Research report

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

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Abstract

The present study sought to examine whether repeated administration of the CB1 receptor agonist WIN55,212-2 affected intracranial self-stimulation (ICSS) behavior and induced phenomena of tolerance or sensitization, similar to typical addictive drugs. Rats received intraperitoneal injections of vehicle for 5 days, vehicle or WIN55,212-2 (0.1, 0.3 or 1 mg/kg) for 20 subsequent days, and vehicle for 5 additional days. Thresholds for ICSS were measured before and after each injection. The initial five injections of vehicle did not affect ICSS thresholds. WIN55,212-2 (1 mg/kg) significantly increased ICSS thresholds from the first day of administration, an effect that remained stable across the subsequent days of administration. During the 5 additional days, where WIN55,212-2 was substituted with vehicle, rats demonstrated a conditioned increase in postinjection thresholds that was significant the first 3 days of this period. These findings indicate that repeated WIN55,212-2 administration elicited a sustained increase in ICSS, i.e., phenomena of tolerance or sensitization were not observed. The present data demonstrate cannabinoid-predictive stimuli that may gain affective salience and play an important role in maintaining cannabinoid administration.

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 Δ9-tetrahydrocannabinol (Δ9-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 Δ9-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 Δ9-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.
Hence, the purpose of the present study was to investigate whether repeated administration of the CB1 receptor agonist WIN55,212-2 affected ICSS behavior in the rat. Since acute administration of WIN55,212-2 has been shown to increase ICSS thresholds, our study was designed to assess not only the effects of daily WIN55,212-2 injections on brain stimulation reward, but also whether a conditioned stimulus associated with chronic WIN55,212-2 would affect brain reward function. To this end, we used a protocol invented by Markou and co-workers that assessed the effects of repeated cocaine administration on ICSS and potential, associated, conditioned phenomena [11].

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 the hypothalamus (2.56 mm anterior to bregma, 1.4 mm lateral from mid sagittal suture, and 8.6 below the outer flat skull), according to Paxinos and Watson [22]. The electrodes were constructed from 0.25 mm stainless-steel wire insulated with Epoxyit 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 pole 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 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 for at least three consecutive days (1 h daily), using stimulation parameters that maintained near maximal barpressing rates. After self-stimulation has been acquired and stabilized for a given pulse frequency, rats 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.

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 plotted the responses of the animals against the various pulse frequencies yielded 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 1 mg/kg, i.p. 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.

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 environmental 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 Δ^2^-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 habitation 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 r-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 saline 0.9%, which was followed by a 50 cc solution of potassium ferrocyanide (3%). 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, 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 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 statistically 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(1, 3, 17) = 5.658, F2(3, 17) = 14.519, F3(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).

4. Discussion

ICSS thresholds remained stable and unaltered when measured before each daily injection of vehicle or WIN55,212-2 over the
whereas in the Fisher strain of rats there was no effect. The same
pared to Sprague-Dawley rats, in which the effect was marginal,
acute regimens[1,2,12,32,34–36]. Our results differ from those of

tolerance or sensitization to this effect over a course of administra-
preinjection baseline the next day, and there was no evidence of
receptor agonist WIN55,212-2 transiently increased ICSS thresh-
maximal lever pressing to one that failed to sustain lever pressing.

30 consecutive days of testing. Furthermore, there was no differ-
ence between pre- and post-injection ICSS thresholds during the
first 5 consecutive days of the baseline phase. However, postin-
jection ICSS thresholds were increased after each WIN55,212-2
injection during the testing phase. This effect was significant for
the highest dose of WIN55,212-2 (1 mg/kg), and the magnitude of
this increase did not vary significantly across the 20 consecutive
days of drug administration. These findings indicate that the CB1
receptor agonist WIN55,212-2 transiently increased ICSS thresh-
hold after each daily injection, whereas ICSS thresholds returned to
preinjection baseline the next day, and there was no evidence of
tolerance or sensitization to this effect over a course of administra-

These findings clearly indicate that chronic WIN55,212-2 did
not facilitate ICSS, but rather increased the ICSS thresholds, a finding
that is in agreement with several previous reports utilizing
acute regimens [1,2,12,32,34–36]. Our results differ from those of
previous studies by Gardner’s group [8,13], whereby acute Δ9-
THC decreased ICSS threshold. These contrasting results could be
attributed to procedural differences. For example, Lepore et al. [13]
found the most pronounced action of Δ9-THC in Lewis rats, com-
pared to Sprague-Dawley rats, in which the effect was marginal,
whereas in the Fisher strain of rats there was no effect. The same
group in their previous studies also used Lewis rats, which in gen-
eral 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 compo-
nent in this action, other studies have shown that Δ9-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], Δ9-
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 Δ9-THC in their study may reflect normal baseline vari-
ation over days. To the contrary, in our study the criterion of stable
responding was defined within 0.1 log units over three consecu-
tive 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 cannabi-
noid 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 adminis-
tration of cannabinoids, such as Δ9-tetrahydrocannabinol and
WIN55,212-2, produces cannabinoid receptor desensitization and
down-regulation, as well as tolerance to some of the cannabino-
mediated effects, such as hypoactivity, 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 adminis-
tration 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 injec-
tion 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
coworkers after repeated cocaine administration [11].

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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 thresholds. Interestingly, exogenous cannabinoid ligands, such as Δ9-tetrahydrocannabinol (THC), have previously shown to exert conditioned place avoidance responses (see, e.g., [5,16,25]). The present data demonstrate cannabinoid-predictive stimuli that may gain affective salience and plausibly act 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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