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RESEARCH PAPER

Cannabinoid CB_1 and CB_2 receptor mechanisms underlie cannabis reward and aversion in rats

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Background and Purpose: Endocannabinoids are critically involved in brain reward functions, mediated by activation of CB_1 receptors, reflecting their high density in the brain. However, the recent discovery of CB_2 receptors in the brain, particularly in the midbrain dopamine neurons, has challenged this view and inspired us to re-examine the roles of both CB_1 and CB_2 receptors in the effects of cannabis.

Experimental Approach: In the present study, we used the electrical intracranial self-stimulation paradigm to evaluate the effects of various cannabinoid drugs on brain reward in laboratory rats and the roles of CB_1 and CB_2 receptors activation in brain reward function(s).

Key Results: Two mixed CB_1 / CB_2 receptor agonists, Δ^9-tetrahydrocannabinol (Δ^9-THC) and WIN55,212-2, produced biphasic effects—mild enhancement of brain-stimulation reward (BSR) at low doses but inhibition at higher doses. Pretreatment with a CB_1 receptor antagonist (AM251) attenuated the low dose-enhanced BSR, while a CB_2 receptor antagonist (AM630) attenuated high dose-inhibited BSR. To confirm these opposing effects, rats were treated with selective CB_1 and CB_2 receptor agonists. These compounds produced significant BSR enhancement and inhibition, respectively.

Conclusions and Implications: CB_1 receptor activation produced reinforcing effects, whereas CB_2 receptor activation was aversive. The subjective effects of cannabis depend on the balance of these opposing effects. These findings not only explain previous conflicting results in animal models of addiction but also explain why cannabis can be either rewarding or aversive in humans, as expression of CB_1 and CB_2 receptors may differ in the brains of different subjects.

1 | INTRODUCTION

Marijuana or cannabis has now been legalized in many states of the United States, although it is still unclear whether cannabis is entirely safe (Schulden, Thomas, & Compton, 2009). In humans, cannabis produces "paradoxical" effects that are often diametrically opposed. For instance, cannabis is well known for its ability to produce euphoria, pleasure, and relaxation (Fattore, Fadda, Spano, Pistis, & Fratta, 2008; Maldonado, Valverde, & Berrendero, 2006; Parsons & Hurd, 2015). However, not all users enjoy cannabis, and some experience dysphoria, anxiety, and depression after its use (D'Souza et al., 2004; Raft, Gregg, Ghia, & Harris, 1977). Even in the same person, cannabis may produce positive effects at one time but negative effects at another (Farris, Zvolensky, Boden, & Bonn-Miller, 2014; Gregg, Small, Moore, Raft, & Toomey, 1976). Similar paradoxical effects of **Δ^9-tetrahydrocannabinol** (Δ^9-THC, the major psychoactive

Abbreviations: ACEA, arachidonyl-2′-chloroethylamide; BSR, brain-stimulation reward; ICSS, intracranial self-stimulation; JWH 133, (6*aR*,10*aR*)-3-(1,1-Dimethylbutyl)-6*a*,7,10,10*a*-tetrahydro-6,6,9-trimethyl-6*H*-dibenzo[*b*,*d*]pyran; NAc, nucleus accumbens; WIN55,212-2, (*R*)-(+)-[2,3-Dihydro-5-methyl-3-(4-morpholinylmethyl)pyrrolo[1,2,3-*de*]-1,4-benzoxazin-6-yl]-1-naphthalenylmethanone; Δ^9-THC, Δ^9-tetrahydrocannabinol

component of cannabis; Gaoni & Mechoulam, 1971) have been found in non-human primates. Specifically, Δ^9-THC is self-administered by squirrel monkeys (Justinova, Tanda, Redhi, & Goldberg, 2003; Tanda, Munzar, & Goldberg, 2000), suggesting that it has rewarding effects, but it is not self-administered in rhesus monkeys (John, Martin, & Nader, 2017; Mansbach, Nicholson, Martin, & Balster, 1994). In rodents (laboratory rats and mice), Δ^9-THC or other cannabinoid compounds can be rewarding, ineffective or aversive (Panagis, Vlachou, & Nomikos, 2008; Vlachou & Panagis, 2014). For example, Δ^9-THC has been reported to facilitate electrical intracranial brain-stimulation reward (BSR; Gardner et al., 1988; Katsidoni, Kastellakis, & Panagis, 2013; Lepore, Liu, Savage, Matalon, & Gardner, 1996), while other groups and/or studies found depression of BSR (Kwilasz & Negus, 2012; Negus & Miller, 2014; Vlachou, Nomikos, Stephens, & Panagis, 2007; Wiebelhaus et al., 2015). Conflicting findings have also been reported in studies using conditioned place preference and intravenous self-administration (Panagis, Vlachou, & Nomikos, 2008; Vlachou & Panagis, 2014). The neurobiological mechanisms underlying such paradoxical effects are poorly understood.

With the identification of cannabinoid **CB_1** and **CB_2** receptors as the major targets of cannabinoids (Matsuda, Lolait, Brownstein, Young, & Bonner, 1990; Munro, Thomas, & Abu-Shaar, 1993) and the finding that CB_1 receptors are highly expressed in the CNS and CB_2 receptors are expressed predominantly in peripheral tissues, it has generally been thought that the neurobehavioural and psychotropic effects of cannabinoids are *mediated by activation of CB_1 receptors not CB_2 receptors* (Mackie, 2005). This hypothesis is supported by electrophysiological and neurochemical evidence demonstrating that activation of CB_1 receptors on **GABA**ergic neurons may increase midbrain dopaminergic neuron activity in the ventral tegmental area (VTA) by **dopamine** neuron disinhibition (Lupica & Riegel, 2005; Szabo, Siemes, & Wallmichrath, 2002) and that Δ^9-THC increases dopamine release in the nucleus accumbens (NAc) as assessed by in vivo microdialysis in rats (Chen, Paredes, Lowinson, & Gardner, 1991; Tanda, Pontieri, & Di Chiara, 1997; although *cf*. Castaneda, Moss, Oddie, & Whishaw, 1991). However, there is no direct behavioural evidence in vivo demonstrating whether a CB_1 receptor-dependent mechanism underlies cannabis reward. Moreover, we have recently reported that activation of CB_1 receptors in glutamatergic neurons by Δ^9-THC produces aversive effects (Han et al., 2017).

In addition to CB_1 receptors, growing evidence indicates that CB_2 receptors are also expressed in the brain although the level is much lower than CB_1 receptors in healthy subjects (Onaivi et al., 2006, 2008). Immunohistochemistry and in situ hybridization assays detect CB_2 receptor-immunostaining or CB_2 receptor mRNA in various brain regions (Aracil-Fernandez et al., 2012; Ashton, Friberg, Darlington, & Smith, 2006; Baek, Zheng, Darlington, & Smith, 2008; Brusco, Tagliaferro, Saez, & Onaivi, 2008; Gong et al., 2006; Liu et al., 2009, 2017; Schmidt, Schafer, Striggow, Frohlich, & Striggow, 2012; Stempel et al., 2016; Van Sickle et al., 2005; Zhang et al., 2019). Notably, CB_2 receptors were recently identified in VTA dopaminergic neurons (Zhang et al., 2014, 2017, 2019) and dopaminergic terminals in the NAc (Foster et al., 2016), two critical brain regions involved in drug reward and addiction. Activation of CB_2 receptors in both brain regions inhibits VTA dopaminergic neuron activity and NAc dopamine release (Xi et al., 2011; Zhang et al., 2014, 2017). In addition, overexpression of brain CB_2 receptors inhibits **cocaine** self-administration and cocaine-enhanced locomotion in mice (Aracil-Fernandez et al., 2012). These findings suggest that CB_2 receptors may also be involved in cannabis reward or aversion.

In the present study, we used the electrical intracranial self-stimulation (ICSS) paradigm to evaluate the effects of various cannabinoid ligands on ICSS and explored the roles of CB_1 and CB_2 receptors in these actions. Specifically, we used a wide range of doses of Δ^9-THC, as well as the synthetic mixed CB_1/CB_2 receptor agonist **WIN55,212-2** and then extended the findings using individual selective CB_1 and CB_2 receptor agonists, as well as pretreatment with selective antagonists.

What is already known

- Cannabis can produce both positive and negative effects in different subjects or at different times.
- The neurobehavioural effects of cannabinoids are generally thought to be mediated by activation of CB_1 receptors.

What this study adds

- In rats, CB_1 receptor activation produces reinforcing effects, whereas CB_2 receptor activation is aversive.
- These differential effects may explain the previous conflicting results of Δ^9-THC treatment in animals.

What is the clinical significance

- The subjective effects of cannabis may depend on the balance of opposing CB_1 and CB_2 receptor effects.

2 | METHODS

Animals

All animal care and experimental procedures outlined in the animal research protocol were approved by the Animal Care and Use Committee of the National Institute on Drug Abuse of the U.S. National Institutes of Health under approved animal use protocol 07-BNRB-47 and were carried out in compliance with applicable U.S. Federal and Maryland state laws and regulations. Animal studies are reported in compliance with the ARRIVE guidelines (Kilkenny et al., 2010) and with the recommendations made by the *British Journal of Pharmacology*. Adult male Long–Evans rats (Charles River Laboratories, Raleigh, NC; RGD Cat# 2308852, RRID:RGD_2308852), 300–325 g at the

time of surgery, were used. Animals were housed individually post-surgery in a climate-controlled environment (70~74°F, humanity 40–50%, reverse 12 h light/dark cycle) with food (TestDiet, St. Louis, MO, USA) and water freely available with the exception of the time spent each day in the test chambers.

2.1 | Surgery

Under 60 mg·kg^{-1} **sodium pentobarbital** anaesthesia, rats were surgically implanted with a unilateral monopolar stainless steel stimulating electrode (Plastics One, Roanoke, VA) targeted at the medial forebrain bundle at the level of the lateral hypothalamus (stereotaxic coordinates from bregma: AP + 2.5 mm, ML + 1.7 mm, and DV − 8.4 mm). A wire wrapped around a jeweller's screw implanted in the skull and connected to a mini-pin in the electrical connector at the top of the electrode was used to accommodate return electrical current. The electrodes were cemented to the skull with acrylic resin cement. Each animal was kept warm and under observation until all effects of the anaesthetic had dissipated. Rats were monitored closely and allowed a minimum of 7 days to recover, prior to the start of experiments.

2.2 | ICSS apparatus

All training and testing occurred in standard operant chambers (MED Associates, Georgia, VT), each of which contained a retractable wall-mounted lever and a cue light immediately above the lever. The operant chambers were enclosed in ventilated, sound-attenuating cabinets. Depression of the operant lever activated a brain stimulator.

2.3 | General ICSS procedure

The general BSR procedures were as reported previously (Pak et al., 2006; Spiller et al., 2008; Xi et al., 2007, 2008). Briefly, rats were allowed to self-train to lever press for BSR. Each press on the operant lever resulted in a 500-ms train of 0.1-ms rectangular cathodal pulses through the electrode, followed by a 500-ms "timeout" in which further presses did not produce brain stimulation. The initial stimulation parameters were 72 Hz and 200 mA. If the animal did not learn to lever press, the stimulation intensity was increased daily by 50 mA until the animal learned to press (45–60 responses per 30 s) or a maximum of 800 mA was reached. Animals (three of 50 rats) that did not lever press at 800 mA or in which the stimulation produced unwanted effects (e.g., head or body movements or vocalization) were removed from the experiment.

2.4 | Rate-frequency ICSS procedure

Following establishment of lever pressing for BSR, animals were presented with a series of 16 different pulse frequencies, ranging from 141 to 25 Hz in descending order. At each pulse frequency, animals responded for two 30-s time periods, with the mean number of lever responses recorded as the response rate. Between frequencies, the lever retracted for 5 s. Animals were run for three sessions per day; within each session, animals were run twice on the full range of stimulation frequency over a 40-min trial. The first session was a "warm up," the second session was the baseline session, and the third session was the test session. The BSR threshold (θ_0) was defined as the minimum frequency at which the animal responded for rewarding stimulation. Y_{max} was defined as the maximal rate of lever responding. The BSR threshold (θ_0) and Y_{max} were mathematically derived for each baseline run and each test session run by analysing each rate-frequency BSR function generated by a given animal over a given descending series of pulse frequencies using best-fit mathematical algorithms as reported previously (Spiller et al., 2009; Xi, Gilbert, et al., 2006; Xi, Newman, et al., 2006).

2.5 | Testing the effects of Δ^9-THC, WIN55,212-2, AM251, AM630, ACEA, or JWH 133 on BSR

Once a baseline value was achieved (<10% variation over five continuous days), the rats were randomly divided into five experimental groups and treated with different test compounds (Table 1) to assess the effects of Δ^9-THC, cocaine, WIN55,212-2, **AM251**, **AM630**, **ACEA**, or **JWH 133** on BSR. All animals were injected, between the baseline and test BSR sessions with an i.p. injection of sterile water, 0.5% Tween-80 or tocrisolve vehicle (i.e., the 0 mg·kg^{-1} dose in each group) or one of various doses of test compounds. Thirty minutes after test compound injection, the test sessions began. After each test, animals received an additional 5–7 days of BSR re-stabilization until a new baseline θ_0 was established. The order of testing of various drug

TABLE 1 Experimental groups and the drug treatments in each group of rats

Group #	Test drugs	Treatment (mg·kg^{-1})[a]
1	Δ^9-THC (n = 14)	Δ^9-THC (0, 0.3, 1, 3, 5), (AM251 + THC), (AM630 + THC)
2	WIN55,212-2 (n = 11)	WIN (0, 0.3, 1, 3), (AM251 + WIN), (AM630 + WIN)
3	ACEA (n = 8)	ACEA (0, 0.1, 0.3, 1), (AM251 + ACEA), (AM630 + ACEA)
4	JWH 133 (n = 7)	JWH (0, 3, 10, 20), (AM251 + JWH), (AM630 + JWH), Δ^9-THC (1)
5	Cocaine (n = 7)	Cocaine (0, 3), Δ^9-THC (1), AM251 (0, 1, 3), AM630 (0, 1, 3, 10)

[a]The order of testing for the various drug doses in each group was counterbalanced according to a Latin square design.

doses was counterbalanced according to a Latin square design. To monitor potential drug effects on motor behaviour, the maximum rate of lever pressing (Y_{max}) was measured, and any treatment that altered this significantly in either direction was eliminated from the study (see also Section 2.8).

2.6 | Testing the effects of AM251 or AM630 pretreatment on drug-enhanced or drug-inhibited BSR

For pretreatment studies, rats were injected, between the baseline and test BSR sessions, with 0.5% Tween-80 vehicle, AM251 (3 mg·kg^{-1}), or AM630 (3 mg·kg^{-1}) 10 min prior to the second drug injection. Then, a dose of the second drug (Δ^9-THC, WIN55,212-2, ACEA, or JWH 133) was administered 30 min before the test session began. After each test, animals received an additional 3–7 days of BSR re-stabilization until a new baseline θ_0 was established.

2.7 | Locomotor activity

Four additional groups of rats (n = 8 each) were used to evaluate the locomotor effects of cannabinoid compounds. These additional drug-naive rats were placed in locomotor detection chambers (Accuscan, Columbus, OH) and habituated for 1 hr. Each group then randomly received one dose of Δ^9-THC (0, 1, 3, or 5 mg·kg^{-1} i.p.), WIN55,212-2 (0, 0.3, 1, or 3 mg·kg^{-1} i.p.), JWH 133 (0, 10, 20 mg·kg^{-1}), or ACEA (0, 0.3, 1, 3 mg·kg^{-1}). The Δ^9-THC and WIN55,212-2 groups of rats were also used to observe the effects of AM251 (3 mg·kg^{-1} i.p.) or AM630 (3 mg·kg^{-1} i.p.) on open-field locomotion. Following injection, locomotor activity was recorded for 2 hr in 10-min intervals. Each animal was tested three to five times with different drug doses in a counterbalanced manner. The time interval was 1–3 days between each test. Distance counts per 10 min bin (cm) were used to evaluate the effects of each cannabinoid compound on locomotion.

2.8 | Data and statistical analysis

The data and statistical analysis comply with the recommendations of the *British Journal of Pharmacology* on experimental design and analysis in pharmacology. Experiments showing biphasic effects on BSR by Δ^9-THC and WIN55,212-2 were performed in two independent groups of rats, with seven to 14 animals per group as shown in figure legends. All other experiments were performed once, with seven to 14 rats per treatment. Though the experimenter was not blinded to the animals' identity and treatment condition during data collection, the data were blinded during analyses. No data points were excluded from the analysis in any experiment. Data were checked for normality using the Shapiro–Wilk method and for equal variance by the Brown–Forsythe method. Statistical significance was determined using paired two-tailed t tests when comparing two groups, and one-way ANOVAs for repeated measures when comparing multiple groups, using SigmaPlot. For significant results by one-way ANOVA, all pairwise multiple comparisons were made using the Holm–Sidak method. A P value of less than 0.05 was considered significant.

2.9 | Materials

Δ^9-THC and cocaine (provided by the National Institute on Drug Abuse, Intramural Research Program, Baltimore, MD) were dissolved in sterile 0.5% Tween-80 (Sigma-Aldrich) and saline, respectively. WIN55,212-2, AM251, AM630, and ACEA (Tocris) were dissolved in sterile 0.5% Tween-80. JWH 133 (Tocris) was dissolved in Tocrisolve™ (Tocris Bioscience brand of Bio-Techne Corporation, Minneapolis, MN).

2.10 | Nomenclature of targets and ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY (Harding et al., 2018), and are permanently archived in the Concise Guide to PHARMACOLOGY 2017/18 (Alexander, Christopoulos et al., 2017).

3 | RESULTS

3.1 | Mixed CB_1 receptor/ CB_2 receptor agonists have biphasic effects on BSR

Systemic administration of a wide range of doses of Δ^9-THC produced biphasic effects (Figure 1a,b). A low dose of Δ^9-THC (1.0 mg·kg^{-1}) significantly enhanced BSR (i.e., reduced the minimum frequency at which the animal responded for rewarding stimulation) by 7–9%, while the highest dose tested (5.0 mg·kg^{-1}) significantly inhibited BSR by about 9%. No dose of Δ^9-THC affected the maximal operant response (Y_{max}; Figure 1a), suggesting no significant sedation or locomotor impairment by Δ^9-THC administration (see also Section 2.8).

Because there has been controversy over the effect of Δ^9-THC on reward, we treated a separate group of rats with the enhancing dose of Δ^9-THC as well as a low dose of the appetitive drug, cocaine, for comparison (Figure 1c). The facilitating effect of cocaine was roughly twice as much as that of Δ^9-THC, even at the dose of 2 mg·kg^{-1}, which is a moderately low dose compared to the doses of cocaine used to produce reward ICSS in other recent studies (Bauer, Banks, & Negus, 2014; Yang et al., 2017). The 1 mg·kg^{-1} dose of Δ^9-THC significantly facilitated ICSS in this second independent cohort of rats, relative to vehicle treatment (difference of means = 8.8%).

Similar to Δ^9-THC, systemic administration of the synthetic high affinity CB_1/CB_2 receptor agonist WIN55,212-2 (Compton, Gold, Ward, Balster, & Martin, 1992) produced biphasic effects (Figure 1d), with similar percent shifts. We also found that doses of

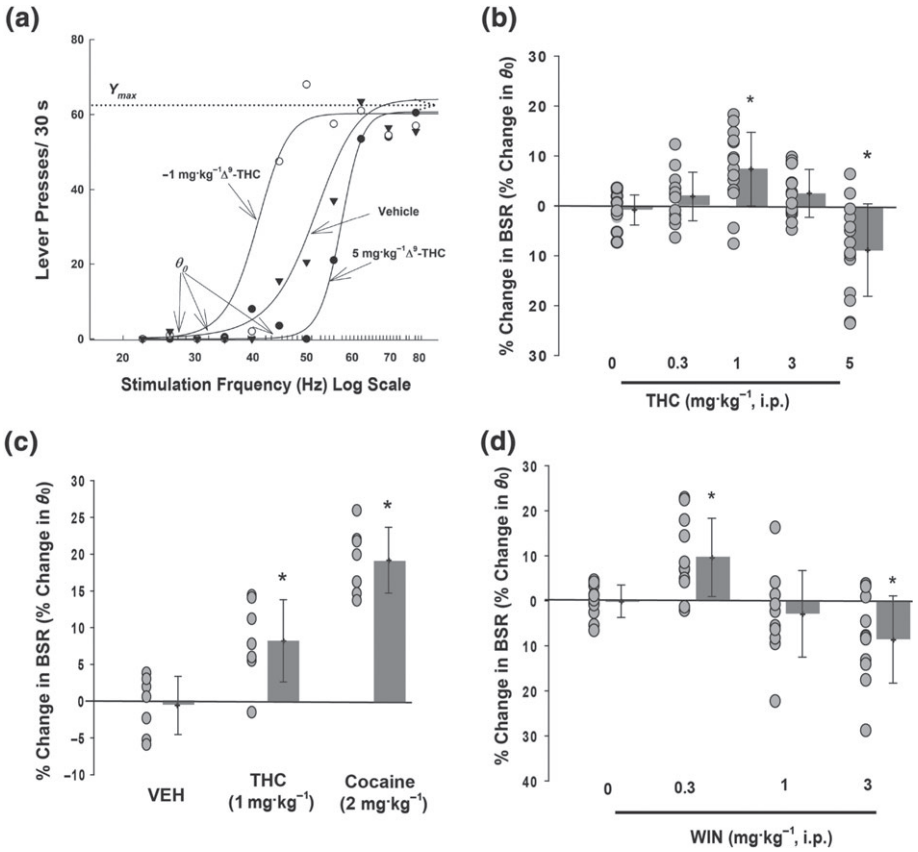

FIGURE 1 Biphasic effects of Δ^9-THC or WIN55,212-2 on electrical BSR —low doses enhance, whereas high doses inhibit BSR. (a) Representative stimulation-response curves, indicating that a low dose of Δ^9-THC (1 mg·kg^{-1} i.p.) shifted the stimulation-response curve to the left and decreased the stimulation threshold (θ_0) value, while a higher dose of Δ^9-THC (5 mg·kg^{-1} i.p.) significantly shifted the curve to the right and increased the stimulation threshold (θ_0). Δ^9-THC did not affect maximal operant responses (Y_{max} level) at any dose tested. (b) Summary of all Δ^9-THC doses tested, with both rewarding and aversive effects apparent as percentage enhancement or inhibition of θ_0 (n = 14, one-way ANOVA for repeated measures, $F_{4, 52}$ = 11.9). (c) The rewarding effects of low dose Δ^9-THC are only about half of those produced by 2 mg·kg^{-1} cocaine (n = 7, one-way ANOVA for repeated measures, $F_{2, 12}$ = 23.6). (d) The synthetic full CB_1/CB_2 receptor agonist WIN55,212-2 had similar effects to Δ^9-THC, wherein a low dose of WIN55,212-2 (0.3 mg·kg^{-1}) enhanced brain reward, and the highest dose tested (3 mg·kg^{-1}) inhibited brain reward (n = 11, $F_{3, 30}$ = 9.3). For all panels, individual data points are shown as black circles, with bars indicating group means ± SD shown to the right. *P < 0.05, significantly different from vehicle treatment groups

5 mg·kg^{-1} i.p. and above of WIN55,212-2 produced a sedative effect in the rats as assessed by a decrease in the maximum frequency of lever pressing (Y_{max}). Those animals were excluded from the study in order to differentiate treatment effects on reward from potentially confounding effects on motor function.

3.2 | Effects of Δ^9-THC or WIN55,212-2 on open-field locomotion

To further determine the potential involvement of locomotor effects, we observed the effects of the same doses of Δ^9-THC or WIN55,212-2 on open-field locomotion in rats. Δ^9-THC produced a trend towards reduction (not significant) in open-field locomotion (Figure 2a,b). However, systemic administration of WIN55,212-2 produced a significant, dose-dependent reduction in basal levels of locomotor activity (Figure 2c,d), suggesting locomotor depression or sedation. Post-hoc individual group comparisons revealed a significant reduction in locomotion only after 3 mg·kg^{-1} WIN55,212-2 administration (Figure 2d).

3.3 | Effects of selective CB_1 or CB_2 receptor antagonists on Δ^9-THC- or WIN-altered BSR

In order to understand the nature of the biphasic effects produced by these mixed CB_1/CB_2 receptor agonists, we next pretreated Δ^9-THC or WIN55,212-2 with the selective CB_1 or CB_2 receptor antagonists AM251 and AM630, respectively. We first confirmed that neither AM251 nor AM630 itself altered BSR or Y_{max} levels at the doses tested (AM251, 1 and 3 mg·kg^{-1} i.p. or AM630, 1, 3, and 10 mg·kg^{-1} i.p.; Figure 3).

We then selected doses in the middle of the ranges that have been previously shown to be effective in antagonizing CB_1 receptors

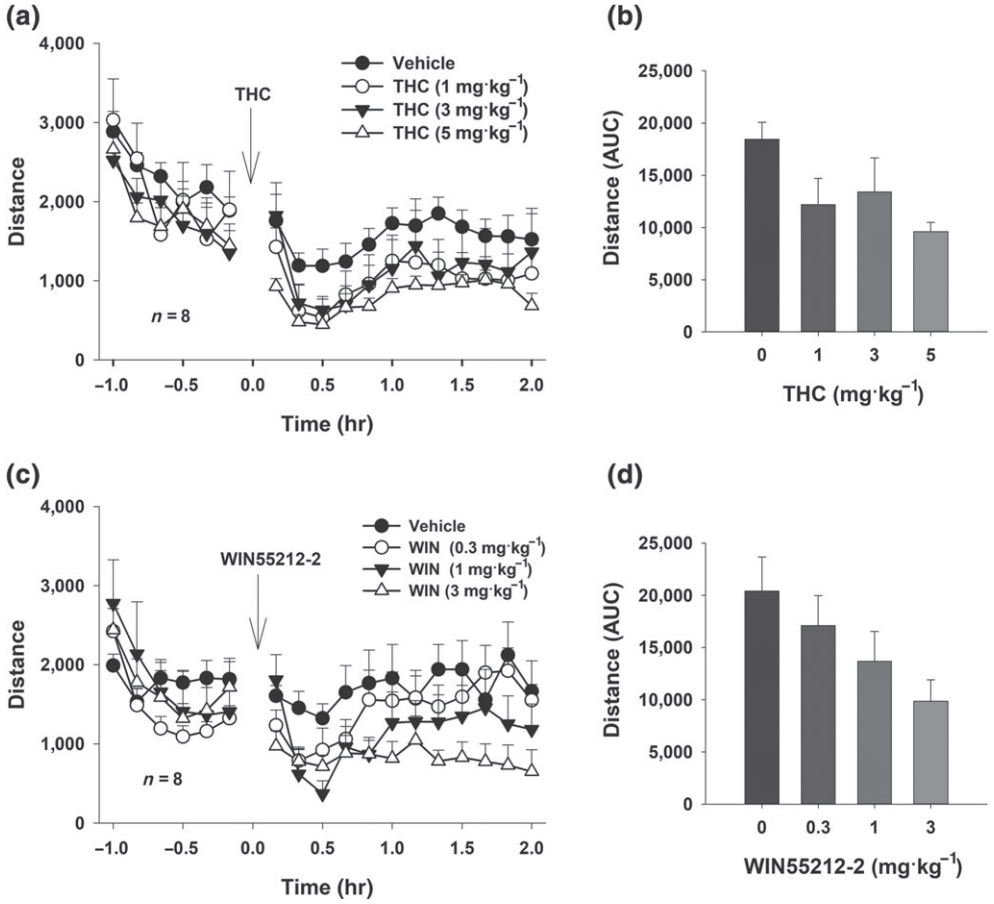

FIGURE 2 High doses of WIN55,212-2 decrease spontaneous locomotion. (a, b) The time course and AUC measurements after systemic administration of different doses of Δ^9-THC or vehicle in the open-field test revealed a significant time main effect (two-way ANOVA for repeated measures, $F_{11, 77} = 5.93$), but no significant Δ^9-THC treatment main effect ($F_{3, 21} = 2.30$) or Treatment × Time interaction ($F_{33, 231} = 0.39$). (c, d) In contrast, WIN55,212-2 administration produced a more pronounced impairment of spontaneous movement in the open-field test (two-way ANOVA for repeated measures revealed a significant Time main effect ($F_{11,77} = 6.35$), treatment main effect ($F_{3,21} = 7.08$), and Treatment X Time interaction ($F_{33,231} = 1.97$)), shown here by the time course for individual doses (c) and the AUC summary data (d)

(3 mg·kg^{-1} of AM251; Xi, Gilbert, et al., 2006) and CB_2 receptors (3 mg·kg^{-1} of AM630; Rahn et al., 2014). Pretreatment with AM251 10 min prior to a 1.0 mg·kg^{-1} Δ^9-THC injection moderately attenuated the Δ^9-THC-enhanced BSR, with a change in BSR facilitation from about 7.4% after the Vehicle + Δ^9-THC treatment to 2.1% after the AM251 + Δ^9-THC treatment (Figure 4a, left panel), although this change was not statistically significant. Given the low level of Δ^9-THC-enhancement in this group of rats, this result may have been due to a floor effect. However, compared to the vehicle control group, 1 mg·kg^{-1} Δ^9-THC-enhanced BSR was clearly blocked by AM251 (Figure 1b vs. Figure 4a). In contrast to Δ^9-THC, AM251 significantly blocked the 0.3 mg·kg^{-1} WIN-enhanced BSR (Figure 4b, left panel), from mean value of 10.5% to 1.2%. AM251 pretreatment had no effect on the inhibition of BSR by the 5.0 mg·kg^{-1} dose of Δ^9-THC (Figure 4a, right panel) or the 3 mg·kg^{-1} dose of WIN55,212-2 (Figure 4b, right panel). These data suggest that the enhancement of BSR by low doses of CB_1/ CB_2 receptor agonists is driven by action on the CB_1 receptor.

To assess the inhibitory effects on ICSS of high dose Δ^9-THC or WIN55,212-2, we pretreated the animals with the selective CB_2 antagonist AM630 (3 mg·kg^{-1} i.p., 10 min prior). The inhibition produced by both 5.0 mg·kg^{-1} Δ^9-THC (Figure 4c) and 3 mg·kg^{-1} WIN55,212-2 (Figure 4d) was attenuated to baseline levels by this pretreatment, with a 13% and 10% shift, respectively, compared to when the rats were pretreated with vehicle. AM630 did not alter the low dose facilitation of ICSS by either compound. Taken together, these results suggest that the biphasic effects of Δ^9-THC or WIN55,212-2 result from differential CB_1 or CB_2 receptor-mediated effects.

3.4 | CB_1 receptor activation is rewarding, whereas CB_2 receptor activation is dysphorogenic

To further investigate our hypothesis that it is the actions on different cannabinoid receptor subtypes that drive Δ^9-THC's and WIN55,212-2's biphasic effects on BSR in rats, we used selective agonists in different groups of rats. First, we treated rats with the highly selective CB_1 receptor agonist ACEA (Figure 5a, 0.1, 0.3, 1.0 mg·kg^{-1}) and found that this treatment produced only a monophasic enhancement of BSR, which was significantly attenuated by pretreatment with

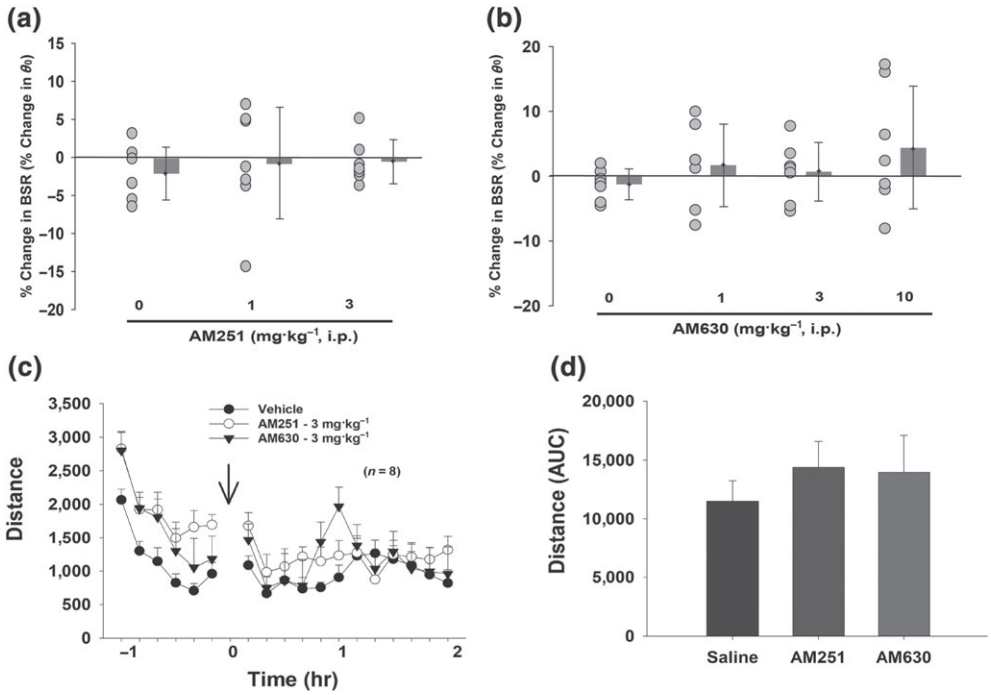

FIGURE 3 Neither AM251 nor AM630 produced a significant alteration in BSR (a, b) or open-field locomotion as assessed by the time course of locomotion (c) or the AUC after each drug administration (d) at any dose tested

AM251 (3 mg·kg^{-1}) but not by the CB_2 receptor antagonist AM630 (Figure 5b). Consistent with these data, the selective CB_2 receptor agonist, JWH 133, increased BSR threshold at 20 mg·kg^{-1} (Figure 5c) by about 14% from baseline. This inhibition was attenuated by AM630 (Figure 5d) but not AM251, confirming the CB_2 receptor specificity of this effect.

3.5 | Effects of ACEA and JWH 133 on open-field locomotion

Finally, we observed the effects of ACEA and JWH 133 on open-field locomotion. We found that systemic administration of the same doses of ACEA that enhanced BSR had no effect (Figure 6a,b), while JWH 133 produced a dose-dependent reduction, on open-field locomotion (Figure 6c,d). Post hoc individual group comparisons revealed a significant reduction in locomotion after 20 mg·kg^{-1} JWH 133 administration (Figure 6d).

4 | DISCUSSION

The major findings in the present study are that systemic administration of Δ^9-THC or the synthetic cannabinoid agonist WIN55,212-2 produced dose-dependent biphasic effects —lower doses enhanced, while high doses inhibited BSR, as assessed by electrical ICSS. The selective CB_1 receptor agonist, ACEA, produced a BSR-enhancing effect, while the selective CB_2 receptor agonist, JWH 133, produced a dose-dependent inhibition of BSR. The BSR-enhancing effect produced by low doses of Δ^9-THC, WIN55,212-2, or ACEA was blocked selectively by the CB_1 receptor selective antagonist, AM251, while the inhibition of BSR produced by high doses of Δ^9-THC or WIN55,212-2 or by JWH 133 was blocked by the selective CB_2 receptor antagonist AM630. Together, these data suggest that brain cannabinoid CB_1 and CB_2 receptors modulate brain reward function in opposite directions, that is, CB_1 receptor activation-producing enhancement and CB_2 receptor activation-producing inhibition of BSR.

It is well known that cannabis can be rewarding or aversive in both humans and experimental animals (Panagis, Vlachou, & Nomikos, 2008; Vlachou & Panagis, 2014). ICSS is a commonly used behavioural paradigm to study brain reward functions (Bauco & Wise, 1997; Peng et al., 2010; Wise, 1996). In this model, animals press a lever to deliver brief electrical pulses to a discrete brain region such as the VTA of the midbrain or the middle forebrain bundle via an implanted electrode. Most drugs of abuse such as cocaine, heroin, or nicotine lower the stimulation threshold for electrical BSR, indicating enhanced BSR and implying a summation between the BSR and the drug reward (Bauco & Wise, 1997; Peng et al., 2010). However, the effects of cannabinoids on BSR have been controversial. In some studies, Δ^9-THC produced a significant reduction in the electrical stimulation threshold in rats (Gardner et al., 1988; Lepore, Liu, Savage, Matalon, & Gardner, 1996), suggesting enhanced BSR. However, in other studies, Δ^9-THC or other cannabinoid agonists either had no effect on electrical BSR (Vlachou et al., 2007) or produced a reduction in electrical BSR (i.e., aversion) in rats (Katsidoni, Kastellakis, & Panagis, 2013; Vlachou, Nomikos, & Panagis, 2005,

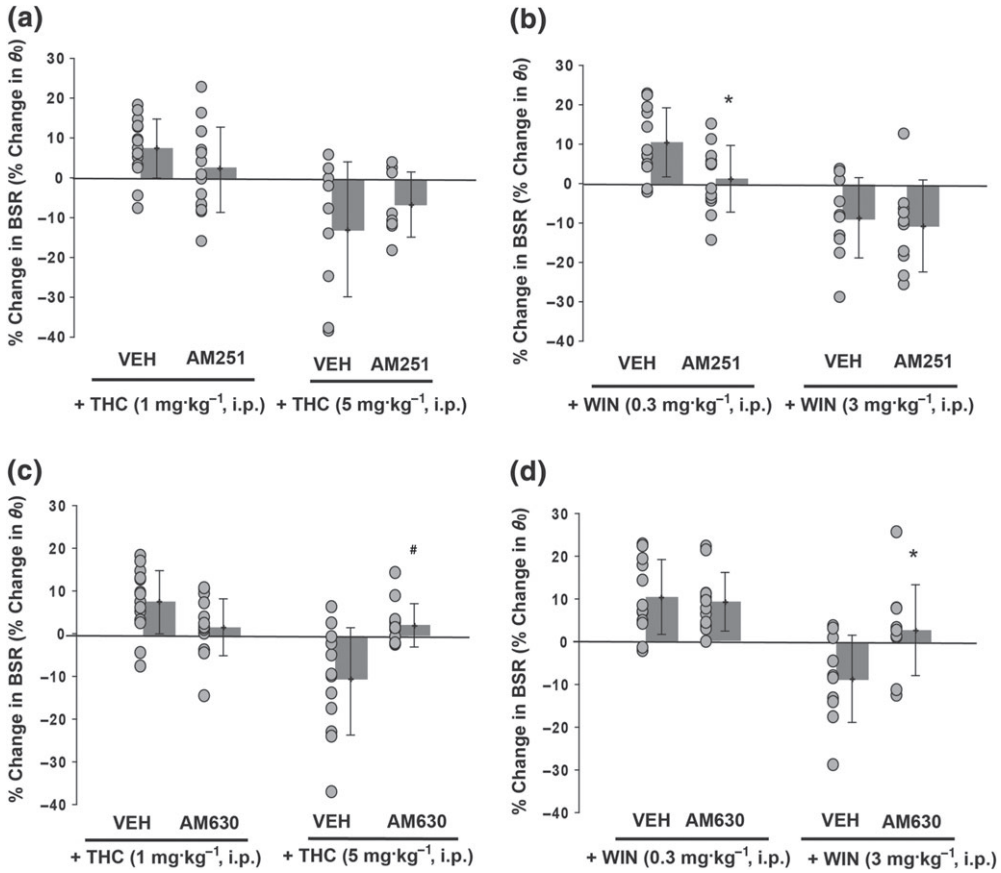

FIGURE 4 Pretreatment with AM251, a selective CB_1 receptor antagonist, blocks low dose WIN55,212-2-induced enhancement of BSR but not high dose-induced BSR inhibition. (a) Pretreatment with AM251 (3 mg·kg^{-1} i.p., 10 min prior to Δ^9-THC) appeared to attenuate Δ^9-THC-mediated enhancement and inhibition of BSR although paired *t* tests did not reveal significant differences. (b) Pretreatment with the same dose of AM251 significantly blocked WIN-enhanced BSR (*n* = 12) but not high dose WIN-induced inhibition of BSR (*n* = 10, paired *t* test). (c, d) Pretreatment with AM630, a selective CB_2 receptor antagonist, blocks high dose Δ^9-THC- or WIN55,212-2-mediated BSR inhibition. When AM630 (3 mg·kg^{-1} i.p.) was injected 10 min prior to Δ^9-THC (c) or WIN55,212-2 treatment (d), the high dose BSR inhibition was significantly attenuated (5 mg·kg^{-1} Δ^9-THC, *n* = 12, paired *t* test 3 mg·kg^{-1} WIN55,212-2, *n* = 10). For all panels, individual data points are shown as black circles, with bars indicating group means ± SD shown to the right. **P* < 0.05, significantly different from vehicle + WIN treatment; #*P* < 0.05, significantly different from vehicle + Δ^9-THC group

2006). An important finding in the present study is that the hedonic effects of cannabis or cannabinoids depend on a drug dose—lower doses are rewarding, while higher doses are aversive. This may in part explain the previous conflicting findings regarding cannabis actions in humans and experimental animals.

We note that our results on the enhancing effects of low dose Δ^9-THC differ from some previous studies showing Δ^9-THC-induced inhibition (Kwilasz & Negus, 2012; Negus & Miller, 2014; Vlachou et al, 2007; Wiebelhaus et al., 2015). This could be related to smaller sample sizes used in these studies. For example, Vlachou and colleagues tested all Δ^9-THC doses (0, 0.5, 1 or 2 mg·kg^{-1} i.p.) using only five animals (Vlachou et al., 2007). The power analysis performed for the present study suggests at least *n* = 7 are needed to detect an 8% change in BSR with a power of 0.79 (α = 0.05). Assuming that the Vlachou et al. study had similar low levels of variability between rats as in the present study, a sample size of five would be underpowered to detect an 8% change.

Nevertheless, because of the negative findings in those previous studies, we repeated the low dose Δ^9-THC treatment in three independent groups of rats (see Table 1, *n* = 7–14). We found the same moderate but significant enhancement in all groups with low dose Δ^9-THC treatment. This is further supported by a similar level of enhancement after treatment with the CB_1 receptor agonist, ACEA. Our results also fit well with observations in other animal models of drug reward, in which CB_1 receptor agonists increase the motivational and reinforcing effects of alcohol, nicotine, and opiates, whereas diminished CB_1 receptor signalling diminishes the rewarding effects of these drugs (Parsons & Hurd, 2015). In all of the figures presented in the present study, we show individual data points for each comparison to allow for greater future reproducibility.

Another important finding in the present study is that different receptor mechanisms may underlie cannabis reward versus aversion. This is supported by several lines of evidence. First, the selective CB_1 receptor agonist, ACEA, enhanced electrical BSR, an effect that

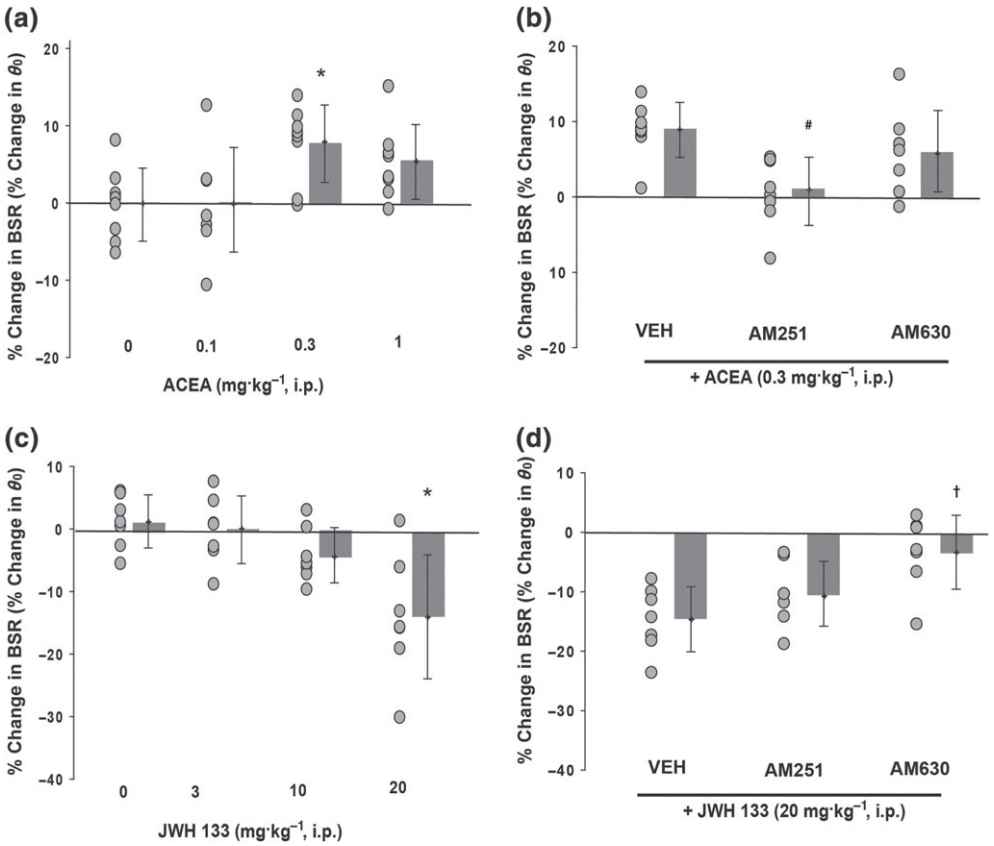

FIGURE 5 Effects of selective CB_1 or CB_2 receptor agonists on electrical brain-stimulation reward. (a) ACEA, a selective CB_1 receptor agonist, produced a significant enhancement in BSR (one-way ANOVA for repeated measures, $F_{3, 21} = 4.6$, $n = 8$). (b) Pretreatment with AM251 (3 mg·kg^{-1}) but not AM630 (3 mg·kg^{-1}) blocked ACEA-enhanced BSR ($n = 8$, one-way ANOVA for repeated measures, $F_{2, 14} = 11.8$). (c) JWH 133, a selective CB_2 receptor agonist, dose-dependently inhibited BSR ($n = 7$, $F_{3, 27} = 12.5$). (d) Pretreatment with AM630 (3 mg·kg^{-1}) but not AM251 (3 mg·kg^{-1}) blocked 20 mg·kg^{-1} JWH 133-induced BSR inhibition ($n = 7$, $F_{2, 12} = 9.5$). For all panels, individual data points are shown as black circles, with bars indicating group means ± SD shown to the right. *$P < 0.05$, significantly different from vehicle; #$P < 0.05$, significantly different from vehicle + ACEA treatment; †$P < 0.05$, significantly different from VEH + JWH 133 treatment

was blocked by the selective CB_1 receptor antagonist AM251 but not by the selective CB_2 receptor antagonist, AM630. Second, the selective CB_2 receptor agonist, JWH 133, dose-dependently inhibited electrical BSR, an effect that was selectively blocked by AM630 not by AM251. Third, the BSR-enhancing effect produced by low doses of WIN55,212-2 was also blocked by AM251 not AM630, while the BSR-suppressing effect produced by higher doses of Δ^9-THC or WIN55,212-2 was blocked by AM630 not AM251. We note that the BSR-enhancing effect produced by low dose Δ^9-THC appeared to be reduced by both AM251 and AM630 (Figure 4a,c). This may be related to the fact that (a) Δ^9-THC-enhanced BSR is moderate (~7%) and marginally significant and (b) brain levels of CB_2 receptors are much lower than those of CB_1 receptors. Thus, AM630 may also bind to brain CB_2 receptors to affect Δ^9-THC-enhanced BSR to a certain extent. Compared to Δ^9-THC, WIN55,212-2 produced more potent biphasic effects on BSR, which were blocked by AM251 and AM630, respectively. WIN55,212-2 also produced more potent locomotor reduction than Δ^9-THC. The mechanisms underlying the different pharmacological efficacies or potencies of Δ^9-THC and WIN55,212-2 on BSR and locomotion are unclear. They may be related to different receptor binding profiles—Δ^9-THC may act as a CB_1/CB_2 receptor partial agonist, while WIN55,212-2 may act as a CB_1/CB_2 receptor full agonist (Paronis, Nikas, Shukla, & Makriyannis, 2012; Pertwee, 2010; Tai & Fantegrossi, 2017). Whatever the mechanisms, the present findings with both Δ^9-THC and WIN55,212-2 suggest that activation of CB_1 receptors is rewarding, while activation of CB_2 receptors is aversive. This means that the final subjective effect of cannabis depends on the balance of two opposite actions on brain reward function. Individual differences in brain CB_1 receptor and CB_2 receptor expression may in part explain why cannabis is rewarding in some subjects but aversive in others. These findings may also relate to our previous reports that both CB_1 receptor antagonists and CB_2 receptor agonists produce inhibitory effects on cocaine self-administration and reinstatement of drug-seeking behaviour (Xi, Gilbert, et al., 2006; Xi et al., 2008, 2011; Zhang et al., 2014, 2015). Similarly, overexpression of CB_2 receptors in the brain inhibits cocaine self-administration and attenuates cocaine-induced locomotor sensitization (Aracil-Fernandez et al., 2012). Consistent with these findings, several recent reports indicate that CB_1 receptors

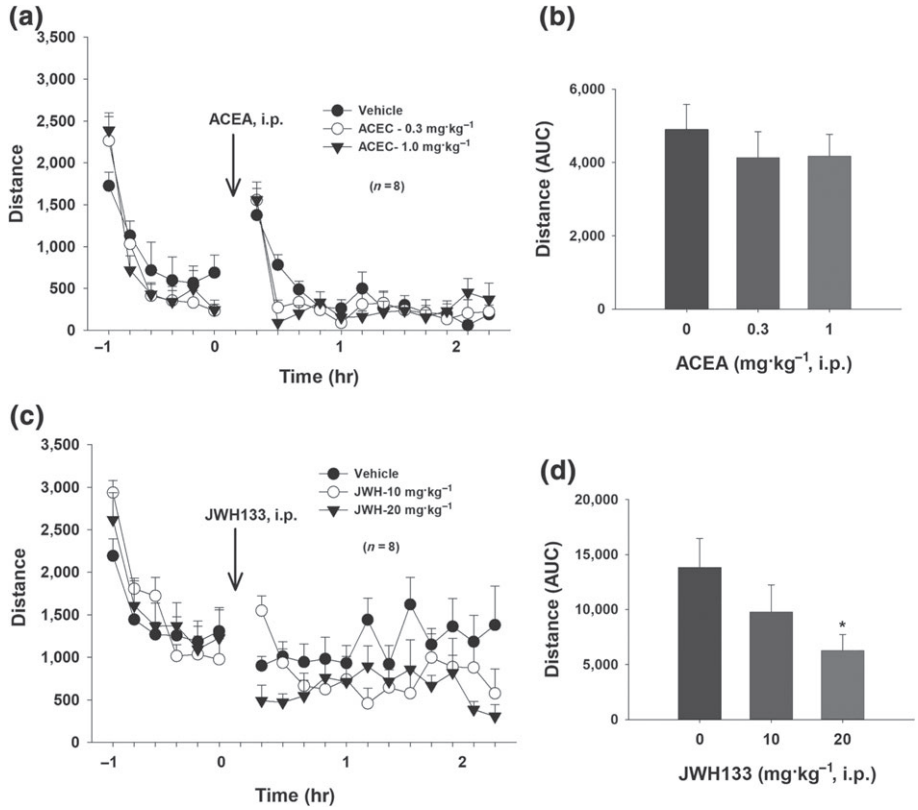

FIGURE 6 Effects of ACEA and JWH 133 on open-field locomotion. (a) Time courses of basal levels of locomotor activity after systemic administration of different doses of ACEA or vehicle, illustrating that ACEA did not significantly alter open-field locomotor activity. A two-way ANOVA for repeated measures over time and drug dose revealed a significant time main effect ($F_{11, 77}$ = 5.93, P < 0.001) but no significant ACEA treatment main effect ($F_{3, 21}$ = 2.230) or Treatment × Time interaction ($F_{33, 231}$ = 0.38). (b) The AUC data from (a) after ACEA administration. A one-way ANOVA for repeated measures over Δ^9-THC dose failed to show a significant treatment main effect ($F_{3, 21}$ = 2.30). (c) Time course of the effects of JWH 133 on open-field locomotion. A two-way ANOVA for repeated measures over time and drug dose revealed a significant treatment main effect ($F_{2,14}$ = 6.74), time main effect ($F_{11, 77}$ = 1.68), and Treatment × Time interaction ($F_{22,154}$ = 2.86). (d) AUC data from (c) after JWH 133 administration. A one-way ANOVA for repeated measures over dose revealed a significant JWH 133 treatment main effect ($F_{2,14}$ = 6.74). *P < 0.05, significantly different from vehicle group

and CB_2 receptors may play opposing roles in modulating cocaine's action, e.g., CB_2 receptor agonism exerting behavioural effects similar to those of CB_1 receptor antagonism on acquisition and expression of cocaine-induced conditioned place preference, cocaine-induced locomotion, cocaine-induced c-Fos expression and MAPK expression (Delis et al., 2017; Garcia-Cabrerizo & Garcia-Fuster, 2016). Such differential CB_1 versus CB_2 receptor effects may also partially explain some of the difficulty in parsing the neurological effects of cannabis use (Filbey et al., 2014).

We note that, while WIN55,212-2 or JWH 133 did produce a significant reduction in open-field locomotion in a dose-dependent manner, neither Δ^9-THC nor ACEA produced such effects, suggesting possible involvement of locomotor suppression in high dose WIN55,212-2- or high dose JWH 133-inhibited BSR. Although we cannot completely exclude it, such a possibility could be low since WIN55,212-2 or JWH 133, at the same high doses, did not alter maximal operant lever responses (Y_{max}). In addition, we have previously reported that JWH 133, at the same high doses (10 and 20 mg·kg^{-1}) did not alter cocaine self-administration under fixed ratio 1 schedule of reinforcement but produced an increase in break point (maximal lever response to receive a drug infusion) for cocaine self-administration under progressive ratio schedule of reinforcement in rats (Zhang et al., 2015). These findings suggest that in the presence of drug or BSR, animals still worked very hard to get the reward and displayed high motivation to overcome drug-induced locomotor inhibition or sedation to get reward.

The cellular mechanisms underlying CB_1 receptor-mediated reward are not fully understood. We have recently reported that CB_1 receptor mRNA is expressed in VTA GABAergic and **glutamatergic** neurons in mice (Han et al., 2017), which may project to VTA dopaminergic neurons and modulate dopaminergic neuron activity (Lupica, Riegel, & Hoffman, 2004). CB_1 receptor-mediated inhibition of VTA GABAergic neurons may disinhibit dopaminergic neuron activity, producing reward-enhancing effects, while CB_1 receptor inhibition of VTA glutamatergic neurons may be aversive by decreasing VTA glutamate release and thereby decreasing dopaminergic neuronal activity (Han et al., 2017). We have hypothesized that the hedonic effect of CB_1 receptor activation may depend on the net

effect of these two opposing actions (Han et al., 2017). In the present study, we found that activation of CB_1 receptors was rewarding in rats. This would be congruent with a supposition that more CB_1 receptors are expressed in VTA GABAergic neurons or GABAergic afferents or that CB_1 receptor-mediated GABAergic disinhibition of VTA dopaminergic neurons is dominant when animals are exposed to low loses of cannabis or Δ^9-THC. Conversely, at high doses, cannabis or Δ^9-THC may activate CB_1 receptors on VTA glutamatergic neurons or glutamatergic afferents, producing aversive or reward-depressing effects.

In addition to CB_1 receptors, CB_2 receptors are found in VTA dopaminergic neurons in both rats and mice (Zhang et al., 2014, 2017). Given that activation of CB_2 receptors inhibits dopaminergic neuronal activity in the VTA, dopamine release in the NAc (Foster et al., 2016; Xi et al., 2011; Zhang et al., 2014, 2017), and dopamine-related behaviours such as intravenous cocaine self-administration, cocaine-induced conditioned place preference, and cocaine-induced hyperactivity (Delis et al., 2017; Liu et al., 2017; Xi et al., 2011), we believe that the aversive or reward-depressing effects produced by the selective CB_2 receptor agonist, JWH 133, or high doses of Δ^9-THC or WIN55,212-2 are mediated at least in part by direct activation of CB_2 receptors on VTA dopaminergic neurons.

In conclusion, CB_1 receptor activation produces reinforcing effects, whereas CB_2 receptor activation is aversive. These opposing effects may not only explain the conflicting findings in previous ICSS studies but also explain why cannabis is rewarding or aversive in different subjects under different circumstances.

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AUTHOR CONTRIBUTIONS

K.S., E.L.G., and Z.-X.X. designed the experiments. K.S., G.-H.B., E.G., and H.Y. conducted the experiments. K.S., G.-H.B., E.G., and Z.-X.X. analysed the data and made the figures. K.S. and Z.-X.X. wrote the manuscript. E.L.G. revised the manuscript.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

DECLARATION OF TRANSPARENCY AND SCIENTIFIC RIGOUR

This Declaration acknowledges that this paper adheres to the principles for transparent reporting and scientific rigour of preclinical research as stated in the *BJP* guidelines for Design & Analysis, and Animal Experimentation, and as recommended by funding agencies, publishers and other organisations engaged with supporting research.

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