JPET Fast Forward. Published on November 14, 2014 as DOI: 10.1124/jpet.114.218677
This article has not been copyedited and formatted. The final version may differ from this version.

Delta-9 tetrahydrocannabinol (THC) and endocannabinoid degradative enzyme inhibitors attenuate intracranial self-stimulation (ICSS) in mice

Jason M. Wiebelhaus, Travis W. Grim, Robert A. Owens, Matthew F. Lazenka, Laura J. Sim-Selley, Rehab A. Abdullah, Micah J. Niphakis, Robert E. Vann, Benjamin F. Cravatt, Jenny L. Wiley, S. Stevens Negus, and Aron H. Lichtman

Department of Pharmacology and Toxicology, Virginia Commonwealth University School of Medicine, Richmond, VA 23298-0613 (J.M.W., T.W.G., R.A.O., M.F.L., L.J.S., R.A.A., R.E.V., S.S.N., A.H.L.); The Skaggs Institute for Chemical Biology and Department of Chemical Physiology, The Scripps Research Institute, 10550 N. Torrey Pines Rd. La Jolla, CA 92037 (M.J.N., B.F.C.); RTI International, 3040 Cornwallis Road, Research Triangle Park, NC 27709-2194 (J.L.W.)

Running Title: THC and Endocannabinoids Attenuate ICSS

Corresponding author:

Jason M. Wiebelhaus

PO Box 980613

Richmond, VA 23298, USA

Phone: (804)828-3697

Fax: (804)828-2117

Email: wiebelhausjm@vcu.edu

Text pages: 43

Tables: 1

Figures: 6 (plus 6 supplemental)

References: 55

Words in abstract: 249

Introduction: 690

Discussion: 1340

Nonstandard Abbreviations

AA: arachidonic acid;

AEA: anandamide, N-arachidonoylethanolamine;

2-AG: 2-arachidonoylglycerol;

CB_1: cannabinoid receptor type 1;

CB_2: cannabinoid receptor type 2;

FAAH: fatty acid amide hydrolase;

JZL184: 4-nitrophenyl 4-(dibenzo[d][1,3]dioxol-5-yl(hydroxy)methyl)piperidine-1-carboxylate;

JPET Fast Forward. Published on November 14, 2014 as DOI: 10.1124/jpet.114.218677
This article has not been copyedited and formatted. The final version may differ from this version.

MAGL: monoacylglycerol lipase;

NAc: nucleus accumbens

OEA: N-oleoylethanolamine;

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;

THC: Δ^9-tetrahydrocannabinol, ((−)-(6aR,10aR)-6,6,9-trimethyl-3-pentyl-6a,7,8,10a-tetrahydro-6H-benzo[c]chromen-1-ol);

VTA: ventral tegmental area

Recommended section: Behavioral Pharmacology

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 attenuated operant responding for ICSS and food, and also reduced spontaneous locomotor activity. In contrast, the FAAH inhibitor PF-3845 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 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), while peak effects of SA-57 were associated with increased levels of both *N*-arachidonoylethanolamine (anandamide; AEA) and 2-AG. The CB_1 receptor antagonist rimonabant, but not the CB_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 non-reinforced behaviors.

Introduction

Cannabis remains the most commonly used illicit drug for decades (SAMSHA, 2012). Marijuana users report positive subjective ratings (Hart et al., 2001; Hart et al., 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 CNS-mediated behavioral effects through the activation of cannabinoid CB_1 receptors (Wiley et al., 1995; Compton et al., 1996). Similarly, the endocannabinoids *N*-arachidonoylethanolamine (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; Cravatt et al., 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).

THC and AEA facilitate dopamine release in the nucleus accumbens (NAc) in rats (Chen et al., 1990; Chen et al., 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 CB_1 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), while 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, CP55,940, levonantradol, nabilone, and HU210, attenuate ICSS (Negus and Miller, 2014). Similarly, the FAAH inhibitor URB597 (Vlachou et al., 2006; Kwilasz et al., 2014) and the purported AEA transport inhibitor AM-404 (Vlachou et al., 2008) reduce ICSS. Moreover, some studies have shown CB_1 receptor antagonists reduced ICSS behavior (Arnold et al., 2001; Deroche-Gamonet et al., 2001; De Vry et al., 2004; Xi et al., 2008), while others have found no effect (Vlachou et al., 2005; Vlachou et al., 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. Towards this end, we examined the dose-response relationships of the FAAH inhibitor PF-3845 (Ahn et al., 2009), the MAGL inhibitor JZL184 (Long et al., 2009a), and the dual FAAH/MAGL inhibitor SA-57 (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 CB_1 and CB_2 receptors have been implicated in modulation of the mesolimbic dopamine system, the selected antagonists of these receptors, rimonabant and SR144528, were used to infer the respective involvement of CB_1 and CB_2 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 inhibitors on AEA, 2-AG, and other relevant lipids in brain regions associated with the mesolimbic dopaminergic system, including VTA, NAc, prefrontal cortex, 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 Jackson Laboratories (Bar Harbor, Maine) served as subjects. Mice were between 8 and 12 weeks of age and weighed between 22 and 30 g at the commencement of each experiment, and were individually housed and maintained on a 12 h light cycle (lights on from 0600 to 1800 h), with free access to food and water, except in experiments assessing operant responding for food reinforcement. All experiments were conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals, and were approved by the Virginia Commonwealth University Institutional Animal Care and Use Committee (IACUC).

Drugs

JZL184 (4-nitrophenyl 4-(dibenzo[d][1,3]dioxol-5-yl(hydroxy)methyl)piperidine-1-carboxylate), rimonabant (5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-N-1-piperidinyl-1H-pyrazole-3-carboxamide), SR144528 (5-(4-chloro-3-methylphenyl)-1-[(4-methylphenyl)methyl]-N-[(1S,2S,4R)-1,3,3-trimethylbicyclo[2.2.1]hept-2-yl]-1H-pyrazole-3-carboxamide), THC ((−)-(6aR,10aR)-6,6,9-trimethyl-3-pentyl-6a,7,8,10a-tetrahydro-6H-benzo[c]chromen-1-ol) (obtained from NIDA drug supply, RTI, Research Triangle Park, NC), SA-57 (synthesized in the Cravatt laboratory at TSRI, 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 (1R,2R,3S,5S,8R)-3-(benzoyloxy)-2-(methoxycarbonyl)-8-

methyl-8-azoniabicyclo[3.2.1]octane chloride) (obtained from NIDA drug supply, RTI, Research Triangle Park, NC) was dissolved in 0.9% saline.

Apparatus

ICSS testing was conducted using eight standard (18 X 18 X 18 cm) mouse operant conditioning chambers (Med Associates Inc., St. Albans, VT). Each chamber was equipped with a retractable lever located in the right-hand position on the front chamber wall, 2 LED stimulus 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 Inc. 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 Inc., St. Albans, VT) 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 commuators and tethers.

Locomotor activity testing was conducted in Plexiglas chambers (17.5 X 8.5 X 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 and colleagues: 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 post-operative care for five days following surgery, including treatment with bacitracin and acetaminophen, and were introduced to operant chambers one week after surgery.

Behavioral Procedures

ICSS. One week after stereotaxic surgery, mice began lever-press training. Subjects were trained on a fixed-ratio 1 (FR1) schedule of reinforcement, in which each lever press delivered a 0.5 s train of square-wave cathodal pulses (0.1 ms 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 30–60 min FR1 training sessions until they maintained stable responding at response rates greater than 35 responses per min. Prior to testing, the

stimulation amplitudes were adjusted individually from 20–300 μA to maintain maximal response rates. After operant responding for ICSS stabilized, mice began frequency-rate training, using a similar procedure employed by Negus and colleagues (Negus et al., 2010). Detailed methods related to rate-frequency training are provided in the supplemental data section.

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 min for cocaine, 30 min for THC, and 120 min 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 CB_1 and CB_2 receptors in the effects of each drug on ICSS, rimonabant or SR144528 was administered 15 min 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. Naïve 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 min training sessions, mice were assigned to a particular nose-poke aperture, while the other aperture on the opposite side of the chamber was blocked with a rubber stopper to prevent spontaneous responding. Responding in the aperture under a fixed ratio 1 (FR-1) schedule of reinforcement resulted in delivery of 1 food pellet reinforcer (Bio-Serv), and FR value was gradually increased to a FR-10

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 min and total number of responses remained within 10% of that of the prior two training days on at least three consecutive training days. These criteria were evaluated before every operant test was conducted, and mice had a minimum of 96 h 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 wash-out period of at least one 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. Naïve mice (n = 7–12 per group) were acclimated to the testing room 24 h 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, Kiel, WI) software. Distance travelled was calculated during the 25 min observation period. Box assignment and time of day were counter-balanced between the different drug/dose conditions. Separate groups of naïve mice were used for each treatment condition.

Endocannabinoid and eicosanoid analysis

AEA, 2-AG, arachidonic acid, oleoylethanolamide (OEA), and palmitoylethanolamide (PEA) were quantified in prefrontal cortex, nucleus accumbens, and amygdala, as well as the control region, the cerebellum. Drug naïve 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) i.p. 2 h prior to sacrifice. Immediately following cervical dislocation and decapitation, brains were removed and dissected, and the prefrontal cortex, nucleus accumbens, 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). Detailed methods on the extraction and quantification of endocannabinoids and eicosanoids are included in the supplemental data section.

Data Analysis

The independent variable measured in the ICSS studies was stimulations per min for each frequency. ICSS data were evaluated using two separate approaches, as previously reported (Negus and Miller, 2014). The first method examined % 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 and 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 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 (% MCR). Repeated-measures two-way ANOVA (treatment x frequency) were 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 the supplemental materials. 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 travelled was observed during locomotor activity studies and analyzed using independent sample one-way ANOVA followed. Response rate (responses/min) was the dependent variable of interest in the food-reinforced operant responding studies, and was analyzed using repeated-measures one-way ANOVA. Brain endocannabinoid and AA 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, while cocaine produced dose-related ICSS facilitation (Fig. 1; Supplemental Figure 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 Figure 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 mg/kg and 17.8 mg/kg (Fig. 1E), providing a positive control for this series of experiments, and frequency-rate curves showed similar results (Supplemental Figure 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 min after administration, and behavior returned to baseline levels by 24 h (Fig. 2A; Fig. S3). Administration of 30 mg/kg PF-3845 (Fig. 2B) or 40.0 mg/kg JZL184 (Fig. 2C) attenuated ICSS up to 48 h after drug administration. SA-57 (10.0 mg/kg) reduced responding for electrical stimulation 8 h 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 Figure 3; other data not shown).

Antagonism of cannabinoid effects on ICSS. In order to infer the involvement of CB_1 and CB_2 receptors, rimonabant or SR144528 were administered prior to cannabinoids and enzyme inhibitors. Rimonabant blocked depression of ICSS produced by THC (10 mg/kg, Fig. 3A; Supplemental Figure 4), JZL184 (40 mg/kg, Fig. 3C;

Supplemental Figure 5), or SA-57 (10 mg/kg, Fig. 3D; Supplemental Figure 6). Frequency-rate curves for rimonabant challenges of THC, JZL184, and SA-57 are shown in Supplemental Figures 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 travelled 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 prefrontal cortex (PFC), nucleus accumbens (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 CB_1 receptor mechanism of action, which is in agreement with reports that CB_1 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 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, while 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, sub-threshold 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, or 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 CB_1 or CB_2 receptor antagonists. While this

depression of ICSS may be mediated through other targets associated with FAAH substrates (e.g., TRPV1 or PPARα receptors), nonspecific effects of this high dose cannot be neglected. However, it should be noted that URB597 and the purported AEA transport inhibitor AM-404 produced CB_1 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 CB_1 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 CB_1 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 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 prefrontal cortex, 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 CB_1

receptor overstimulation by endocannabinoids, each of these effects was blocked by rimonabant.

Here, we also found that rimonabant alone did not affect ICSS in mice. Likewise, CB_1 receptor antagonists given alone did not affect ICSS in rats (De Vry et al., 2004; Vlachou et al., 2005; Vlachou et al., 2006; Xi et al., 2008; Kwilasz and Negus, 2012). In other studies, however, rimonabant reduced ICSS in rats at moderate doses (1 and 3 mg/kg) or high doses (10 or 20 mg/kg). While these latter findings are consistent with the idea that endocannabinoids tonically modulate ICSS in rats, CB_1 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 CB_2 receptors are dispensable in this behavior. There are no previous studies of which we are aware that have examined the consequences of CB_2 receptor antagonists on ICSS. The results of the present study indicate that CB_1 and CB_2 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 CB_1 receptor activation in mice are not limited to ICSS. Accordingly, motor suppression may, in part, contribute to CB_1 receptor-mediated disruption of ICSS. Employment of methods that dependent less on response 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 (Kwilasz and Negus, 2012).

CB_1 receptors are expressed on glutamatergic (Melis et al., 2004; Fernandez-Ruiz et al., 2010), cholinergic, and GABAergic neuronal terminals. The finding that CB_1 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%, which is considerably less than that induced by cocaine (about 400% of basal levels). While 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 psychological factors such as 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 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 s.c. 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 CB_1 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. While 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 CB_1 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.

JPET Fast Forward. Published on November 14, 2014 as DOI: 10.1124/jpet.114.218677
This article has not been copyedited and formatted. The final version may differ from this version.

Acknowledgements

The authors thank Mr. Justin Poklis for quantifying brain endocannabinoid levels.

Authorship Contributions

Participated in research design: Wiebelhaus, Grim, Owens, Cravatt, Niphakis, Sim-Selley, Vann, Negus, Lichtman

Conducted experiments: Wiebelhaus, Grim, Lazenka, Owens, Abdullah

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, Lazerwith S, Stiff C, Kamtekar S, Bhattacharya K, Zhang Y, Swaney S, Van Becelaere K, Stevens RC and Cravatt BF (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 (Berl)* **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 U S A* **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 (Berl)* **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 U S A* **99**:10819-10824.

Fernandez-Ruiz J, Hernandez M and Ramos JA (2010) Cannabinoid-dopamine interaction in the pathophysiology and treatment of CNS disorders. *CNS Neurosci Ther* **16**:e72-91.

Filbey FM, Schacht JP, Myers US, Chavez RS and Hutchison KE (2009) Marijuana craving in the brain. *Proc Natl Acad Sci U S A* **106**:13016-13021.

Fish EW, Riday TT, McGuigan MM, Faccidomo S, Hodge CW and Malanga CJ (2010) Alcohol, cocaine, and brain stimulation-reward in C57Bl6/J and DBA2/J 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 (Berl)* **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 and Franklin TR (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 (Berl)* **181**:237-243.

Hart CL, Ilan AB, Gevins 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 Delta9-tetrahydrocannabinol on brain stimulation reward and motor activity. *Int J Neuropsychopharmacol*:1-12.

Koob GF and Volkow ND (2010) Neurocircuitry of addiction. *Neuropsychopharmacology* **35**:217-238.

Kwilasz 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.

Kwilasz AJ and Negus SS (2012) Dissociable effects of the cannabinoid receptor agonists Delta9-tetrahydrocannabinol and CP55940 on pain-stimulated versus pain-depressed behavior in rats. *J Pharmacol Exp Ther* **343**:389-400.

Landsman RS, Burkey TH, Consroe P, Roeske WR and Yamamura HI (1997) SR141716A is an inverse agonist at the human cannabinoid CB1 receptor. *Eur J Pharmacol* **334**:R1-2.

Lazenka MF, Selley DE and Sim-Selley LJ (2014) DeltaFosB induction correlates inversely with CB(1) receptor desensitization in a brain region-dependent manner following repeated Delta(9)-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**:PL365-372.

Long JZ, Li W, Booker L, Burston JJ, Kinsey SG, Schlosburg JE, Pavon FJ, Serrano AM, Selley DE, Parsons LH, Lichtman AH and Cravatt BF (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 and Cravatt BF (2009b) Dual blockade of FAAH and MAGL identifies behavioral processes regulated by endocannabinoid crosstalk in vivo. *Proc Natl Acad Sci U S A* **106**:20270-20275.

Mechoulam R, Ben-Shabat S, Hanus L, Ligumsky M, Kaminski NE, Schatz AR, Gopher A, Almog S, Martin BR, Compton DR and 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-arachidonoyl-glycerol-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* **in press**.

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 (Berl)* **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, Schetters D, Pattij T, Roitman MF, Lichtman AH and Cheer JF (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 Harbor perspectives in medicine* **2**.

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 (Berl)* **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. *Behavioural Brain Research* **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 (Berl)* **179**:498-508.

Vlachou S, Nomikos GG and Panagis G (2006) Effects of endocannabinoid neurotransmission modulators on brain stimulation reward. *Psychopharmacology (Berl)* **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. *Current pharmaceutical design* **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.

Footnotes

a) Financial support was provided by the National Institutes of Health grants [T32DA007027, F31DA030869, P01DA009789, P01DA017259, P30DA033934, R01DA026449, R01DA032933, R01DA030404] and a Toni Rosenberg Fellowship.

b) This work was part of a thesis conducted and written by Jason M. Wiebelhaus in the department of Psychology at Virginia Commonwealth University. Sections of this work were presented at scientific meetings listed below:

Wiebelhaus, JM. (2013) THC and endocannabinoid catabolic enzyme inhibitors attenuate ICSS in C57BL/6 mice. Oral presentation at the Carolina Cannabinoid Collaborative (CCC) meeting

Wiebelhaus, JM, Grim, TW & Lichtman, AH. (2012). Investigating the effects monoacylglycerol lipase inhibition using intracranial self-stimulation (ICSS) in mice. Invited poster presentation at the NIDA mini-convention, Frontiers in Addiction Research, held in conjunction with the Society for Neuroscience (SFN) Annual Meeting

c) Jason M. Wiebelhaus email: wiebelhausjm@vcu.edu
address: Virginia Commonwealth University, School of Medicine, Department of Pharmacology and Toxicology 1112 E. Clay St. Suite 100 P.O. Box 980613, Richmond, VA, 23298-0613

Figure Legends

Fig. 1. THC and endocannabinoid catabolic enzyme inhibitors significantly attenuated ICSS, while cocaine facilitated ICSS. **Panel A**. THC administered 30 min prior to testing significantly decreased operant responding for ICSS ($F_{6, 30} = 24.1$, $p < 0.001$; n = 7). **Panel B**. PF-3845 administered 2 h prior to testing significantly reduced ICSS ($F_{4, 24} = 4.48$, $p < 0.01$; n = 9), though only an excessively high dose (30 mg/kg) significantly reduced ICSS. **Panel C**. JZL184 administered 2 h prior to testing significantly decreased ICSS ($F_{4, 24} = 4.48$, $p < 0.01$; n = 7). **Panel D**. SA-57 administered 2 h prior to testing significantly decreased ICSS ($F_{4, 24} = 49.25$, $p < 0.001$) with a magnitude similar to that THC. Panel **Panel E**. Cocaine given 10 min prior to testing significantly facilitated ICSS ($F_{4, 20} = 15.37$, $p < 0.001$; n = 6). *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ vs. vehicle (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 ± S.E.M.

Fig. 2. Time course of decreased operant responding for ICSS produced by THC (10 mg/kg, n = 7 mice/group; **Panel A**), PF-3845 (30 mg/kg, n = 8 mice/group; **Panel B**), JZL184 (40 mg/kg, n = 6 mice/group; **Panel C**), and SA-57(10 mg/kg, n = 7 mice/group; **Panel D**). Two way ANOVAs (time-point X 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 ($F_{5, 25} = 33.30$, $p < 0.001$). *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ vs. appropriate vehicle (VEH) group (Holm-Sidak's *post hoc* test). All values reflect mean ± S.E.M.

Fig. 3. Evaluation of whether CB_1 receptors mediate the disruptive effects of THC and endocannabinoid catabolic enzyme inhibitors on ICSS. Rimonabant significantly

JPET Fast Forward. Published on November 14, 2014 as DOI: 10.1124/jpet.114.218677
This article has not been copyedited and formatted. The final version may differ from this version.

blocked ICSS depression produced by THC (10 mg/kg; two-way ANOVA interaction: $F_{1, 6} = 90.8$, $p < 0.001$; n = 7; **Panel A**), JZL184 (40 mg/kg; two-way ANOVA interaction: $F_{1, 7} = 7.66$, $p < 0.05$; n = 8; **Panel C**), and SA-57 (10 mg/kg; two-way ANOVA interaction: $F_{2, 10} = 25.90$, $p < 0.05$; n = 6; **Panel D**). **Panel 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 rimonabant, and no interaction between PF-3845 and rimonabant; n = 6). *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ vs. VEH + VEH or VEH + RIM condition (Holm-Sidak's *post hoc* test). All values represent mean ± S.E.M.

Fig. 4. CB_2 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 min prior to the test compound did not alter decreased operant responding for ICSS produced by: **Panel A**. THC (10 mg/kg; main effect of drug, only: $F_{1, 4} = 43.66$, $p < 0.01$; n = 5), **Panel B.** PF-3845 (30 mg/kg; main effect of drug, only: $F_{1, 5} = 7.96$, $p < 0.05$; n = 6), **Panel 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 SR2 (10 mg/kg) or VEH (Holm-Sidak's post-hoc test); n = 8), or **Panel D.** SA-57 (10 mg/kg; main effect of drug, only: $F_{1, 5} = 153.6$, $p < 0.001$; n = 6). *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ vs. VEH + VEH and VEH + SR2. Data reflect mean ± S.E.M.

Fig. 5. Food-maintained operant responding was attenuated by THC, JZL184, and SA-57, but PF-3845 had no effect. **Panel 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). **Panel B.** PF-3845 did not alter operant responding for food (*F*

JPET Fast Forward. Published on November 14, 2014 as DOI: 10.1124/jpet.114.218677
This article has not been copyedited and formatted. The final version may differ from this version.

$_{3, 28}$ = 0.50, p = 0.69; n = 8). **Panel C**. JZL184 attenuated operant responding for food (F $_{4, 32}$ = 9.89, p < 0.001; n = 9). **Panel 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 vs. respective VEH group (Dunnett's *post hoc* test). All values represent mean ± S.E.M.

Fig. 6. Spontaneous locomotor activity was reduced by THC and endocannabinoid catabolic enzyme inhibitors. **Panel A**. THC significantly reduced locomotor activity (F $_{4, 31}$ = 7.04, p < 0.001; n = 7–8). **Panel B**. PF-3845 significantly reduced activity (F $_{3, 28}$ = 3.14, p < 0.05; n = 8). **Panel C**. JZL184 significantly decreased spontaneous locomotor activity (F $_{3, 31}$ = 8.9, p < 0.001; n = 7-12). **Panel 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 vs. appropriate vehicle (VEH) group (Dunnett's *post hoc* test). All values represent mean ± S.E.M.

Table 1

Summary of AEA, 2-AG, arachidonic acid (AA), OEA and PEA levels in brain regions mediating ICSS after treatment with enzyme inhibitors. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ vs. vehicle (VEH) as determined by Dunnett's *post hoc* test. Values represent mean ± SEM.

Drug	Region	Dose (mg/kg)	AEA (pm/g)	2-AG (nm/g)	AA (nm/g)	OEA (pm/g)	PEA (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***

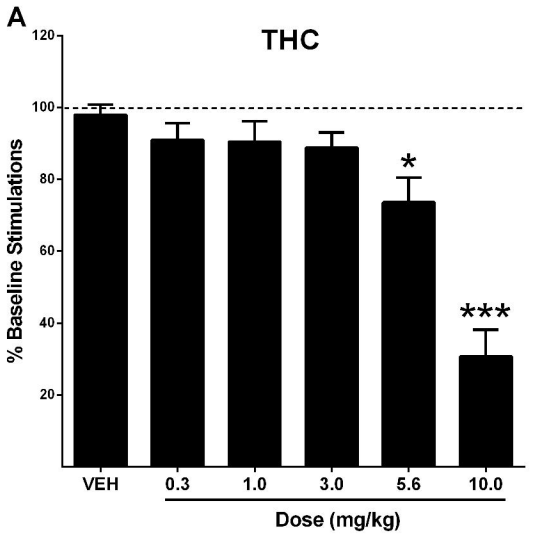
	30	47.8 ± 3.82	12.9 ± 1.42	166 ± 3.93	850 ± 36.8***	926 ± 84.2***
Cerebellum	0 (VEH)	6.33 ± 0.57	6.64 ± 0.21	77.3 ± 3.06	190 ± 23.4	228 ± 26.4
	10	44.5 ± 3.44***	6.92 ± 0.82	77.2 ± 3.90	1306 ± 48.0***	1159 ± 104***
	30	39.4 ± 3.02***	8.31 ± 0.84	76.6 ± 2.54	1405 ± 43.7***	1175 ± 91.5***
JZL184 (MAGL 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

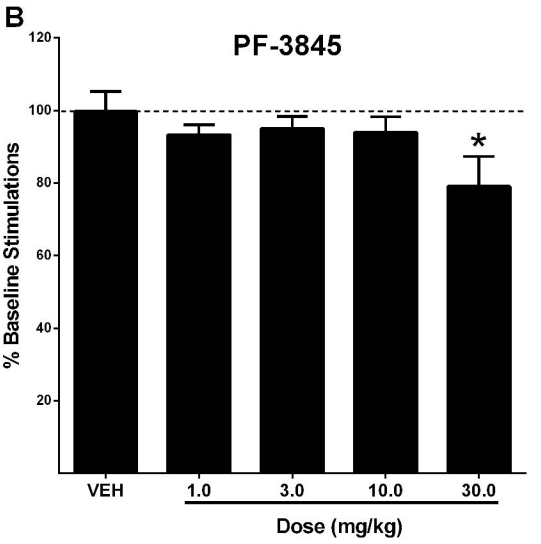
	40	12.7 ± 2.23	139 ± 11.0***	119 ± 8.52**	59.8 ± 6.82	57.9 ± 1.02
Cerebellum	0 (VEH)	9.0 ± 1.28	13.0 ± 1.67	76.6 ± 2.92	136 ± 5.15	153 ± 3.30
	4	8.1 ± 0.80	28.6 ± 2.58***	73.8 ± 2.58	140 ± 3.98	160 ± 6.70
	40	7.1 ± 0.62	50.8 ± 2.18***	52.7 ± 1.33***	136 ± 6.77	165 ± 9.38
SA-57						
(FAAH/MAGL inhibitor)						
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***

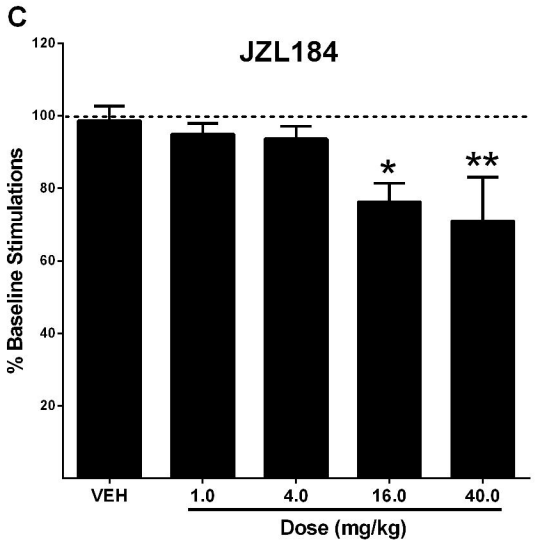
Cerebellum	0 (VEH)	5.34 ± 0.78	4.67 ± 0.22	66.7 ± 3.81	134 ± 3.73	161 ± 10.3
	1	29.2 ± 2.91***	10.6 ± 0.67	69.1 ± 2.91	1325 ± 53.1***	1312 ± 87.7***
	10	33.1 ± 3.97***	51.7 ± 3.38***	38.2 ± 2.15***	1231 ± 38.8***	1190 ± 78.9***

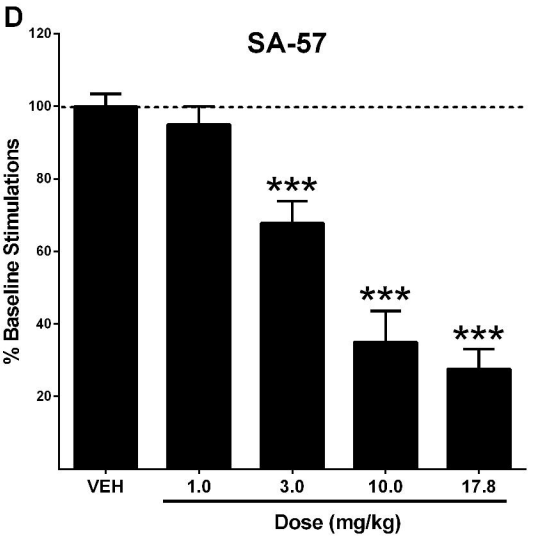
PFC, prefrontal cortex; NAc, nucleus accumbens; ventral midbrain includes the VTA

Figure 1


A
THC
% Baseline Stimulations
120
100
80
60
40
20
*
***
VEH
0.3
1.0
3.0
5.6
10.0
Dose (mg/kg)


B
PF-3845
% Baseline Stimulations
120
100
80
60
40
20
*
VEH
1.0
3.0
10.0
30.0
Dose (mg/kg)


C
JZL184
% Baseline Stimulations
120
100
80
60
40
20
*
**
VEH
1.0
4.0
16.0
40.0
Dose (mg/kg)


D
SA-57
% Baseline Stimulations
120
100
80
60
40
20
***
***
***
VEH
1.0
3.0
10.0
17.8
Dose (mg/kg)

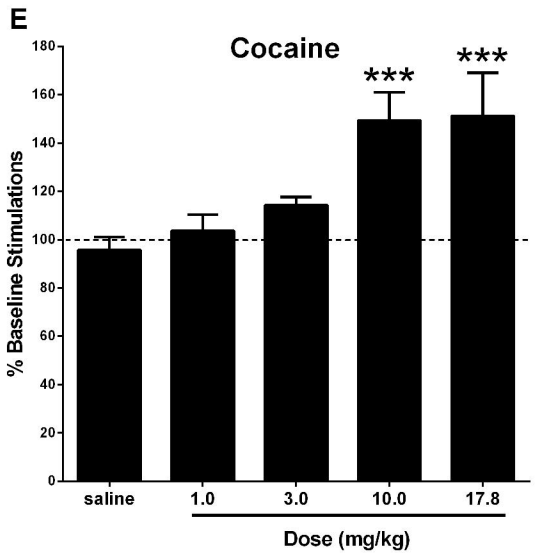

E
Cocaine
% Baseline Stimulations
180
160
140
120
100
80
60
40
20
0
***
***
saline
1.0
3.0
10.0
17.8
Dose (mg/kg)

Figure 2

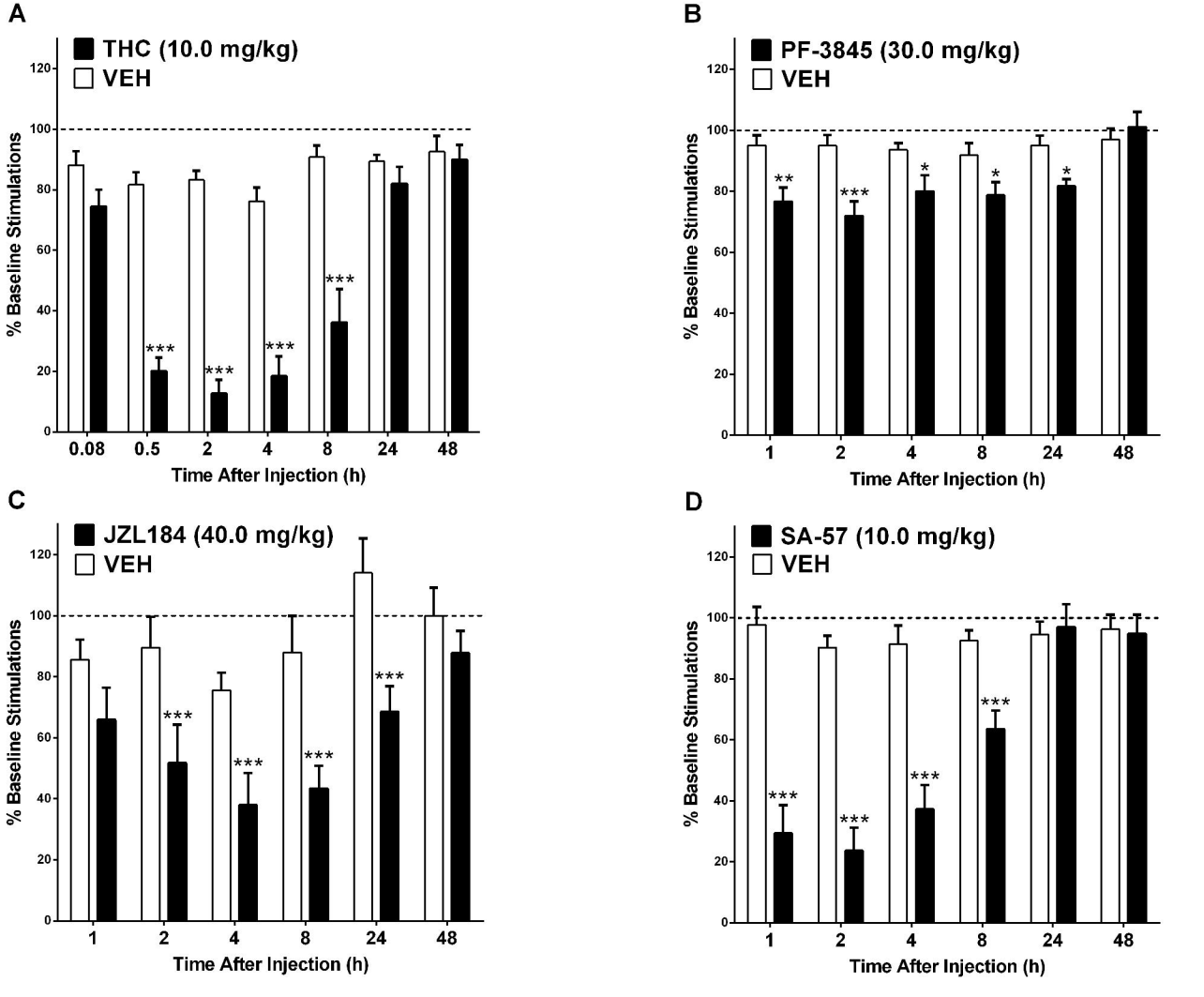

A
THC (10.0 mg/kg)
VEH
% Baseline Stimulations
Time After Injection (h)
0.08
0.5
2
4
8
24
48
B
PF-3845 (30.0 mg/kg)
VEH
% Baseline Stimulations
Time After Injection (h)
1
2
4
8
24
48
C
JZL184 (40.0 mg/kg)
VEH
% Baseline Stimulations
Time After Injection (h)
D
SA-57 (10.0 mg/kg)
VEH
% Baseline Stimulations
Time After Injection (h)

Figure 3

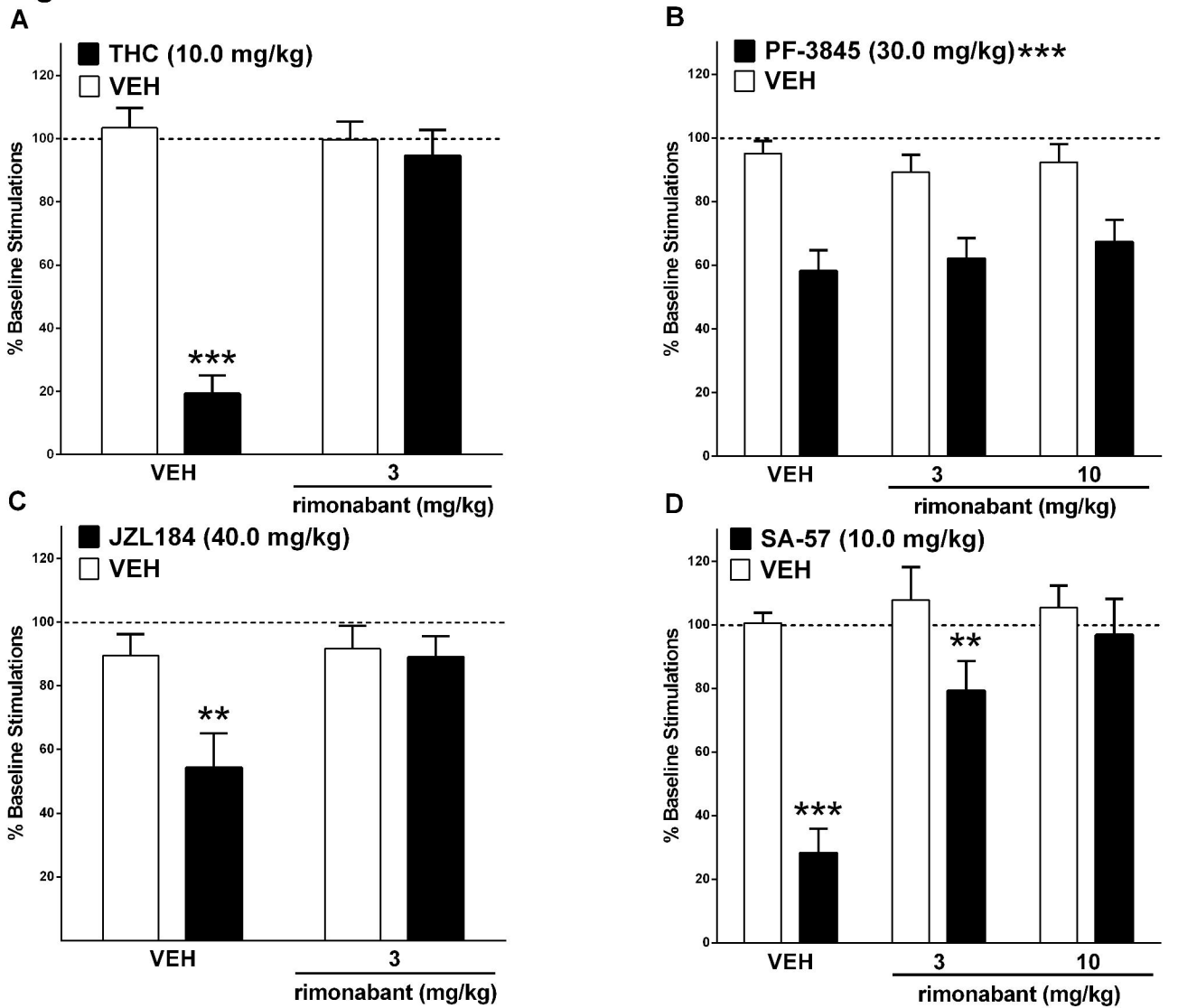

A
THC (10.0 mg/kg)
VEH
% Baseline Stimulations
***
VEH
3
rimonabant (mg/kg)
B
PF-3845 (30.0 mg/kg)***
VEH
% Baseline Stimulations
VEH
3
10
rimonabant (mg/kg)
C
JZL184 (40.0 mg/kg)
VEH
% Baseline Stimulations
**
VEH
3
rimonabant (mg/kg)
D
SA-57 (10.0 mg/kg)
VEH
% Baseline Stimulations
***
**
VEH
3
10
rimonabant (mg/kg)

Figure 4

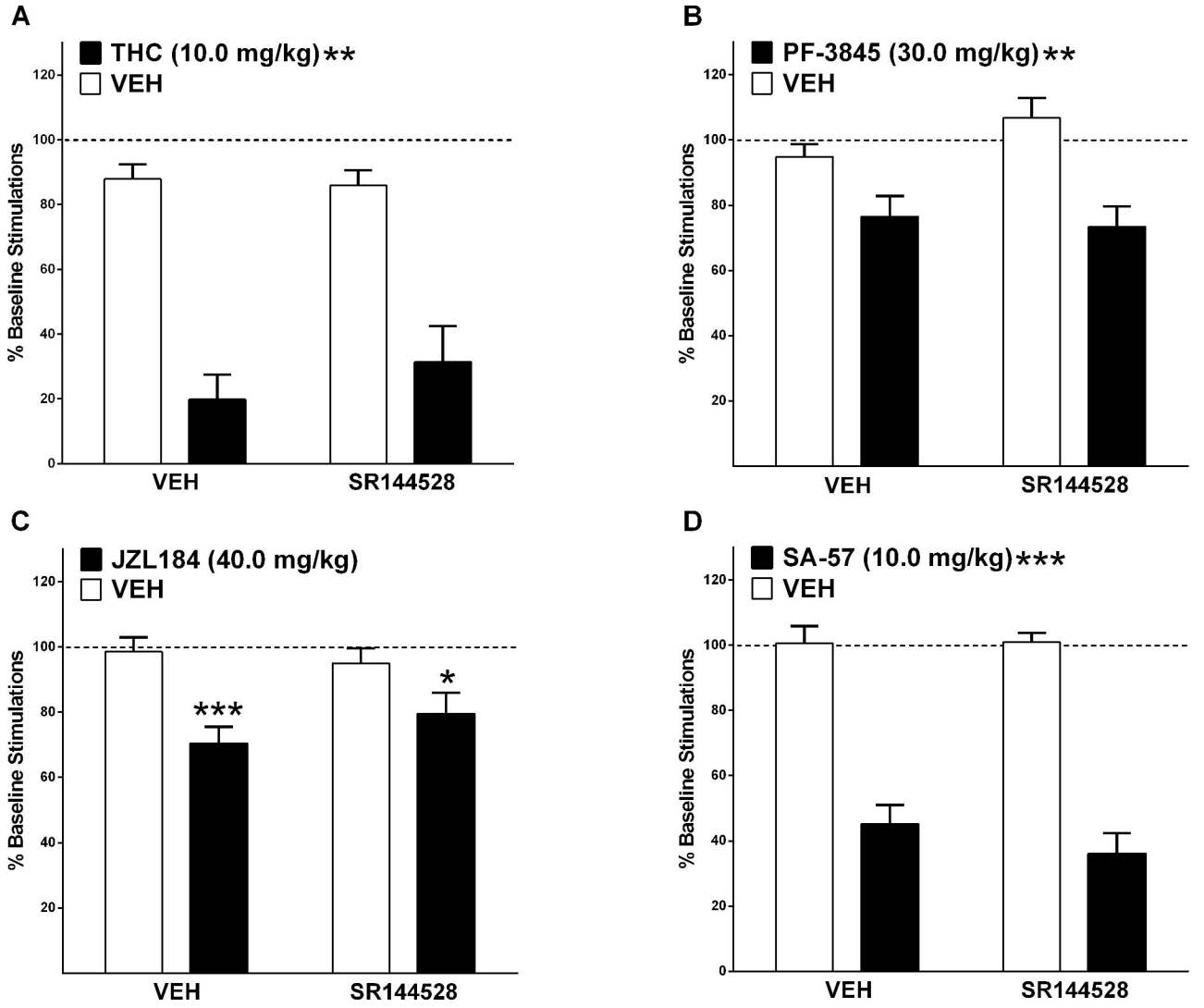

A
THC (10.0 mg/kg)**
VEH
% Baseline Stimulations
120
100
80
60
40
20
0
VEH
SR144528
B
PF-3845 (30.0 mg/kg)**
VEH
% Baseline Stimulations
120
100
80
60
40
20
VEH
SR144528
C
JZL184 (40.0 mg/kg)
VEH
% Baseline Stimulations
120
100
80
60
40
20
***
*
VEH
SR144528
D
SA-57 (10.0 mg/kg)***
VEH
% Baseline Stimulations
120
100
80
60
40
20
0
VEH
SR144528

Figure 5

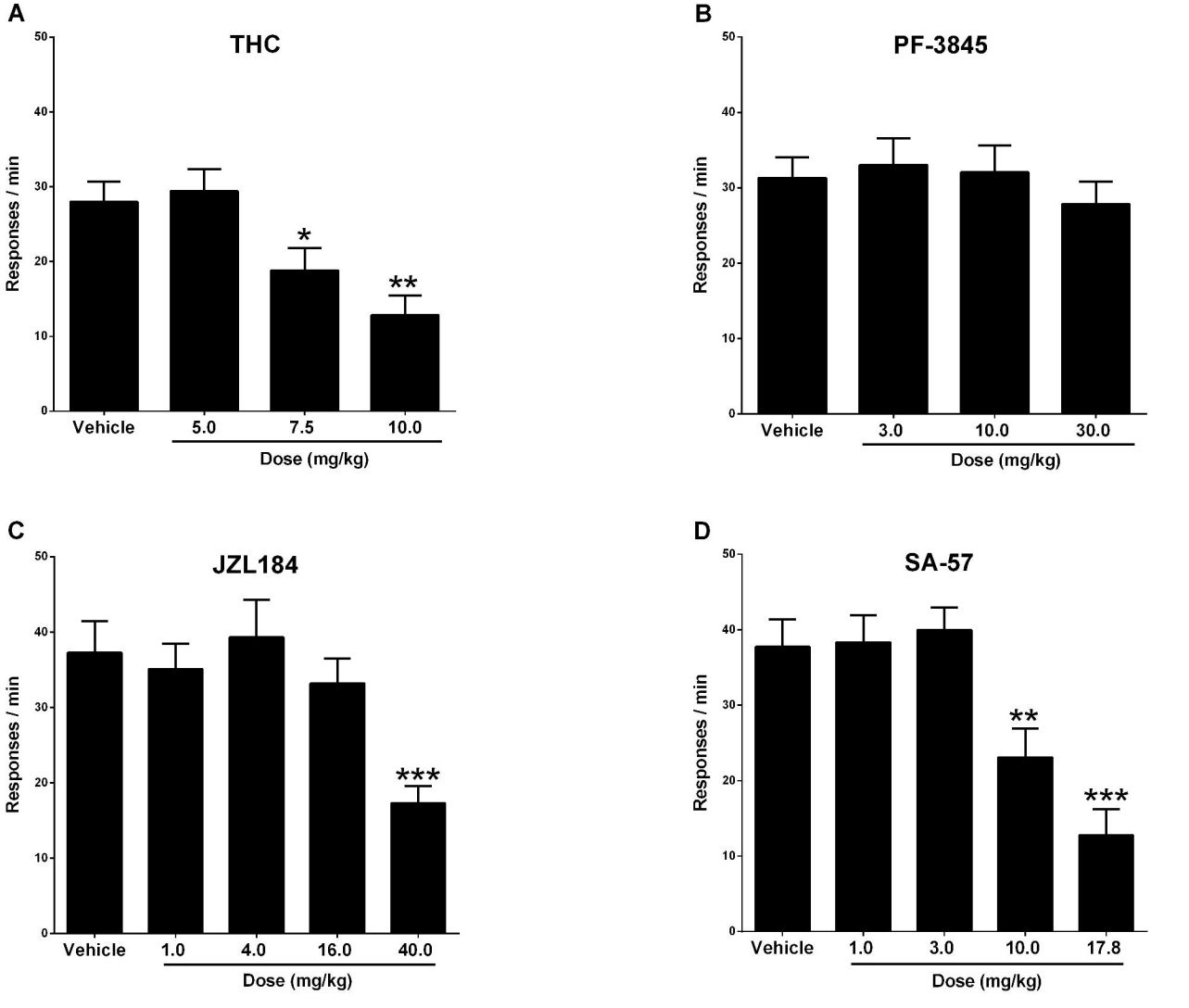
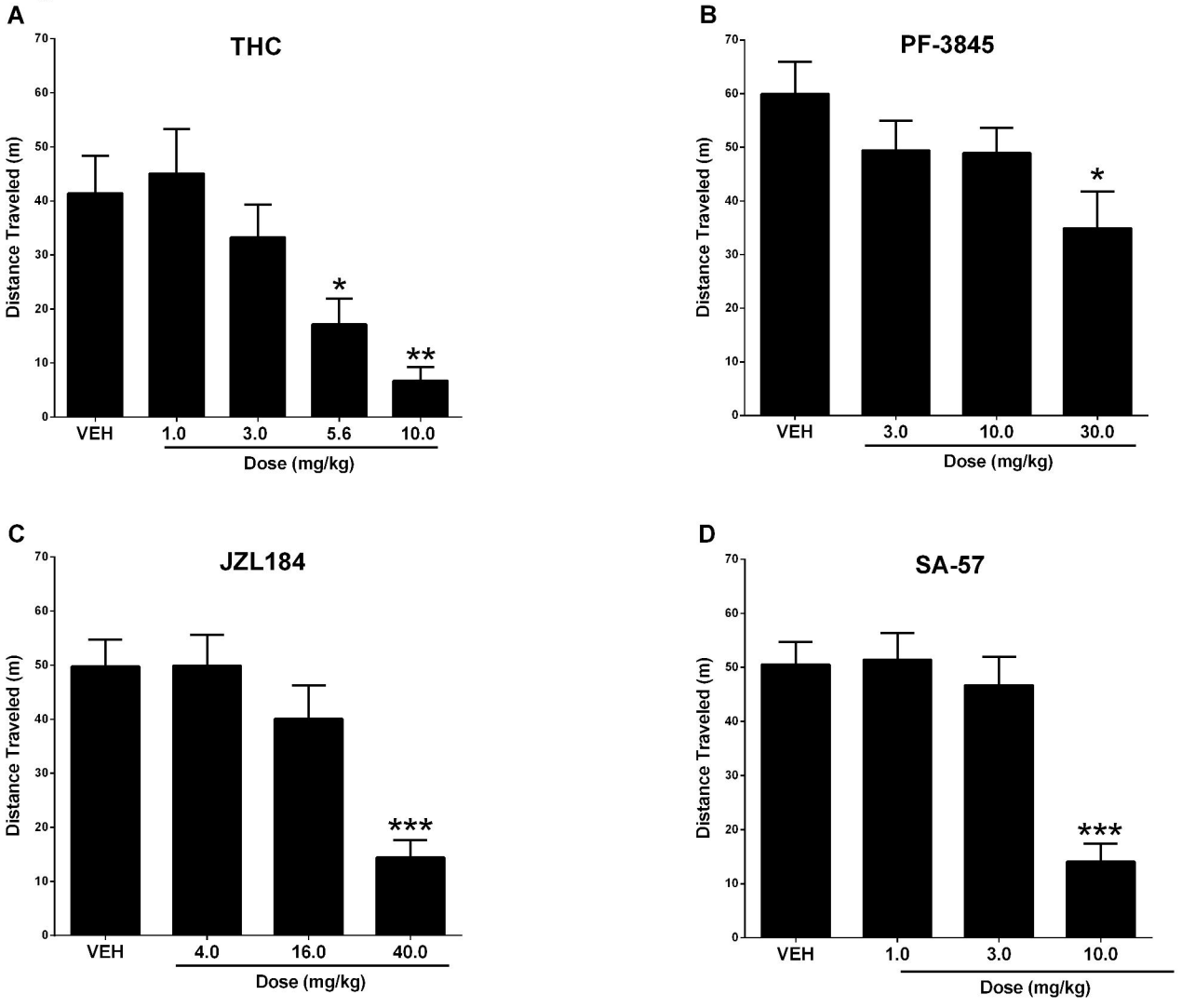

A
THC
Responses / min
50
40
30
20
10
0
*
**
Vehicle
5.0
7.5
10.0
Dose (mg/kg)
B
PF-3845
Responses / min
Vehicle
3.0
10.0
30.0
Dose (mg/kg)
C
JZL184
Responses / min
***
Vehicle
1.0
4.0
16.0
40.0
Dose (mg/kg)
D
SA-57
Responses / min
**
***
Vehicle
1.0
3.0
10.0
17.8
Dose (mg/kg)

Figure 6


A
THC
Distance Traveled (m)
0
10
20
30
40
50
60
70
VEH
1.0
3.0
5.6
10.0
*
**
Dose (mg/kg)
B
PF-3845
Distance Traveled (m)
VEH
3.0
10.0
30.0
*
Dose (mg/kg)
C
JZL184
Distance Traveled (m)
VEH
4.0
16.0
40.0
***
Dose (mg/kg)
D
SA-57
Distance Traveled (m)
VEH
1.0
3.0
10.0
***
Dose (mg/kg)