Δ^9 -Tetrahydrocannabinol and Endocannabinoid Degradative Enzyme Inhibitors Attenuate Intracranial Self-Stimulation in Mice[§]

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ABSTRACT

A growing body of evidence implicates endogenous cannabinoids as modulators of the mesolimbic dopamine system and motivated behavior. Paradoxically, the reinforcing effects of Δ^9 -tetrahydrocannabinol (THC), the primary psychoactive constituent of cannabis, have been difficult to detect in preclinical rodent models. In this study, we investigated the impact of THC and inhibitors of the endocannabinoid hydrolytic enzymes fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase (MAGL) on operant responding for electrical stimulation of the medial forebrain bundle [intracranial self-stimulation (ICSS)], which is known to activate the mesolimbic dopamine system. These drugs were also tested in assays of operant responding for food reinforcement and spontaneous locomotor activity. THC and the MAGL inhibitor JZL184 (4-[bis(1,3-benzodioxol-5-yl)hydroxymethyl]-1-piperidinecarboxylic acid 4-nitrophenyl ester) attenuated operant responding for ICSS and food, and also reduced spontaneous locomotor activity. In contrast, the FAAH inhibitor PF-3845 (*N*-3-pyridinyl-4-[[3-[[5-(trifluoromethyl)-

2-pyridinyl]oxy]phenyl]methyl]-1-piperidinecarboxamide) was largely without effect in these assays. Consistent with previous studies showing that combined inhibition of FAAH and MAGL produces a substantially greater cannabimimetic profile than single enzyme inhibition, the dual FAAH-MAGL inhibitor SA-57 (4-[2-(4-chlorophenyl)ethyl]-1-piperidinecarboxylic acid 2-(methylamino)-2-oxoethyl ester) produced a similar magnitude of ICSS depression as that produced by THC. ICSS attenuation by JZL184 was associated with increased brain levels of 2-arachidonoylglycerol (2-AG), whereas peak effects of SA-57 were associated with increased levels of both *N*-arachidonylethanolamine (anandamide) and 2-AG. The cannabinoid receptor type 1 receptor antagonist rimonabant, but not the cannabinoid receptor type 2 receptor antagonist SR144528, blocked the attenuating effects of THC, JZL184, and SA-57 on ICSS. Thus, THC, MAGL inhibition, and dual FAAH-MAGL inhibition not only reduce ICSS, but also decrease other reinforced and nonreinforced behaviors.

Introduction

Cannabis remains the most commonly used illicit drug for decades (SAMSHA, 2012). Marijuana users report positive subjective ratings (Hart et al., 2001, 2010), and marijuana-dependent individuals presented with marijuana cues show activation of reward-related brain regions [e.g., ventral tegmental

area (VTA) and amygdala] (Filbey et al., 2009; Goldman et al., 2013). Although cannabis acts as a behavioral reinforcer in humans, preclinical studies examining the rewarding or reinforcing effects of its primary psychoactive constituent, Δ^9 -tetrahydrocannabinol (THC), have yielded mixed results (Vlachou and Panagis, 2014).

THC produces the majority of its central nervous system-mediated behavioral effects through the activation of cannabinoid receptor type 1 (CB₁) (Wiley et al., 1995; Compton et al., 1996). Similarly, the endocannabinoids *N*-arachidonylethanolamine [anandamide (AEA)] and 2-arachidonoylglycerol (2-AG) bind and activate cannabinoid receptors (Devane et al., 1992; Mechoulam et al., 1995; Sugiura et al., 1995), but they are rapidly degraded by the respective enzymes fatty acid amide hydrolase (FAAH) (Cravatt et al., 1996, 2001) and monoacylglycerol lipase (MAGL) (Dinh et al., 2002). The endogenous cannabinoid system has been implicated in reward processes (Vlachou and Panagis, 2014) and has been shown to modulate neural substrates of reinforced behavior, including the mesolimbic dopamine system (Oleson and Cheer, 2012).

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THC and AEA facilitate dopamine release in the nucleus accumbens (NAc) in rats (Chen et al., 1990, 1991; Tanda et al., 1997; Cheer et al., 2004; Solinas et al., 2006; Oleson et al., 2012) and mice (Robledo et al., 2007). These findings are consistent with the idea that CB₁ receptor stimulation enhances reinforced behavior mediated by the mesolimbic dopamine system. However, the effects of THC on intracranial self-stimulation (ICSS), a reinforced behavior mediated through activation of the mesolimbic pathway, have been mixed. Some studies report that THC attenuated ICSS or had no effect (Stark and Dews, 1980; Vlachou et al., 2007; Kwilasz and Negus, 2012), whereas others indicate THC facilitated ICSS under a narrow range of conditions (Gardner et al., 1988; Lepore et al., 1996; Katsidoni et al., 2013). Attenuation of ICSS is an effect that is opposite to that of many drugs of abuse (e.g., cocaine, opiates), which facilitate ICSS (Negus and Miller, 2014). Other cannabinoid receptor agonists, including WIN55212-2 [(R)-(+)-[2,3-dihydro-5-methyl-3-(4-morpholinylmethyl)pyrrolo[1,2,3-de]-1,4-benzoxazin-6-yl]-1-naphthalenylmethanone], CP55,940 [(–)-*cis*-3-[2-hydroxy-4-(1,1-dimethylheptyl)phenyl]-*trans*-4-(3-hydroxypropyl)cyclohexanol], levonantradol, nabilone, and HU210 [(6*aR*)-*trans*-3-(1,1-dimethylheptyl)-6*a*,7,10,10*a*-tetrahydro-1-hydroxy-6,6-dimethyl-6*H*-dibenzo[*b,d*]pyran-9-methanol], attenuate ICSS (Negus and Miller, 2014). Similarly, the FAAH inhibitor URB597 [3'-(aminocarbonyl)-[1,1'-biphenyl]-3-yl ester; Vlachou et al., 2006; Kwilasz et al., 2014] and the purported AEA transport inhibitor AM-404 [*N*-(4-hydroxyphenyl)-5*Z*,8*Z*,11*Z*,14*Z*-eicosatetraenamide; Vlachou et al., 2008] reduce ICSS. Moreover, some studies have shown CB₁ receptor antagonists reduced ICSS behavior (Arnold et al., 2001; Deroche-Gamonet et al., 2001; De Vry et al., 2004; Xi et al., 2008), whereas others have found no effect (Vlachou et al., 2005, 2006; Kwilasz and Negus, 2012).

The primary goal of the present study was to test the hypothesis that THC and inhibitors of FAAH and MAGL alone and in combination will facilitate ICSS in mice. The published studies of which we are aware have evaluated THC or FAAH inhibitors using rat ICSS procedures. No studies have evaluated the effects of MAGL inhibitors or dual FAAH and MAGL inhibitors in ICSS. Toward this end, we examined the dose-response relationships of the FAAH inhibitor PF-3845 [*N*-3-pyridinyl-4-[[3-[[5-(trifluoromethyl)-2-pyridinyl]oxy]phenyl]methyl]-1-piperidinecarboxamide; Ahn et al., 2009], the MAGL inhibitor JZL184 [4-[[bis(1,3-benzodioxol-5-yl)hydroxymethyl]-1-piperidinecarboxylic acid 4-nitrophenyl ester; Long et al., 2009a], and the dual FAAH/MAGL inhibitor SA-57 [4-[2-(4-chlorophenyl)ethyl]-1-piperidinecarboxylic acid 2-(methylamino)-2-oxoethyl ester; Niphakis et al., 2012]. SA-57 inhibits FAAH more potently than MAGL, resulting in elevated levels of AEA and other FAAH substrates at low doses, and increased 2-AG levels with higher doses (Niphakis

et al., 2012). Cocaine was included as a comparison drug, because it is well established to increase ICSS (Fish et al., 2010; Straub et al., 2010). Because both CB₁ and cannabinoid receptor type 2 (CB₂) have been implicated in modulation of the mesolimbic dopamine system, the selected antagonists of these receptors, rimonabant and SR144528 [5-(4-chloro-3-methylphenyl)-1-[(4-methylphenyl)methyl]-*N*-[(1*S*,2*S*,4*R*)-1,3,3-trimethylbicyclo[2.2.1]hept-2-yl]-1*H*-pyrazole-3-carboxamide], were used to infer the respective involvement of CB₁ and CB₂ receptors. The dose relationships of THC and the enzyme inhibitors were also evaluated in a food-reinforced operant task and spontaneous locomotor activity. The final study determined the impact of each enzyme inhibitor on AEA, 2-AG, and other relevant lipids in brain regions associated with the mesolimbic dopaminergic system, including VTA, NAc, prefrontal cortex (PFC), and amygdala.

Materials and Methods

Subjects

A total of 308 male C57BL/6J mice (21 for ICSS studies, 13 for operant studies, 135 for endocannabinoid measurement, and 139 for locomotor activity studies) obtained from The Jackson Laboratory (Bar Harbor, ME) served as subjects. Mice were between 8 and 12 weeks of age; weighed between 22 and 30 g at the commencement of each experiment; and were individually housed and maintained on a 12-hour light/dark cycle (lights on from 6:00 AM to 6:00 PM), with free access to food and water, except in experiments assessing operant responding for food reinforcement. All experiments were conducted in accordance with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals*, and were approved by the Virginia Commonwealth University Institutional Animal Care and Use Committee.

Drugs

JZL184, rimonabant [5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-*N*-1-piperidinyl-1*H*-pyrazole-3-carboxamide], SR144528, THC [(–)-(6*aR*,10*aR*)-6,6,9-trimethyl-3-pentyl-6*a*,7,8,10*a*-tetrahydro-6*H*-benzo[*c*]chromen-1-ol] (obtained from National Institute on Drug Abuse drug supply, Research Triangle Institute, Research Triangle Park, NC), SA-57 (synthesized in the Cravatt laboratory at Scripps Research Institute, La Jolla, CA), and PF-3845 (obtained from Organix, Woburn, MA) were dissolved in a vehicle (VEH) consisting of 5% ethanol, 5% alkamuls-620 (Rhone-Poulenc, Princeton, NJ), and 90% saline (0.9%). Cocaine HCl [(1*R*,2*R*,3*S*,5*S*,8*R*)-3-(benzoyloxy)-2-(methoxycarbonyl)-8-methyl-8-azoniabicyclo[3.2.1]octane chloride] (obtained from National Institute on Drug Abuse drug supply, Research Triangle Institute) was dissolved in 0.9% saline.

Apparatus

ICSS testing was conducted using eight standard (18 × 18 × 18 cm) mouse operant conditioning chambers (Med Associates, St. Albans, VT). Each chamber was equipped with a retractable lever located in the right-hand position on the front chamber wall, two LED stimulus

ABBREVIATIONS: 2-AG, 2-arachidonoylglycerol; AEA, anandamide; AM-404, *N*-(4-hydroxyphenyl)-5*Z*,8*Z*,11*Z*,14*Z*-eicosatetraenamide; ANOVA, analysis of variance; CB₁, cannabinoid receptor type 1; CB₂, cannabinoid receptor type 2; CP55,940, (–)-*cis*-3-[2-hydroxy-4-(1,1-dimethylheptyl)phenyl]-*trans*-4-(3-hydroxypropyl)cyclohexanol; FAAH, fatty acid amide hydrolase; FR, fixed ratio; HU210, (6*aR*)-*trans*-3-(1,1-dimethylheptyl)-6*a*,7,10,10*a*-tetrahydro-1-hydroxy-6,6-dimethyl-6*H*-dibenzo[*b,d*]pyran-9-methanol; ICSS, intracranial self-stimulation; JZL184, 4-[[bis(1,3-benzodioxol-5-yl)hydroxymethyl]-1-piperidinecarboxylic acid 4-nitrophenyl ester; MAGL, monoacylglycerol lipase; NAc, nucleus accumbens; OEA, *N*-oleylethanolamine; PEA, *N*-palmitoylethanolamine; PF-3845, *N*-3-pyridinyl-4-[[3-[[5-(trifluoromethyl)-2-pyridinyl]oxy]phenyl]methyl]-1-piperidinecarboxamide; PFC, prefrontal cortex; SA-57, 4-[2-(4-chlorophenyl)ethyl]-1-piperidinecarboxylic acid 2-(methylamino)-2-oxoethyl ester; SR144528, 5-(4-chloro-3-methylphenyl)-1-[(4-methylphenyl)methyl]-*N*-[(1*S*,2*S*,4*R*)-1,3,3-trimethylbicyclo[2.2.1]hept-2-yl]-1*H*-pyrazole-3-carboxamide; THC, Δ⁹-tetrahydrocannabinol; URB597, 3'-(aminocarbonyl)-[1,1'-biphenyl]-3-yl ester; VEH, vehicle; VTA, ventral tegmental area; WIN55212-2, (R)-(+)-[2,3-dihydro-5-methyl-3-(4-morpholinylmethyl)pyrrolo[1,2,3-de]-1,4-benzoxazin-6-yl]-1-naphthalenylmethanone.

lights, a chamber house light, and a tone generator. The outside of each chamber was equipped with a suspended electrical commutator connected to a shock generator, as well as to a tether that was fed through a hole in the top of the chamber. The operant conditioning chambers were enclosed within sound- and light-attenuating chambers equipped with exhaust fans. MED Associates software was used to control manipulations in the operant chambers and to record data during training and testing sessions.

Operant responding for food was conducted in mouse operant conditioning chambers (Med Associates) similar to those described above. Each chamber was equipped with two nose poke apertures (in place of levers) and a food hopper to deliver sweetened food pellets (Bio-Serv, Frenchtown, NJ) to a recessed well within the chamber. All other aspects of the chamber were identical to those used for ICSS testing, except equipment related to electrical stimulation was omitted, including commutators and tethers.

Locomotor activity testing was conducted in Plexiglas chambers ($17.5 \times 8.5 \times 8$ in) with plastic floor inserts and clear Plexiglas lids that were enclosed within sound- and light-attenuating chambers. Locomotor activity was recorded during tests using Unibrain Fire-I digital cameras and analyzed using ANY-maze software (Stoelting, Kiel, WI) to track and quantify movements.

Stereotaxic Surgery

Surgical procedures for implanting electrodes in mice for ICSS studies were similar to those previously reported (Carlezon and Chartoff, 2007). Mice were anesthetized with isoflurane and received constant isoflurane delivery during surgical procedures. Bipolar twisted stainless steel electrodes (part number 8IMS3033SPCE; Plastics One, Roanoke, VA) were implanted into the right medial forebrain bundle of the mice using coordinates reported by Straub et al. (2010): 2.0 mm posterior of bregma, 0.8 mm lateral from midline, and 4.8 mm ventral to dura. After the hole for the electrode was drilled, three holes were bored into the skull in the surrounding area, and anchoring screws were secured. Electrodes were inserted and fixed to the anchoring screws using dental cement. Mice were given postoperative care for 5 days following surgery, including treatment with bacitracin and acetaminophen, and were introduced to operant chambers 1 week after surgery.

Behavioral Procedures

ICSS. One week after stereotaxic surgery, mice began lever-press training. Subjects were trained on a fixed ratio (FR) 1 schedule of reinforcement, in which each lever press delivered a 0.5-second train of square-wave cathodal pulses (0.1 millisecond pulse duration) at a frequency of 141 Hz, which was accompanied by a stimulus light, house light, and tone cue presentation. Amplitudes of stimulation were adjusted for each mouse on an individual basis to produce maximal responding at this stage. Mice were exposed to daily 30- to 60-minute FR1 training sessions until they maintained stable responding at response rates greater than 35 responses per minute. Prior to testing, the stimulation amplitudes were adjusted individually from 20 to 300 μ A to maintain maximal response rates. After operant responding for ICSS stabilized, mice began frequency-rate training, using a similar procedure employed by Negus et al. (2010). Details related to rate-frequency training are provided in the Supplemental Methods.

On test days, mice were removed from the operant chambers after baseline testing, injected with drug or VEH, and returned to home cages. Testing commenced following pretreatment times of 10 minutes for cocaine; 30 minutes for THC; and 120 minutes for PF-3845, JZL184, and SA-57. The selection of these pretreatment times was based on previous reports with enzyme inhibitors and preliminary studies with cocaine and THC (Ahn et al., 2009; Long et al., 2009b; Niphakis et al., 2012). To investigate the role of CB₁ and CB₂ receptors in the effects of each drug on ICSS, rimonabant or SR144528 was administered 15 minutes prior to each test compound in a

separate set of experiments. These pretreatment times were used for all other behavioral and analytical measurements, except for ICSS time-course evaluations. Following injections, mice were returned to the operant chambers to respond during two additional series of frequency presentations, which were considered the test series.

Food-Maintained Operant Responding. Naive mice ($n = 8$ –13 per group) were food-deprived to reach 85% of their free-feeding weight before exposure to operant chambers. During the 15-minute training sessions, mice were assigned to a particular nose-poke aperture (i.e., right or left), whereas the other aperture was blocked with a rubber stopper to prevent spontaneous responding. Responding in the aperture under a FR1 schedule of reinforcement resulted in delivery of one food pellet reinforcer (Bio-Serv), and FR value was gradually increased to a FR10 schedule of reinforcement. The FR10 schedule was used to produce comparable rates of responding to ICSS, as it limits inter-response pauses for consumption and satiation is less of a factor than with lower FR schedules (Sidman and Stebbins, 1954). A computer with a logic interface and MED-PC software (Med Associates) was used to program the schedule of reinforcement and to record data from training and test sessions. Mice qualified for testing when they maintained at least 20 responses per minute and total number of responses remained within 10% of that of the prior 2 training days on at least 3 consecutive training days. These criteria were evaluated before every operant test was conducted, and mice had a minimum of 96 hours between testing conditions. The dose-response relationships of THC, PF-3845, JZL184, and SA-57 were evaluated on operant responding for food using the same pretreatment times as described above. Subjects were given a washout period of at least 1 week before testing a new compound. On test days, mice were given their appropriate injections and returned to home cages before testing. VEH tests were conducted at the beginning and end of every dose-effect curve to assess response rate stability over time. Dose conditions were tested using a quasi-randomized design to control for order effects.

Locomotor Activity. Naive mice ($n = 7$ –12 per group) were acclimated to the testing room 24 hours before testing began. Following injections, subjects were returned to home cages for the appropriate pretreatment time, and then immediately placed in Plexiglas activity observation boxes and recorded using Any-maze (Stoelting) software. Distance traveled was calculated during the 25-minute observation period. Box assignment and time of day were counterbalanced between the different drug/dose conditions. Separate groups of naive mice were used for each treatment condition.

Endocannabinoid and Eicosanoid Analysis

AEA, 2-AG, arachidonic acid, oleoylethanolamide (OEA), and palmitoylethanolamide (PEA) were quantified in PFC, NAc, and amygdala, as well as the control region, the cerebellum. Drug naive subjects were injected with vehicle, PF-3845 (10.0 or 30.0 mg/kg), JZL184 (4.0 or 40.0 mg/kg), or SA-57 (1.0 or 10.0 mg/kg) intraperitoneally 2 hours prior to sacrifice. Immediately following cervical dislocation and decapitation, brains were removed and dissected, and the PFC, NAc, amygdala, and cerebellum were collected, as previously described (Lazenka et al., 2014). After isolation of the amygdala, a cut was made just anterior to the mammillary bodies and anterior to the middle cerebellar peduncle. From this slice, the interpeduncular nucleus/mammillary bodies, located ventrally, and the substantia nigra, located laterally, were removed. The remaining regions ventral to the red nucleus were dissected and were comprised primarily of the ventral tegmental area and interfascicular nucleus. Samples were processed and substrates quantified in a similar manner to previous studies (Wise et al., 2012). Details on the extraction and quantification of endocannabinoids and eicosanoids are included in the Supplemental Methods.

Data Analysis

The independent variable measured in the ICSS studies was stimulations per minute for each frequency. ICSS data

were evaluated using two separate approaches, as previously reported (Negus and Miller, 2014). The first method examined percentage of baseline stimulations by dividing the total number of stimulations during the test series by the total number of responses during the baseline series, and multiplying the quotient by 100. This transformation effectively collapsed all the separate frequency stimulation rates into an overall stimulation count, allowed for comparison of data between days/treatments, and was performed for all doses of each drug and VEH/saline; and values were analyzed using repeated-measures one-way analysis of variance (ANOVA). Dunnett's post hoc test was used following significant ANOVA results to compare treatment groups with VEH controls. Throughout this report, the data are primarily depicted as percent baseline stimulations.

The second method expressed the data as frequency-rate curves in which the data were normalized to the highest response rate recorded during the baseline frequency-rate curve generated for each individual mouse to yield the percent maximum control rate. Repeated-measures two-way ANOVA (treatment $\times$ frequency) was used to analyze the data. Holm-Šidák tests were used for post hoc analyses of frequency-rate results, which allowed multiple comparisons at all frequencies. These data analyses are presented in Supplemental Methods. In the vast majority of comparisons, the percent baseline stimulations data analyses revealed significant differences for the same doses that yielded significant differences in frequency-rate analyses.

Distance traveled was observed during locomotor activity studies and analyzed using independent sample one-way ANOVA. Response rate (responses/min) was the dependent variable of interest in the food-reinforced operant responding studies, and was also analyzed using repeated-measures one-way ANOVA. Brain endocannabinoid and arachidonic acid levels (pm/g or nm/g) were analyzed using one-way ANOVA for each brain region and substrate measured. Significant ANOVA results were assessed using Dunnett's post hoc test.

Results

Effects of THC, Enzyme Inhibitors, and Cocaine on ICSS. THC, PF-3845, JZL184, and SA-57 attenuated ICSS, whereas cocaine produced dose-related ICSS facilitation (Fig. 1; Supplemental Fig. 1). Significant depression of ICSS was produced by THC (5.6 mg/kg and 10.0 mg/kg; Fig. 1A), PF-3845 (30 mg/kg; Fig. 1B), JZL184 (16.0 and 40.0 mg/kg; Fig. 1C), and SA-57 (3.0, 10.0, and 17.8 mg/kg; Fig. 1D). As can be seen by the frequency-rate curves for THC (Supplemental Fig. 1), no dose of THC facilitated ICSS at any frequency. Similar results were obtained with the enzyme inhibitors (data not shown). Cocaine facilitated ICSS at 10.0 and 17.8 mg/kg (Fig. 1E), providing a positive control for this series of experiments, and frequency-rate curves showed similar results (Supplemental Fig. 2).

Time Course of Effects on ICSS. Maximally effective doses of THC and the enzyme inhibitors were administered to determine the time course for inhibition of ICSS. THC (10.0 mg/kg) attenuated ICSS 30 minutes after administration, and behavior returned to baseline levels by 24 hours (Fig. 2A; Supplemental Fig. 3). Administration of 30 mg/kg PF-3845 (Fig. 2B) or 40.0 mg/kg JZL184 (Fig. 2C) attenuated ICSS up to 48 hours after drug administration. SA-57 (10.0 mg/kg)

reduced responding for electrical stimulation 8 hours after administration (Fig. 2D). Analysis of frequency-rate curves found that none of the cannabinoids facilitated ICSS at any frequency at any time (THC data shown in Supplemental Fig. 3; other data not shown).

Antagonism of Cannabinoid Effects on ICSS. To infer the involvement of CB₁ and CB₂ receptors, rimonabant or SR144528 was administered prior to cannabinoids and enzyme inhibitors. Rimonabant blocked depression of ICSS produced by THC (10 mg/kg; Fig. 3A; Supplemental Fig. 4), JZL184 (40 mg/kg; Fig. 3C; Supplemental Fig. 5), or SA-57 (10 mg/kg; Fig. 3D; Supplemental Fig. 6). Frequency-rate curves for rimonabant challenges of THC, JZL184, and SA-57 are shown in Supplemental Figs. 4–6. Rimonabant did not attenuate decreases in ICSS produced by PF-3845 (30 mg/kg; Fig. 3B). In contrast, SR144528 (3.0 or 10.0 mg/kg) did not block attenuation of ICSS produced by THC (Fig. 4A), PF-3845 (Fig. 4B), JZL184 (Fig. 4C), or SA-57 (Fig. 4D).

Cannabinoids Reduce Other Behaviors. THC (7.5 and 10 mg/kg; Fig. 5A), JZL184 (40.0 mg/kg; Fig. 5C), and SA-57 (10 and 17.8 mg/kg; Fig. 5D) significantly reduced operant responding for food, but PF-3845 was without effect at doses up to 30 mg/kg (Fig. 5B). In addition, all drugs reduced spontaneous locomotor activity. THC (5.6 and 10 mg/kg; Fig. 6A), PF-3845 (30 mg/kg; Fig. 6B), JZL184 (40 mg/kg; Fig. 6C), and SA-57 (10 mg/kg; Fig. 6D) reduced the distance traveled by the mice.

Brain Levels of Endocannabinoids and Eicosanoids. Table 1 shows the effects of acute administration of PF-3845 (10 and 30 mg/kg), JZL184 (4 and 40 mg/kg), and SA-57 (1 and 10 mg/kg) on AEA, 2-AG, arachidonic acid, OEA, and PEA levels in the PFC, NAc, ventral midbrain (which included the VTA), and amygdala, as well as the cerebellum, which was included for comparison. Administration of the FAAH inhibitor PF-3845 increased AEA, OEA, and PEA in all brain regions with the exception of AEA in the amygdala, but did not alter 2-AG or arachidonic acid levels in any region examined. The MAGL inhibitor JZL184 increased 2-AG in all brain regions assessed, and the highest dose (40 mg/kg) also reduced AEA in the dopamine terminal field regions PFC and NAc. JZL184 (40 mg/kg) also decreased arachidonic acid levels in all brain regions except for the NAc ($P = 0.05$). Administration of the dual FAAH/MAGL inhibitor SA-57 (1 and 10 mg/kg) increased AEA, OEA, and PEA levels in all brain regions, but increased 2-AG concentrations in all brain regions only at the 10 mg/kg dose. SA-57 (10 mg/kg) decreased arachidonic acid concentrations in the PFC, amygdala, and cerebellum, but not in the NAc or ventral midbrain.

Discussion

In the present study, we found no evidence of cannabinoid-induced facilitation of ICSS in mice, which is in agreement with the majority of previous rat studies (Negus and Miller, 2014). THC and inhibitors of MAGL or combined inhibition of MAGL and FAAH attenuated ICSS through a CB₁ receptor mechanism of action, which is in agreement with reports that CB₁ receptors are required for THC-induced attenuation of ICSS in rats (Vlachou et al., 2007; Kwilasz and Negus, 2012; Katsidoni et al., 2013). In contrast, initial studies reported that THC facilitated ICSS in the Lewis rat, but not in Fisher 344 or Sprague-Dawley rats, suggesting that strain is an

Fig. 1. THC and endocannabinoid catabolic enzyme inhibitors significantly attenuated ICSS, whereas cocaine facilitated ICSS. (A) THC administered 30 minutes prior to testing significantly decreased operant responding for ICSS ($F_{6, 30} = 24.1$, $P < 0.001$; $n = 7$). (B) PF-3845 administered 2 hours prior to testing significantly reduced ICSS ($F_{4, 24} = 4.48$, $P < 0.01$; $n = 9$), although only an excessively high dose (30 mg/kg) significantly reduced ICSS. (C) JZL184 administered 2 hours prior to testing significantly decreased ICSS ($F_{4, 24} = 4.48$, $P < 0.01$; $n = 7$). (D) SA-57 administered 2 hours prior to testing significantly decreased ICSS ($F_{4, 24} = 49.25$, $P < 0.001$) with a magnitude similar to that THC. (E) Cocaine given 10 minutes prior to testing significantly facilitated ICSS ($F_{4, 20} = 15.37$, $P < 0.001$; $n = 6$). * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ versus VEH as determined by Dunnett's post hoc test. Ordinate indicates percentage of total baseline stimulations (total stimulations during test/total stimulations during baseline multiplied by 100). All values reflect mean $\pm$ S.E.M.

Fig. 2. Time course of decreased operant responding for ICSS produced by THC (10 mg/kg, $n = 7$ mice/group; A), PF-3845 (30 mg/kg, $n = 8$ mice/group; B), JZL184 (40 mg/kg, $n = 6$ mice/group; C), and SA-57 (10 mg/kg, $n = 7$ mice/group; D). Two-way ANOVAs (time point $\times$ treatment) yielded significant interactions between time point and drug for THC ($F_{6, 36} = 17.09$, $P < 0.001$), PF-3845 ($F_{5, 35} = 3.75$, $P < 0.01$), JZL184 ($F_{5, 25} = 2.61$, $P < 0.05$), and SA-57 ($F_{5, 25} = 33.30$, $P < 0.001$). * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ versus appropriate VEH group (Holm-Sidak's post hoc test). All values reflect mean $\pm$ S.E.M.

important factor (Gardner et al., 1988; Lepore et al., 1996). Another report indicated a biphasic effect, in which a single low dose of THC (0.1 mg/kg) facilitated ICSS, whereas 1.0 mg/kg THC reduced responding in Sprague-Dawley rats (Katsidoni et al., 2013). However, in the present study, a 10-fold dose range of THC below those that did not reduce ICSS also did not facilitate ICSS in the mouse. Likewise, subthreshold doses of the enzyme inhibitors did not facilitate ICSS. The disruptive effects of THC and enzyme inhibitors on ICSS were also observed in separate behavioral assays for operant responding for food and spontaneous locomotor activity, indicating that this effect is not specific to ICSS behavior.

Although 10 mg/kg PF-3845 maximally inhibits FAAH (see Table 1), it did not affect ICSS, reduce operant responding for food reinforcement, nor alter spontaneous locomotor activity. Similarly, SA-57 (1 mg/kg) produced comparable increases in AEA, but did not elevate 2-AG, and did not affect any of the behaviors tested. Increasing the dose of PF-3845 by a half-log step did not elevate brain AEA levels beyond that of the 10 mg/kg dose (see Table 1), but produced a small, but significant attenuation of ICSS (21%) that was not prevented by CB₁ or CB₂ receptor antagonists. Although this depression of ICSS may be mediated through other targets associated with FAAH substrates (e.g., TRPV1 or PPAR α receptors), nonspecific

Fig. 3. Evaluation of whether CB₁ receptors mediate the disruptive effects of THC and endocannabinoid catabolic enzyme inhibitors on ICSS. Rimonabant significantly blocked ICSS depression produced by THC (10 mg/kg; two-way ANOVA interaction: $F_{1,6} = 90.8$, $P < 0.001$; $n = 7$; A), JZL184 (40 mg/kg; two-way ANOVA interaction: $F_{1,7} = 7.66$, $P < 0.05$; $n = 8$; C), and SA-57 (10 mg/kg; two-way ANOVA interaction: $F_{2,10} = 25.90$, $P < 0.05$; $n = 6$; D). (B) The disruptive effects of high-dose PF-3845 (30 mg/kg) on ICSS were not blocked by rimonabant [main effect of PF-3845 ($F_{2,10} = 6.10$, $P < 0.001$), but no main effect of rimonabant, and no interaction between PF-3845 and rimonabant; $n = 6$]. ** $P < 0.01$; *** $P < 0.001$ versus VEH + VEH or VEH + rimonabant condition (Holm-Sidak's post hoc test). Asterisks next to figure keys indicate significant main effects of drug treatment in the absence of an interaction; *** $P < 0.001$. All values represent mean $\pm$ S.E.M.

effects of this high dose cannot be neglected. However, it should be noted that URB597 and the purported AEA transport inhibitor AM-404 produced CB₁ receptor-mediated reductions responding in rat ICSS experiments. Thus, species-dependent effects may influence the ability of FAAH inhibitors to alter ICSS.

In contrast to the predominant inactivity of PF-3845 in each of the three assays, full inhibition of MAGL by JZL184, which increased 2-AG levels in all brain regions assessed, elicited

a small CB₁ receptor-dependent decrease in ICSS (29%), decreased operant responding for food, and reduced spontaneous locomotor activity. Combined inhibition of FAAH and MAGL produced a more dramatic CB₁ receptor-mediated attenuation of ICSS (72%) than single enzyme inhibition, and was similar in magnitude to that produced by THC (69%). SA-57 also reduced spontaneous locomotor activity and food-reinforced operant responding. Each of the three enzyme inhibitors was more potent in disrupting ICSS than in

Fig. 4. CB₂ receptors are dispensable for the attenuation of ICSS produced by THC (10 mg/kg), PF-3845 (30 mg/kg), JZL184 (40 mg/kg), and SA-57 (10 mg/kg). Vehicle or SR144528 (SR2) given 15 minutes prior to the test compound did not alter decreased operant responding for ICSS produced by the following: (A) THC (10 mg/kg; main effect of drug, only: $F_{1,4} = 43.66$, $P < 0.01$; $n = 5$); (B) PF-3845 (30 mg/kg; main effect of drug, only: $F_{1,5} = 7.96$, $P < 0.05$; $n = 6$); (C) JZL184 [40 mg/kg, significant interaction between drug and SR144528: $F_{1,7} = 7.17$, $P < 0.05$; ICSS was attenuated in both groups that received JZL184 whether given a pretreatment with SR144528 (10 mg/kg) or VEH (Holm-Sidak's post-hoc test); $n = 8$]; or (D) SA-57 (10 mg/kg; main effect of drug, only: $F_{1,5} = 153.6$, $P < 0.001$; $n = 6$). * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ versus VEH + VEH and VEH + SR144528. Asterisks next to figure keys indicate significant main effects of drug treatment in the absence of an interaction; ** $P < 0.01$; *** $P < 0.001$. Data reflect mean $\pm$ S.E.M.

decreasing the other behaviors, indicating that ICSS is somewhat more sensitive than the other measures to these compounds.

It is noteworthy that SA-57 significantly elevated AEA and 2-AG in PFC, NAc, ventral midbrain, and amygdala, which represent the underlying circuitry mediating the acute reinforcing effects of drugs of abuse (Koob and Volkow, 2010). Dual FAAH/MAGL blockade produces other THC-like effects, such as impaired short-term spatial memory (Wise et al., 2012), catalepsy, and enhanced analgesia, as well as full substitution for THC in a mouse drug discrimination procedure (Long et al., 2009b). Consistent with the idea that

FAAH and MAGL serve as regulators that prevent CB₁ receptor overstimulation by endocannabinoids, each of these effects was blocked by rimonabant.

In this study, we also found that rimonabant alone did not affect ICSS in mice. Likewise, CB₁ receptor antagonists given alone did not affect ICSS in rats (De Vry et al., 2004; Vlachou et al., 2005, 2006; Xi et al., 2008; Kwilas and Negus, 2012). In other studies, however, rimonabant reduced ICSS in rats at moderate doses (1 and 3 mg/kg) (Deroche-Gamonet et al., 2001; De Vry et al., 2004) or high doses (10 or 20 mg/kg) (Arnold et al., 2001; Xi et al., 2008). Although these latter findings are consistent with the idea that endocannabinoids tonically

Fig. 5. Food-maintained operant responding was attenuated by THC, JZL184, and SA-57, but PF-3845 had no effect. (A) THC produced a significant dose-related reduction of operant responding, as shown by a main effect of drug treatment ($F_{3, 36} = 11.06$, $P < 0.001$; $n = 13$). (B) PF-3845 did not alter operant responding for food ($F_{3, 28} = 0.50$, $P = 0.69$; $n = 8$). (C) JZL184 attenuated operant responding for food ($F_{4, 32} = 9.89$, $P < 0.001$; $n = 9$). (D) SA-57 significantly reduced operant responding for food ($F_{4, 32} = 19.03$, $P < 0.001$; $n = 9$). * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ versus respective VEH group (Dunnett's post hoc test). All values represent mean $\pm$ S.E.M.

modulate ICSS in rats, CB₁ receptor inverse agonist properties of rimonabant cannot be ruled out (Landsman et al., 1997). SR144528 did not alter operant responding for brain stimulation alone and did not antagonize the ICSS-attenuating effects of the other drugs, suggesting that CB₂ receptors are dispensable in this behavior. There are no previous studies of which we are aware that have examined the consequences of CB₂ receptor antagonists on ICSS. The results of the present study indicate that CB₁ and CB₂ receptors do not play a tonic role in modulating ICSS in C57BL/6 mice.

The results of this study suggest that THC and endocannabinoid catabolic enzyme inhibitors do not potentiate ICSS in mice. Thus, the conditions used in this assay may not be optimized to infer rewarding effects of cannabinoids. Suppression of behaviors other than ICSS (e.g., spontaneous locomotor activity and operant nose-poking for food) suggests that the depressive effects of CB₁ receptor activation in mice are not limited to ICSS. Accordingly, motor suppression may, in part, contribute to CB₁ receptor-mediated disruption of ICSS. Employment of methods that depend less on response

Fig. 6. Spontaneous locomotor activity was reduced by THC and endocannabinoid catabolic enzyme inhibitors. (A) THC significantly reduced locomotor activity ($F_{4, 31} = 7.04$, $P < 0.001$; $n = 7-8$). (B) PF-3845 significantly reduced activity ($F_{3, 28} = 3.14$, $P < 0.05$; $n = 8$). (C) JZL184 significantly decreased spontaneous locomotor activity ($F_{3, 31} = 8.9$, $P < 0.001$; $n = 7-12$). (D) SA-57 significantly attenuated spontaneous activity ($F_{3, 32} = 14.78$, $P < 0.001$; $n = 8-12$). * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ versus appropriate VEH group (Dunnett's post hoc test). All values represent mean $\pm$ S.E.M.

rate, such as a discrete trials procedure, could be useful to unmask potential rewarding properties of cannabinoids in ICSS. One strategy to reveal reinforcing effects with other drugs of abuse is implementing repeated dosing to induce tolerance to the motor-impairing effects (Negus and Miller, 2014). However, repeated THC dosing in rats did not reveal reinforcement-potentiating properties of THC (Kwilas and Negus, 2012).

CB₁ receptors are expressed on glutamatergic (Melis et al., 2004; Fernandez-Ruiz et al., 2010), cholinergic, and GABAergic neuronal terminals. The finding that CB₁ receptor activation on glutamatergic neurons reduces spontaneous dopaminergic neuronal firing is consistent with the reduction of ICSS

observed in this study. THC has been shown to increase extracellular dopamine in the NAc by approximately 50% (Tanda et al., 1997), which is considerably less than that induced by cocaine (about 400% of basal levels) (Panos and Baker, 2010). Whereas endocannabinoids (e.g., 2-AG) are capable of modulating dopaminergic neurotransmission, they do so to a lesser extent than other drugs of abuse. Furthermore, the rewarding effects of cannabinoids may not be related to the activation of the mesolimbic system, but rather driven by other psychologic factors, including stress relief mediated through brain regions such as the amygdala.

When considering the ICSS-attenuating effects of THC, it is important to note that cannabis contains more than 100

TABLE 1

Summary of AEA, 2-AG, arachidonic acid (AA), OEA, and PEA levels in brain regions mediating ICSS after treatment with enzyme inhibitors

Drug	Region	Dose	AEA	2-AG	AA	OEA	PEA
			mg/kg pm/g	nm/g		pm/g	
PF-3845 (FAAH inhibitor)	PFC	0 (VEH)	23.7 ± 2.76	8.69 ± 1.32	207 ± 5.41	72.4 ± 6.10	85.9 ± 11.1
		10	57.0 ± 4.67***	9.36 ± 0.77	208 ± 2.48	824 ± 23.6***	918 ± 94.4***
		30	47.5 ± 3.49**	7.74 ± 1.14	204 ± 7.78	859 ± 30.3***	836 ± 72.3***
	NAc	0 (VEH)	25.9 ± 5.10	10.1 ± 0.77	121 ± 4.84	128 ± 9.20	146 ± 12.1
		10	56.0 ± 8.43**	9.06 ± 0.83	124 ± 9.73	1062 ± 39.4***	1177 ± 83.6***
		30	42.3 ± 1.90	9.72 ± 0.39	131 ± 3.95	1031 ± 38.8***	1125 ± 81.5***
	Ventral midbrain	0 (VEH)	11.0 ± 2.98	14.8 ± 2.13	52.1 ± 5.14	266 ± 67.9	365 ± 79.3
		10	62.2 ± 6.55**	13.0 ± 1.62	49.0 ± 3.14	1511 ± 132***	1541 ± 125***
		30	64.8 ± 10.9**	13.1 ± 1.33	52.9 ± 5.62	1676 ± 77.1***	1718 ± 165***
	Amygdala	0 (VEH)	35.6 ± 5.48	15.1 ± 1.28	171 ± 10.3	74.9 ± 6.31	87.6 ± 10.7
		10	52.4 ± 5.25	16.6 ± 1.01	166 ± 12.6	747 ± 46.3***	837 ± 100***
		30	47.8 ± 3.82	12.9 ± 1.42	166 ± 3.93	850 ± 36.8***	926 ± 84.2***
	Cerebellum	0 (VEH)	6.33 ± 0.57	6.64 ± 0.21	77.3 ± 3.06	190 ± 23.4	228 ± 26.4
		10	44.5 ± 3.44***	6.92 ± 0.82	77.2 ± 3.90	1306 ± 48.0***	1159 ± 104***
		30	39.4 ± 3.02***	8.31 ± 0.84	76.6 ± 2.54	1405 ± 43.7***	1175 ± 91.5***
JZL184 (MAGL inhibitor)	PFC	0 (VEH)	18.1 ± 3.23	10.8 ± 1.03	205 ± 8.29	50.1 ± 4.08	54.4 ± 5.28
		4	15.7 ± 2.15	19.9 ± 1.30	194 ± 3.73	46.4 ± 4.47	58.5 ± 5.31
		40	8.8 ± 0.77*	72.9 ± 4.95***	160 ± 7.15***	52.4 ± 2.12	63.6 ± 4.24
	NAc	0 (VEH)	19.9 ± 3.31	8.69 ± 0.73	155 ± 18.3	125 ± 10.8	127 ± 21.3
		4	11.7 ± 2.25	37.6 ± 3.20***	139 ± 8.26	105 ± 11.7	104 ± 7.32
		40	9.2 ± 0.84*	90.6 ± 5.90***	104 ± 7.0	106 ± 9.0	123 ± 14.6
	Ventral midbrain	0 (VEH)	18.7 ± 3.75	9.60 ± 0.97	88.6 ± 8.38	228 ± 36.6	273 ± 37.5
		4	23.4 ± 5.16	48.1 ± 5.47**	70.7 ± 8.79	179 ± 23.6	244 ± 38.5
		40	18.7 ± 3.52	85.6 ± 9.94***	56.2 ± 5.91*	205 ± 23.1	326 ± 66.5
	Amygdala	0 (VEH)	24.2 ± 3.39	17.3 ± 2.19	182 ± 5.90	74.2 ± 7.33	70.4 ± 5.39
		4	23.8 ± 4.28	45.0 ± 3.74*	188 ± 18.1	77.1 ± 8.58	75.3 ± 6.01
		40	12.7 ± 2.23	139 ± 11.0***	119 ± 8.52**	59.8 ± 6.82	57.9 ± 1.02
	Cerebellum	0 (VEH)	9.0 ± 1.28	13.0 ± 1.67	76.6 ± 2.92	136 ± 5.15	153 ± 3.30
		4	8.1 ± 0.80	28.6 ± 2.58***	73.8 ± 2.58	140 ± 3.98	160 ± 6.70
		40	7.1 ± 0.62	50.8 ± 2.18***	52.7 ± 1.33***	136 ± 6.77	165 ± 9.38
SA-57 (FAAH/MAGL inhibitor)	PFC	0 (VEH)	13.6 ± 4.42	7.89 ± 0.66	174 ± 10.3	51.3 ± 2.55	65.8 ± 6.54
		1	39.7 ± 2.91**	13.8 ± 0.88	178 ± 6.51	783 ± 11.5***	899 ± 36.7***
		10	34.1 ± 7.23*	30.9 ± 9.40***	115 ± 16.1**	779 ± 22.9***	871 ± 45.7***
	NAc	0 (VEH)	12.7 ± 3.60	8.03 ± 0.41	104 ± 7.10	86.5 ± 5.03	108 ± 11.9
		1	36.4 ± 5.28**	12.4 ± 1.22	115 ± 8.81	1054 ± 34.9***	1277 ± 80.7***
		10	31.6 ± 4.66*	103 ± 3.26***	77.4 ± 12.1	938 ± 50.5***	1106 ± 67.4***
	Ventral midbrain	0 (VEH)	7.28 ± 3.10	12.2 ± 1.37	67.8 ± 22.5	165 ± 11.2	243 ± 16.5
		1	37.3 ± 7.07*	16.0 ± 2.90	66.5 ± 19.3	1413 ± 216***	1768 ± 319***
		10	58.3 ± 12.2**	80.9 ± 13.8***	63.4 ± 17.7	1368 ± 70.9***	1756 ± 156***
	Amygdala	0 (VEH)	14.9 ± 3.08	13.4 ± 1.50	144 ± 5.25	55.8 ± 1.80	75.0 ± 8.69
		1	51.4 ± 8.22**	31.4 ± 4.67	145 ± 9.68	753 ± 22.2***	1027 ± 61.2***
		10	33.2 ± 4.47	148 ± 10.9***	66.4 ± 8.10***	727 ± 75.7***	865 ± 90.7***
	Cerebellum	0 (VEH)	5.34 ± 0.78	4.67 ± 0.22	66.7 ± 3.81	134 ± 3.73	161 ± 10.3
		1	29.2 ± 2.91***	10.6 ± 0.67	69.1 ± 2.91	1325 ± 53.1***	1312 ± 87.7***
		10	33.1 ± 3.97***	51.7 ± 3.38***	38.2 ± 2.15***	1231 ± 38.8***	1190 ± 78.9***

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ versus VEH as determined by Dunnett's post hoc test. Values represent mean ± S.E.M.

cannabinoids (albeit in trace amounts in most cases), as well as other chemicals that could contribute to its subjective effects and abuse potential in humans. Moreover, pure THC is not typically used for recreational purposes by humans and can elicit aversive effects (Calhoun et al., 1998) or only modest reinforcing effects in experienced marijuana users (Hart et al., 2005; Vandrey et al., 2013). In light of these studies, the rightward shift in the frequency-rate curves of ICSS produced by subcutaneous injection of THC may accurately represent a low abuse liability in humans. Future studies assessing inhaled marijuana, cannabis extracts, or synthetic cannabinoids in similar assays are warranted.

In summary, the current study showed THC and degradative endocannabinoid enzyme inhibitors produced CB₁ receptor-mediated, dose- and time-dependent attenuation

of ICSS, a behavioral assay known to be mediated by the mesolimbic reward system, as well as other behavioral tasks. When considering the attenuating effects of THC and the enzyme inhibitors on ICSS, it is difficult to distinguish between reinforcement-specific processes and motor processes. Whereas FAAH inhibition had little or no effect on these measures, combined FAAH and MAGL inhibition with SA-57 produced a substantial attenuation of ICSS that was qualitatively more similar to THC than either JZL184 or PF-3845. Attenuation of ICSS was associated with both large increases in 2-AG brain levels and combined increases of AEA and 2-AG. Collectively, these data suggest that activation of CB₁ receptors elicited by THC, MAGL inhibition, or combined FAAH/MAGL inhibition attenuates reinforcement maintained by operant responding for stimulation of the medial forebrain

bundle, and inhibits other reinforced and nonreinforced behaviors.

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Authorship Contributions

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Contributed new reagents or analytic tools: Cravatt, Niphakis.

Performed data analysis: Wiebelhaus.

Wrote or contributed to the writing of the manuscript: Wiebelhaus, Lazenka, Abdullah, Vann, Negus, Lichtman, Sim-Selley, Wiley.

References

- Ahn K, Johnson DS, Mileni M, Beidler D, Long JZ, McKinney MK, Weerapana E, Sadagopan N, Liimatta M, Smith SE, et al. (2009) Discovery and characterization of a highly selective FAAH inhibitor that reduces inflammatory pain. *Chem Biol* **16**:411–420.
- Arnold JC, Hunt GE, and McGregor IS (2001) Effects of the cannabinoid receptor agonist CP 55,940 and the cannabinoid receptor antagonist SR 141716 on intracranial self-stimulation in Lewis rats. *Life Sci* **70**:97–108.
- Calhoun SR, Galloway GP, and Smith DE (1998) Abuse potential of dronabinol (Marinol). *J Psychoactive Drugs* **30**:187–196.
- Carlezon WA, Jr and Chartoff EH (2007) Intracranial self-stimulation (ICSS) in rodents to study the neurobiology of motivation. *Nat Protoc* **2**:2987–2995.
- Cheer JF, Wassum KM, Heien ML, Phillips PE, and Wightman RM (2004) Cannabinoids enhance subsecond dopamine release in the nucleus accumbens of awake rats. *J Neurosci* **24**:4393–4400.
- Chen JP, Paredes W, Li J, Smith D, Lowinson J, and Gardner EL (1990) Delta 9-tetrahydrocannabinol produces naloxone-blockable enhancement of presynaptic basal dopamine efflux in nucleus accumbens of conscious, freely-moving rats as measured by intracerebral microdialysis. *Psychopharmacology* **102**:156–162.
- Chen JP, Paredes W, Lowinson JH, and Gardner EL (1991) Strain-specific facilitation of dopamine efflux by delta 9-tetrahydrocannabinol in the nucleus accumbens of rat: an in vivo microdialysis study. *Neurosci Lett* **129**:136–180.
- Compton DR, Aceto MD, Lowe J, and Martin BR (1996) In vivo characterization of a specific cannabinoid receptor antagonist (SR141716A): inhibition of delta 9-tetrahydrocannabinol-induced responses and apparent agonist activity. *J Pharmacol Exp Ther* **277**:586–594.
- Cravatt BF, Demarest K, Patricelli MP, Bracey MH, Giang DK, Martin BR, and Lichtman AH (2001) Supersensitivity to anandamide and enhanced endogenous cannabinoid signaling in mice lacking fatty acid amide hydrolase. *Proc Natl Acad Sci USA* **98**:9371–9376.
- Cravatt BF, Giang DK, Mayfield SP, Boger DL, Lerner RA, and Gilula NB (1996) Molecular characterization of an enzyme that degrades neuromodulatory fatty-acid amides. *Nature* **384**:83–87.
- De Vry J, Schreiber R, Eckel G, and Jentzsch KR (2004) Behavioral mechanisms underlying inhibition of food-maintained responding by the cannabinoid receptor antagonist/inverse agonist SR141716A. *Eur J Pharmacol* **483**:55–63.
- Deroche-Gamonet V, Le Moal M, Piazza PV, and Soubrie P (2001) SR141716, a CB1 receptor antagonist, decreases the sensitivity to the reinforcing effects of electrical brain stimulation in rats. *Psychopharmacology* **157**:254–259.
- Devane WA, Hanus L, Breuer A, Pertwee RG, Stevenson LA, Griffin G, Gibson D, Mandelbaum A, Etinger A, and Mechoulam R (1992) Isolation and structure of a brain constituent that binds to the cannabinoid receptor. *Science* **258**:1946–1949.
- Dinh TP, Carpenter D, Leslie FM, Freund TF, Katona I, Sensi SL, Kathuria S, and Piomelli D (2002) Brain monoglyceride lipase participating in endocannabinoid inactivation. *Proc Natl Acad Sci USA* **99**:10819–10824.
- Fernández-Ruiz J, Hernández M, and Ramos JA (2010) Cannabinoid-dopamine interaction in the pathophysiology and treatment of CNS disorders. *CNS Neurosci Ther* **16**:e72–e91.
- Filbey FM, Schacht JP, Myers US, Chavez RS, and Hutchison KE (2009) Marijuana craving in the brain. *Proc Natl Acad Sci USA* **106**:13016–13021.
- Fish EW, Riday TT, McGuigan MM, Faccidomo S, Hodge CW, and Malanga CJ (2010) Alcohol, cocaine, and brain stimulation-reward in C57Bl/6J and DBA/2J mice. *Alcohol Clin Exp Res* **34**:81–89.
- Gardner EL, Paredes W, Smith D, Donner A, Milling C, Cohen D, and Morrison D (1988) Facilitation of brain stimulation reward by delta 9-tetrahydrocannabinol. *Psychopharmacology* **96**:142–144.
- Goldman M, Szucs-Reed RP, Jagannathan K, Ehrman RN, Wang Z, Li Y, Suh JJ, Kampman K, O'Brien CP, Childress AR, et al. (2013) Reward-related brain response and craving correlates of marijuana cue exposure: a preliminary study in treatment-seeking marijuana-dependent subjects. *J Addict Med* **7**:8–16.
- Hart CL, Haney M, Vosburg SK, Comer SD, and Foltin RW (2005) Reinforcing effects of oral Delta9-THC in male marijuana smokers in a laboratory choice procedure. *Psychopharmacology* **181**:237–243.
- Hart CL, Ilan AB, Gervins A, Gunderson EW, Role K, Colley J, and Foltin RW (2010) Neurophysiological and cognitive effects of smoked marijuana in frequent users. *Pharmacol Biochem Behav* **96**:333–341.
- Hart CL, van Gorp W, Haney M, Foltin RW, and Fischman MW (2001) Effects of acute smoked marijuana on complex cognitive performance. *Neuropsychopharmacology* **25**:757–765.
- Katsidoni V, Kastellakis A, and Panagis G (2013) Biphasic effects of Δ9-tetrahydrocannabinol on brain stimulation reward and motor activity. *Int J Neuropsychopharmacol* **16**:2273–2284.
- Koob GF and Volkow ND (2010) Neurocircuitry of addiction. *Neuropsychopharmacology* **35**:217–238.
- Kwilas AJ, Abdullah RA, Poklis JL, Lichtman AH, and Negus SS (2014) Effects of the fatty acid amide hydrolase inhibitor URB597 on pain-stimulated and pain-depressed behavior in rats. *Behav Pharmacol* **25**:119–129.
- Kwilas AJ and Negus SS (2012) Dissociable effects of the cannabinoid receptor agonists Δ9-tetrahydrocannabinol and CP55940 on pain-stimulated versus pain-depressed behavior in rats. *J Pharmacol Exp Ther* **343**:389–400.
- Landsman RS, Burkley TH, Consroe P, Roeske WR, and Yamamura HI (1997) SR141716A is an inverse agonist at the human cannabinoid CB1 receptor. *Eur J Pharmacol* **334**:R1–R2.
- Lazenka MF, Selley DE, and Sim-Selley LJ (2014) ΔFosB induction correlates inversely with CB1 receptor desensitization in a brain region-dependent manner following repeated Δ⁹-THC administration. *Neuropharmacology* **77**:224–233.
- Lepore M, Liu X, Savage V, Matalon D, and Gardner EL (1996) Genetic differences in delta 9-tetrahydrocannabinol-induced facilitation of brain stimulation reward as measured by a rate-frequency curve-shift electrical brain stimulation paradigm in three different rat strains. *Life Sci* **58**:PL365–PL372.
- Long JZ, Li W, Booker L, Burston JJ, Kinsey SG, Schlosburg JE, Pavón FJ, Serrano AM, Selley DE, Parsons LH, et al. (2009a) Selective blockade of 2-arachidonoylglycerol hydrolysis produces cannabinoid behavioral effects. *Nat Chem Biol* **5**:37–44.
- Long JZ, Nomura DK, Vann RE, Walentiny DM, Booker L, Jin X, Burston JJ, Sim-Selley LJ, Lichtman AH, Wiley JL, et al. (2009b) Dual blockade of FAAH and MAGL identifies behavioral processes regulated by endocannabinoid crosstalk in vivo. *Proc Natl Acad Sci USA* **106**:20270–20275.
- Mechoulam R, Ben-Shabat S, Hanus L, Ligumsky M, Kaminski NE, Schatz AR, Gopher A, Almog S, Martin BR, Compton DR, et al. (1995) Identification of an endogenous 2-monoglyceride, present in canine gut, that binds to cannabinoid receptors. *Biochem Pharmacol* **50**:83–90.
- Melis M, Perra S, Muntoni AL, Pillolla G, Lutz B, Marsicano G, Di Marzo V, Gessa GL, and Pistis M (2004) Prefrontal cortex stimulation induces 2-arachidonoylglycerol-mediated suppression of excitation in dopamine neurons. *J Neurosci* **24**:10707–10715.
- Negus SS and Miller LL (2014) Intracranial self-stimulation to evaluate abuse potential of drugs. *Pharmacol Rev* **66**:869–917.
- Negus SS, Morrissey EM, Rosenberg M, Cheng K, and Rice KC (2010) Effects of kappa opioids in an assay of pain-depressed intracranial self-stimulation in rats. *Psychopharmacology* **210**:149–159.
- Niphakis MJ, Johnson DS, Ballard TE, Stiff C, and Cravatt BF (2012) O-hydroxyacetamide carbamates as a highly potent and selective class of endocannabinoid hydrolase inhibitors. *ACS Chem Neurosci* **3**:418–426.
- Oleson EB, Beckert MV, Morra JT, Lansink CS, Cachope R, Abdullah RA, Loriaux AL, Schettens D, Pattij T, Roitman MF, et al. (2012) Endocannabinoids shape accumbal encoding of cue-motivated behavior via CB1 receptor activation in the ventral tegmentum. *Neuron* **73**:360–373.
- Oleson EB and Cheer JF (2012) A brain on cannabinoids: the role of dopamine release in reward seeking. *Cold Spring Harb Perspect Med* **2**:pii: a012229.
- Panos JJ and Baker LE (2010) An in vivo microdialysis assessment of concurrent MDMA and cocaine administration in Sprague-Dawley rats. *Psychopharmacology (Berl)* **209**:95–102.
- Robledo P, Trigo JM, Panayi F, de la Torre R, and Maldonado R (2007) Behavioural and neurochemical effects of combined MDMA and THC administration in mice. *Psychopharmacology* **195**:255–264.
- SAMSHA (2012) Results from the 2011 National Survey on Drug Use and Health: Summary of National Findings, in *H-44* (NSDUH ed), NSDUH, Rockville, MD.
- Sidman M and Stebbins WC (1954) Satiation effects under fixed-ratio schedules of reinforcement. *J Comp Physiol Psychol* **47**:114–116.
- Solinas M, Justinova Z, Goldberg SR, and Tanda G (2006) Anandamide administration alone and after inhibition of fatty acid amide hydrolase (FAAH) increases dopamine levels in the nucleus accumbens shell in rats. *J Neurochem* **98**:408–419.
- Stark P and Dews PB (1980) Cannabinoids. I. Behavioral effects. *J Pharmacol Exp Ther* **214**:124–130.
- Straub CJ, Carlezon WA, Jr, and Rudolph U (2010) Diazepam and cocaine potentiate brain stimulation reward in C57BL/6J mice. *Behav Brain Res* **206**:17–20.
- Sugiura T, Kondo S, Sukagawa A, Nakane S, Shinoda A, Itoh K, Yamashita A, and Waku K (1995) 2-Arachidonoylglycerol: a possible endogenous cannabinoid receptor ligand in brain. *Biochem Biophys Res Commun* **215**:89–97.
- Tanda G, Pontieri FE, and Di Chiara G (1997) Cannabinoid and heroin activation of mesolimbic dopamine transmission by a common mu1 opioid receptor mechanism. *Science* **276**:2048–2050.
- Vandrey R, Stitzer ML, Mintzer MZ, Huestis MA, Murray JA, and Lee D (2013) The dose effects of short-term dronabinol (oral THC) maintenance in daily cannabis users. *Drug Alcohol Depend* **128**:64–70.
- Vlachou S, Nomikos GG, and Panagis G (2005) CB1 cannabinoid receptor agonists increase intracranial self-stimulation thresholds in the rat. *Psychopharmacology* **179**:498–508.
- Vlachou S, Nomikos GG, and Panagis G (2006) Effects of endocannabinoid neurotransmission modulators on brain stimulation reward. *Psychopharmacology* **188**:293–305.
- Vlachou S, Nomikos GG, Stephens DN, and Panagis G (2007) Lack of evidence for appetitive effects of Delta 9-tetrahydrocannabinol in the intracranial self-stimulation and conditioned place preference procedures in rodents. *Behav Pharmacol* **18**:311–319.
- Vlachou S and Panagis G (2014) Regulation of brain reward by the endocannabinoid system: a critical review of behavioral studies in animals. *Curr Pharm Des* **20**:2072–2088.

- Vlachou S, Stamatopoulou F, Nomikos GG, and Panagis G (2008) Enhancement of endocannabinoid neurotransmission through CB1 cannabinoid receptors counteracts the reinforcing and psychostimulant effects of cocaine. *Int J Neuropsychopharmacol* **11**:905–923.
- Wiley JL, Lowe JA, Balster RL, and Martin BR (1995) Antagonism of the discriminative stimulus effects of delta 9-tetrahydrocannabinol in rats and rhesus monkeys. *J Pharmacol Exp Ther* **275**:1–6.
- Wise LE, Long KA, Abdullah RA, Long JZ, Cravatt BF, and Lichtman AH (2012) Dual fatty acid amide hydrolase and monoacylglycerol lipase blockade produces THC-like Morris water maze deficits in mice. *ACS Chem Neurosci* **3**: 369–378.
- Xi ZX, Spiller K, Pak AC, Gilbert J, Dillon C, Li X, Peng XQ, and Gardner EL (2008) Cannabinoid CB1 receptor antagonists attenuate cocaine's rewarding effects: experiments with self-administration and brain-stimulation reward in rats. *Neuropsychopharmacology* **33**:1735–1745.

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