formalin solution. The brains were then removed and stored in 10% formalin for 3 days, and 2 days in a 30% sucrose solution. Finally, the brains were sliced in a cryostat microtome and the sections containing the electrode tract were mounted on slides and stained with cresyl violet. Only the rats in which tracks from the electrode were verified to be located in the MFB were included in this study (n = 21).

3. Results

In the testing phase (Fig. 2), two-way ANOVA with repeated measures performed on the changes in self-stimulation thresholds showed a statistical significant drug effect [F(3,17) = 14.848, p < 0.001], but neither significant interaction of time and drug nor time effect, indicating neither tolerance nor sensitization. Furthermore, paired-samples t-test indicated statistical significant difference between the groups receiving 1 mg/kg and vehicle (p < 0.001) across all 20 days of administration. In contrast, 0.1 and 0.3 mg/kg of WIN55,212-2 did not induce statistically significant effects. To the contrary with the changes in self-stimulation thresholds, two-way ANOVA with repeated measures on the asymptotic rate of responding data showed no statistically significant drug, time or interaction of drug and time effects (p > 0.05).

In the washout phase (Fig. 3), two-way ANOVA with repeated measures on the changes in ICSS thresholds showed a statistical significant interaction of time and drug [F(15,85) = 1.800, p < 0.01]. One-way ANOVA indicated no differences between groups at B5. One-way ANOVAs on days 1–3 indicated significant differences between groups [F(3, 17) = 5.658, F(2, 3, 17) = 14.519, F(3, 17) = 13.637, p < 0.01 for each day]. Paired-samples t-tests indicated statistical significant differences only between the groups administered with 1 mg/kg and vehicle on days 1–3 (p < 0.05 for each day). One-way ANOVAs on days 4 and 5 indicated no significant differences. However, two-way ANOVA with repeated measures on the asymptotic rate of responding showed no statistically significant drug, time or interaction of drug and time effects (p > 0.05). Fig. 4 depicts rate–frequency functions from representative animals obtained before and after administration of WIN55,212-2 and vehicle from the days 1 and 20 of the testing phase and the day 1 of the washout phase. As indicated in the figure WIN55,212-2 administration produced a parallel curve-shift to the right, indicating a clear decrease in the reinforcing efficacy of the stimulation.

4. Discussion

ICSS thresholds remained stable and unaltered when measured before each daily injection of vehicle or WIN55,212-2 over the
group in their previous studies also used Lewis rats, which in general appear to be more sensitive and vulnerable to the effects of addictive drugs [8]. Although one can speculate that the reward facilitating effect of cannabinoids on ICSS may only be obtained in certain strains of rat, suggesting an important genetic component in this action, other studies have shown that ∆9-THC produces aversion in the conditioned place preference paradigm in the same strain of rats [22]. Secondly, in the study by Lepore et al. [13], ∆9-THC could only shift the rate–frequency function to the left by approximately 0.05 log units. Interestingly, these authors adopt a very strict criterion of stable responding (i.e., 0.01 log units for three consecutive days), which increases the likelihood that the observed effect of ∆9-THC in their study may reflect normal baseline variation over days. To the contrary, in our study the criterion of stable responding was defined within 0.1 log units over three consecutive days, in agreement with most other studies utilizing the ICSS paradigm (see, e.g., [2]).

Although in the present study we did not assess the role of CB1 receptors in the inhibitory effects of WIN55,212-2 on ICSS, we have previously shown that CB1 receptors do indeed mediate the observed increases in ICSS thresholds after acute WIN55,212-2 [34]. It appears worthwhile to examine the role of specific CB1 receptor stimulation in the sustained and conditioned anhedonia in response to repeated administration of a non-selective cannabinoid receptor agonist, such as the WIN55,212-2, that is observed here for the first time.

According to our results, vehicle- and WIN55,212-2-administered rats did not present any statistical significant differences in the maximum rate of responding, and chronic administration of WIN55,212-2 did not affect the asymptotic rate of responding (see Fig. 2). Thus, the increased postinjection ICSS thresholds observed after each WIN55,212-2 (1 mg/kg) injection during the testing phase seem to be independent of motor impairment, and the effects of chronic WIN55,212-2 on ICSS thresholds were not confounded by performance effects. This is consistent with previous reports on homologous findings with acute administration of CB1 receptor agonists [34,35].

Pharmacological studies have shown that chronic administration of cannabinoids, such as ∆9-tetrahydrocannabinol and WIN55,212-2, produces cannabinoid receptor desensitization and down-regulation, as well as tolerance to some of the cannabinoid-mediated effects, such as hypothermia, antinociception and hypothermia [20,26–28,30,37]. Our behavioral observations are not congruent with the aforementioned behavioral and biochemical findings. That is, ICSS thresholds increased after the first injection of WIN55,212-2 and remained stable across the 20 days of drug treatment. We speculate that the effects of chronic cannabinoids on brain reward function may be independent from changes in sensitivity, number or function of central cannabinoid receptors. Alternatively, downstream effects elicited by chronic administration of cannabinoids may counteract any ensuing change in cannabinoid receptors resulting in a sustained decrease in ICSS reward.

Interestingly, in the washout phase, in which the WIN55,212-2 injection was substituted with a vehicle injection and thresholds were assessed for 5 more days, the postinjection threshold was significantly increased compared with preinjection threshold. This effect was statistically significant for the days 1, 2 and 3 of the group that received 1 mg/kg WIN55,212-2 during the testing phase. This finding demonstrates that after being repeatedly paired with WIN55,212-2 administration, a previously neutral vehicle injection increased ICSS threshold in a manner similar to that induced by WIN55,212-2 administration. A similar phenomenon in the washout (withdrawal) phase, although in the opposite direction considering the testing phase, has been described by Markou and co-workers after repeated cocaine administration [11].

![Rate–frequency functions taken from representative animals. Each plot represents data from a single animal under pre-drug (filled circles) and drug (open circles) conditions. Rate–frequency functions were obtained by logarithmically decreasing the frequency of the stimulation pulses from a value that sustained maximal lever pressing to one that failed to sustain lever pressing.](image-url)
The conditioned increase in ICSS thresholds observed in the present study probably reflects an aversive conditioning phenomenon (i.e., conditioned anhedonia). It has become increasingly apparent that some drug effects can be elicited by environmental cues or neutral stimuli that have been repeatedly paired with drug administration. That is, certain drug effects can be conditioned. The contribution of such drug-predictive environmental cues or neutral stimuli can be explained by the Pavlovian conditioning model. Thus, in our study, cannabinoid-associated conditioned stimuli can inhibit brain reward function, as indicated by the increased ICSS phenomenon (i.e., conditioned anhedonia). It has become increasingly clear that cannabinoids by acting as discriminative stimuli may play an important role in maintaining cannabinoid self-administration in a subpopulation of cannabis-dependent individuals, by acting as discriminative stimuli and affect/drive subsequent cannabinoid administration or relapse to drug-taking behavior.

In conclusion, repeated administration of CB1 receptor agonist WIN55,212-2 did not increase the reinforcing efficacy of the stimulation. To the contrary, the highest dose increased ICSS threshold, indicating a clear anhedonic effect, which did not undergo tolerance or sensitization but appeared stable with chronic administration. The increase in ICSS thresholds produced by cannabinoid-predictive stimuli may play an important role in maintaining cannabinoid self-administration in a subpopulation of cannabis-dependent individuals, by acting as discriminative stimuli.

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References