



Virginia Commonwealth University  
**VCU Scholars Compass**

---

Theses and Dissertations

Graduate School

---

2013

## Cannabinoid Modulation of Reinforcement Maintained by Stimulation of the Medial Forebrain Bundle in C57Bl/6J Mice

Jason Wiebelhaus  
*Virginia Commonwealth University*

Follow this and additional works at: <https://scholarscompass.vcu.edu/etd>



Part of the [Psychology Commons](#)

© The Author

---

Downloaded from

<https://scholarscompass.vcu.edu/etd/555>

This Dissertation is brought to you for free and open access by the Graduate School at VCU Scholars Compass. It has been accepted for inclusion in Theses and Dissertations by an authorized administrator of VCU Scholars Compass. For more information, please contact [libcompass@vcu.edu](mailto:libcompass@vcu.edu).

© Jason Michael Wiebelhaus, 2013

All Rights Reserved

Cannabinoid Modulation of Reinforcement Maintained by Stimulation of the Medial Forebrain  
Bundle in C57Bl/6J Mice

Dissertation to be submitted in partial fulfillment of the requirements for the degree of Doctor of  
Philosophy at Virginia Commonwealth University

By: Jason M. Wiebelhaus  
Master of Science, Virginia Commonwealth University, 2009  
Bachelor of Science, University of WI-Eau Claire, 2006

Co-Directors: Aron H. Lichtman<sup>1</sup>, Ph.D. and Joseph H. Porter<sup>2</sup>, Ph.D.  
<sup>1, 2</sup>Professors  
<sup>1</sup>Department of Pharmacology and Toxicology, <sup>2</sup>Department of Psychology

Virginia Commonwealth University  
Richmond, VA  
September, 2013

## **Acknowledgement**

I owe my thanks to many people who helped me throughout the process of conducting these experiments and writing my dissertation. First and foremost I would like to thank my mentor and dissertation co-director director Aron H. Lichtman for his guidance and patience throughout my years of graduate school at Virginia Commonwealth University. I would also like to thank my committee members Joseph H. Porter, Robert J. Hamm, Laura J. Sim-Selley, S. Stevens Negus, Scott R. Vrana and Laura E. Wise for their teachings, suggestions and support that led to vast improvements with these studies. I must also extend my gratitude to fellow graduate students and colleagues including, Matt Walentiny, Tom Gamage, Sudeshna Ghosh, Travis Grim, Allen Owens, Matt Lazenka, Andrew Kwilas, and Jon Warner for their stimulating ideas and contributions to this work. They have provided me with many insights and words of advice that contributed to the success of these studies. I would also like to acknowledge my undergraduate research advisor Dr. David C. Jewett for teaching me the foundations behavioral pharmacology, and spurring my interest in becoming a scientist. Last, but not least, I would like to thank my parents, Ron and Eileen Wiebelhaus, my brother, David and my sister Noelle for their endless support, and especially their willingness to listen. This work would not have been possible without all of their contributions.

## Table of Contents

	Page
Acknowledgement .....	ii
List of Tables .....	v
List of Figures .....	vi
Abstract .....	viii
 I. Introduction .....	 1
Endogenous Cannabinoid System.....	1
Endocannabinoids .....	2
AEA and 2-AG: Distinct Endocannabinoids .....	6
Behavioral Effects of MAGL Inhibition.....	8
Behavioral Effects of FAAH inhibition.....	10
Behavioral Effects of Combined FAAH and MAGL inhibition.....	11
Cannabinoids and Reward .....	12
Preclinical Models of Reinforcement and Reward-Associated Behavior.....	13
Self-administration.....	13
Conditioned Place Preference .....	14
Intracranial Self-Stimulation.....	15
Cannabinoid Activity in the Mesolimbic System .....	24
 II. Rationale.....	 28
 III. Methods.....	 31
Subjects.....	31
Drugs.....	32
Apparatus.....	32
Stereotaxic Surgery.....	33
Behavioral Procedures .....	34
Spontaneous Locomotor Activity .....	34
Operant Responding for Food.....	34
ICSS .....	35
Analytical Procedures .....	37
Tissue Extraction and Quantification of AEA, 2-AG and AA .....	37
Data Analysis .....	39
 IV. Results.....	 40
Cocaine ICSS Dose-Response Assessment.. ..	40
THC ICSS Experiments.....	43
THC Dose-Response Assessment.....	43
THC Time-Course Assessment.....	49
THC: Evaluation of CB <sub>1</sub> Receptors .....	54
THC: Evaluation of CB <sub>2</sub> Receptors .....	58

THC: Operant Responding for Food Assessment.....	61
JZL184 ICSS Experiments .....	63
JZL184 Dose-Response Assessment .....	63
JZL184 Time-Course Assessment .....	68
JZL184: Evaluation of CB <sub>1</sub> receptors .....	71
JZL184: Evaluation of CB <sub>2</sub> receptors .....	75
JZL184 Operant Responding for Food Assessment .....	78
JZL184 Spontaneous Locomotor Activity Assessment .....	79
JZL184 Endocannabinoid Quantification .....	82
PF-3845 ICSS Experiments .....	85
PF-3845 Dose-Response Assessment .....	85
PF-3845 Time-Course Assessment .....	90
PF-3845: Evaluation of CB <sub>1</sub> Receptors .....	93
PF-3845: Evaluation of CB <sub>2</sub> Receptors .....	97
SA-57 ICSS Experiments .....	101
SA-57 Dose-Response Assessment .....	101
SA-57 Time-Course Assessment .....	105
SA-57: Evaluation of CB <sub>1</sub> receptors .....	109
SA-57: Evaluation of CB <sub>2</sub> receptors .....	113
SA-57 Spontaneous Locomotor Activity Assessment .....	116
SA-57 Endocannabinoid Quantification .....	118
 V. Discussion.....	 122
Cocaine .....	123
CB <sub>1</sub> and CB <sub>2</sub> Antagonists .....	125
THC.....	126
Cannabinoid Catabolic Enzyme Inhibitors .....	130
JZL184 .....	130
PF-3845 .....	131
SA-57.....	132
Endocannabinoids in Brain.....	132
General Discussion .....	134
 VI. List of References .....	 141
VII. Vita .....	163

## List of Tables

Table	Page
Table 1: Summary of Cannabinoids Agonist, Antagonist, and Endocannabinoid Modulating Drug Effects in ICSS.....	20
Table 2: Summary of Cannabinoid Results.....	124

## List of Figures

Figure	Page
Figure 1: Depiction of endocannabinoid system at synapse.....	5
Figure 2: Depiction of cannabinoid interaction with dopamine neurons in the VTA.....	28
Figure 3: Cocaine ICSS assesement.....	42
Figure 4: THC ICSS dose-response assessment.....	46
Figure 5: Baseline ICSS tests.....	48
Figure 6: THC ICSS percent baseline stimulations.....	49
Figure 7: THC ICSS time-course evaluation.....	52
Figure 8: THC ICSS percent baseline stimulations time-course evaluation.....	54
Figure 9: THC CB <sub>1</sub> antagonism assessment.....	57
Figure 10: THC CB <sub>2</sub> antagonism assessment.....	60
Figure 11: THC operant responding for food.....	62
Figure 12: JZL184 ICSS dose-response assessment.....	65
Figure 13: JZL184 ICSS percent baseline stimulations.....	67
Figure 14: JZL184 ICSS time-course evaluation.....	70
Figure 15: JZL184 CB <sub>1</sub> antagonism assessment.....	74
Figure 16: JZL184 CB <sub>2</sub> antagonism assessment.....	77
Figure 17: JZL184 operant responding for food.....	79
Figure 18: JZL184 spontaneous locomotor activity.....	81
Figure 19: JZL184 endocannabinoid and AA concentrations.....	84
Figure 20: PF-3845 dose-response assessment.....	87
Figure 21: PF-3845 ICSS percent baseline stimulations.....	89



Figure 22: PF-3845 ICSS time-course evaluation.....	92
Figure 23: PF-3845 CB <sub>1</sub> antagonism assessment.....	96
Figure 24: PF-3845 CB <sub>2</sub> antagonism assessment.....	100
Figure 25: SA-57 ICSS dose-response assessment.....	103
Figure 26: SA-57 ICSS percent baseline stimulations.....	105
Figure 27: SA-57 ICSS time-course evaluation.....	108
Figure 28: SA-57 CB <sub>1</sub> antagonism assessment.....	112
Figure 29: SA-57 CB <sub>2</sub> antagonism assessment.....	115
Figure 30: SA-57 spontaneous locomotor activity.....	117
Figure 31: SA-57 endocannabinoid and AA concentrations.....	121

## **Abstract**

### **CANNABINOID MODULATION OF REINFORCEMENT MAINTAINED BY STIMULATION OF THE MEDIAL FOREBRAIN BUNDLE IN C57BL/6J MICE**

By Jason Wiebelhaus

A dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy at Virginia Commonwealth University.

Virginia Commonwealth University, 2013

Co-Directors: Aron H. Lichtman<sup>1</sup>, Ph.D. and Joseph H. Porter<sup>2</sup>, Ph.D.

<sup>1,2</sup>Professors

<sup>1</sup>Department of Pharmacology and Toxicology, <sup>2</sup>Department of Psychology

Cannabinoid agonists, including delta-9-tetrahydrocannabinol (THC), are found rewarding by humans. In addition to human self-reports and experimental studies that show marijuana is rewarding, contributions from preclinical studies also have implicated cannabinoid receptors in reward-motivated behavior. One way to assess these preclinical effects of cannabinoids is intracranial self-stimulation (ICSS), where an animal performs a response to receive electrical stimulation of a specific brain area or circuit known to be involved in

rewarding activities. Drugs of abuse, such as psychomotor stimulants, facilitate responding for ICSS. While a few studies have shown facilitating effects of cannabinoids in rats, several have shown the opposite effect, and no studies so far have evaluated cannabinoids in mouse ICSS. Furthermore there are no studies evaluating specific inhibitors of endocannabinoid catabolic enzymes in ICSS in any species. In these studies we assessed the cannabinoid agonist THC, as well as the fatty acid amide hydrolase (FAAH) inhibitor, PF-3845, the monoacylglycerol lipase (MAGL) inhibitor JZL184, and the combined FAAH/MAGL inhibitor SA-57 in ICSS of the medial forebrain bundle in C57BL/6 mice. Additionally, we assessed the psychomotor stimulant cocaine as a positive control to facilitate ICSS. These studies were complimented with spontaneous locomotor activity and food-maintained operant experiments to assess the sensitivity of ICSS to cannabinoids. Additionally, brain endocannabinoid levels were measured in brain regions associated with the mesolimbic system after enzyme inhibitor treatments.

THC, JZL184, and SA-57 all produced time-dependent reductions in ICSS that were mediated through CB<sub>1</sub> receptors, as they were blocked by pre-treatment with the CB<sub>1</sub> antagonist rimonabant, but not with the CB<sub>2</sub> antagonist SR144528. PF-3845 also reduced ICSS, but did so independent of CB<sub>1</sub> and CB<sub>2</sub> receptors, and only with one dose (30.0 mg/kg) that has not been assessed previously *in vivo*. We showed that ICSS was more sensitive to the rate-reducing effects of cannabinoids than other measures of behavior with motor components including spontaneous locomotor activity and operant nose-poking for food, and that the reduction of ICSS produced by both JZL184 and SA-57 is accompanied by increases in 2-AG in mesolimbic brain areas. Thus, cannabinoids do not facilitate ICSS in C57BL/6 mice over a range of doses and pre-treatment times, similar to most studies with rats. These data suggest that cannabinoids may produce rewarding effects through non-mesolimbic areas of the brain.

## Cannabinoid Modulation of Reinforcement Maintained by Stimulation of the Medial Forebrain Bundle in C57Bl/6J Mice

### **Endogenous Cannabinoid System**

The endocannabinoid system is composed of two known cannabinoid receptor types: cannabinoid receptor types 1 and 2 (CB<sub>1</sub> and CB<sub>2</sub>) (Gerard, Mollereau, Vassart, & Parmentier, 1991; Matsuda, Lolait, Brownstein, Young, & Bonner, 1990) as well as the endogenous ligands that bind to these receptors. Cannabinoid receptors are G-protein-coupled and activating them inhibits calcium influx into cells and reduces the accumulation of cyclic adenosine monophosphate (cAMP), ultimately producing an inhibitory effect (Howlett et al., 1990; Pertwee et al., 2010). CB<sub>1</sub> receptors are located on pre-synaptic terminals of several neuron types including GABAergic, glutamatergic, cholinergic, serotonergic, and noradrenergic neurons, and their stimulation inhibits the release of neurotransmitter into the synapse (Cadogan, Alexander, Boyd, & Kendall, 1997; Katona et al., 2000; Robbe, Alonso, Duchamp, Bockaert, & Manzoni, 2001). Most behavioral effects produced by cannabinoids have been found to be mediated through CB<sub>1</sub> receptors. For example the classical tetrad of behavioral effects (hypolocomotion, hypothermia, catalepsy, and antinociception) and discriminative stimulus effects have been shown to be mediated through CB<sub>1</sub> receptors, as they were blocked by the CB<sub>1</sub> receptor antagonist, rimonabant, in different species including mice, rats, and rhesus monkeys (Compton, Aceto, Lowe, & Martin, 1996; Rinaldi-Carmona et al., 1994; Wiley, Lowe, Balster, & Martin, 1995).

Some recent studies have demonstrated behavioral effects related to CB<sub>2</sub> receptor activation. CB<sub>2</sub> receptors have been thought to be predominantly located in the periphery and on immune cells during states of inflammation, but are also located on subpopulations of neurons and microglial cells (Ashton, Friberg, Darlington, & Smith, 2006; Baek, Zheng, Darlington, &

Smith, 2008; Gong et al., 2006; Onaivi, 2006; Onaivi et al., 2006; Stella, 2010; Van Sickle et al., 2005). However, there is controversy over whether CB<sub>2</sub> receptors are present on neurons, as the antibodies used to detect them are not highly selective. Recent studies have implicated CB<sub>2</sub> receptors as mediators of cocaine reinforcement and nicotine reward (Ignatowska-Jankowska, Muldoon, Lichtman, & Damaj, 2013; Xi et al., 2011). Xi and colleagues found that the CB<sub>2</sub> receptor agonist, JWH133, dose-dependently inhibited cocaine self-administration, cocaine-induced activation of locomotor activity, and cocaine-induced increases in dopamine in the nucleus accumbens (Xi, et al., 2011). These effects were all prevented by treatment with the CB<sub>2</sub> antagonist, AM630, or when assessed in CB<sub>2</sub> -/- mice (Xi, et al., 2011). Ignatowska-Jankowska and colleagues found that nicotine conditioned place preference (CPP) was blocked by pre-treatment with the CB<sub>2</sub> antagonist, SR144528; and the CB<sub>2</sub> agonist 0-1966 enhanced nicotine CPP (Ignatowska-Jankowska, Muldoon, et al., 2013). These are some of the first studies implicating CB<sub>2</sub> receptor activation in drug reward. Interestingly, CB<sub>2</sub> receptor activity produces paradoxical effects on cocaine and nicotine behavior, as agonists block cocaine reward and enhance nicotine reward, and antagonists reverse blockade of cocaine self-administration and block nicotine reward. Taken together, these findings suggest that CB<sub>2</sub> receptors may be involved in drug reward, and their effects seem to be specific to distinct drugs of abuse.

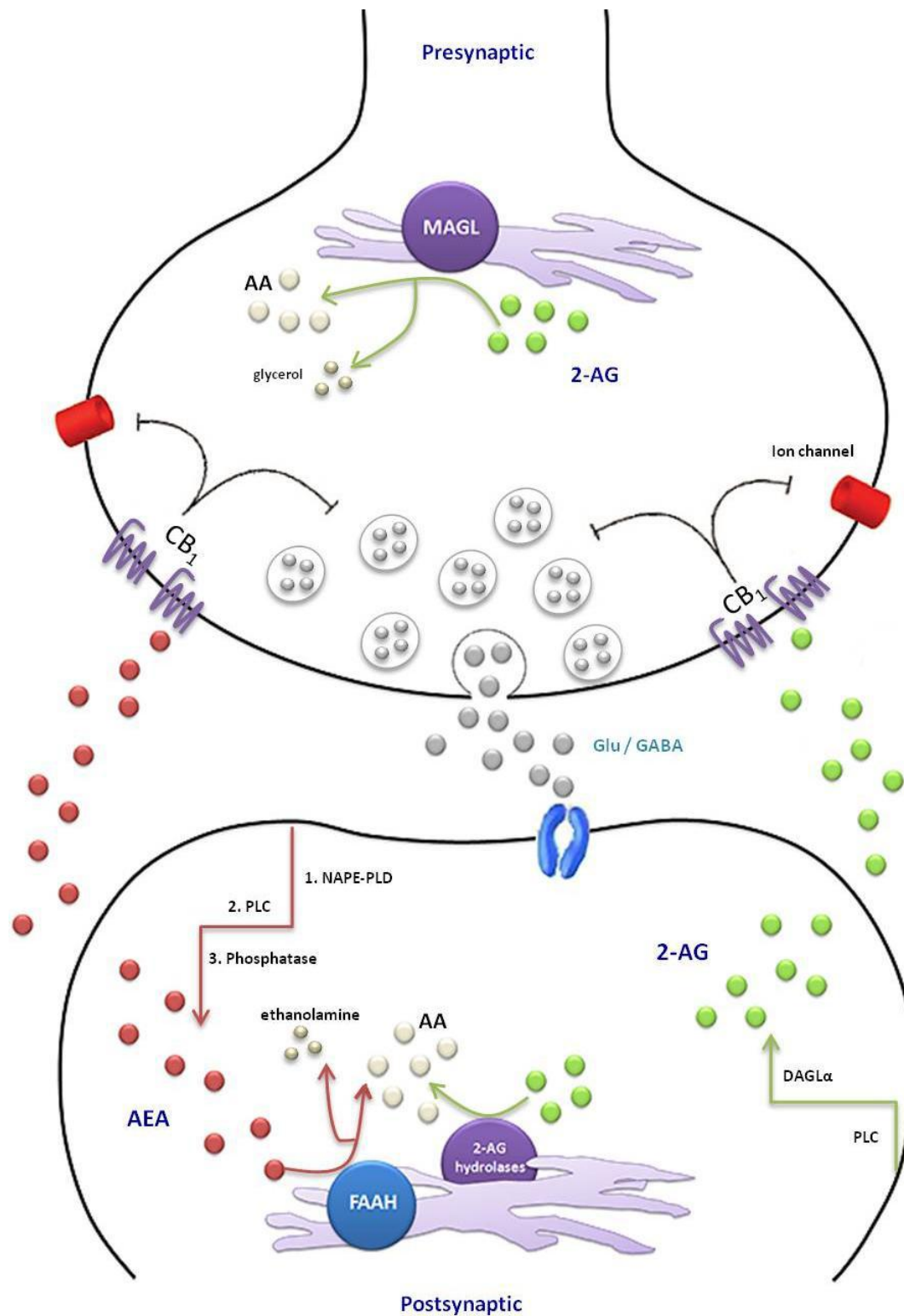
## **Endocannabinoids**

The endogenous cannabinoids (endocannabinoids) include the lipid molecules N-arachidonylethanolamine (anandamide; AEA), 2-arachidonoylglycerol (2-AG) (Devane et al., 1992; Mechoulam et al., 1995; Sugiura et al., 1995) as well as arachidonyl dopamine, nolandin ether, and virodhamine (Bisogno et al., 2000; Hanus et al., 2001; Porter et al., 2002). This dissertation focuses on 2-AG and AEA, as they are the predominant endocannabinoid ligands,

and pharmacological tools are available to evaluate them. AEA was discovered in 1992, and was the first endocannabinoid isolated, while screening endogenous ligands for CB<sub>1</sub> receptor binding (Devane, et al., 1992). 2-AG was discovered simultaneously by two groups in 1995, when it was isolated from canine intestinal tissue (Mechoulam, et al., 1995) and rat brain tissue (Sugiura, et al., 1995), and found to bind to CB<sub>1</sub> and CB<sub>2</sub> receptors. AEA is thought to be primarily synthesized through phosphodiesterase activity or through cleavage of phospholipase C and a phosphatase (Liu et al., 2006). It was originally proposed that AEA is created primarily through the cleavage of N-arachidonylphosphatidylethanolamine (NAPE) by NAPE-phospholipase D (PLD), but a study by Leung and colleagues suggested that NAPE-PLD is not the major biosynthetic pathway for AEA, as NAPE-PLD knock-out mice were found to have similar amounts of AEA as wild-type controls (Leung, Saghatelian, Simon, & Cravatt, 2006). 2-AG is primarily synthesized through the cleavage of diacylglycerol (DAG) by the enzyme DAG lipase- $\alpha$  (DAGL $\alpha$ ) (Gao et al., 2010; Tanimura et al., 2010). This was confirmed by the observation that DAGL $\alpha$  knock-out mice have significantly lower levels of 2-AG in brain compared to wild-type controls. 2-AG and AEA are primarily metabolized by the respective degradative enzymes monoacylglycerol lipase (MAGL) and fatty acid amide hydrolase (FAAH).

As shown on Fig. 1, endocannabinoids are released from post-synaptic neuron terminals and move across the synapse and bind to CB<sub>1</sub> receptors located on pre-synaptic neurons (Ahn, McKinney, & Cravatt, 2008). AEA and 2-AG are rapidly metabolized soon after they are released. FAAH is located within the post-synaptic terminal (Gulyas et al., 2004) and is primarily responsible for the degradation of AEA into arachidonic acid and ethanolamine. FAAH also metabolizes roughly thirteen other fatty acid amides including oleamide, oleylethanolamide (OEA) and palmitoylethanolamide (PEA) (Cravatt et al., 1995). Other

enzymes have the ability to metabolize AEA including FAAH-2 and *N*-acylethanolamine-hydrolyzing acid amidase (NAAA), though the former is only found in human and primates. Both are found in higher relative abundance in peripheral tissues and are inferior to FAAH in their ability to metabolize AEA (Tsuboi et al., 2005; Ueda, Yamanaka, & Yamamoto, 2001; Wei, Mikkelsen, McKinney, Lander, & Cravatt, 2006). Similarly, 2-AG can be hydrolyzed by a number of different enzymes including FAAH, neuropathy target esterase (NTE), hormone sensitive lipase (HSL), alpha/beta hydrolase 6 and 12 (ABHD6, ABHD12), cyclooxygenase-2 (COX2), or MAGL, but the majority of 2-AG (85%) is degraded by the enzyme MAGL located within the presynaptic terminal. Any remaining 2-AG is typically deactivated by enzymes alpha/beta ABHD6 or ABHD12 and converted to arachidonic acid (AA) and glycerol (Belfrage, Jergil, Stralfors, & Tornqvist, 1977; Blankman, Simon, & Cravatt, 2007; Dinh, Freund, & Piomelli, 2002; Goparaju, Ueda, Yamaguchi, & Yamamoto, 1998; Kim & Alger, 2004; van Tienhoven, Atkins, Li, & Glynn, 2002). In addition to being the main enzyme responsible for breaking down 2-AG, MAGL is also the major biosynthetic enzyme for arachidonic acid and its metabolites (Nomura et al., 2011). Hydrolysis of 2-AG produces arachidonic acid (AA) and glycerol; disruption of MAGL activity significantly decreases free AA, as well as the eicosanoids derived from it, including prostaglandins E2 (PGE2), PGD2, PGF2, and thromboxane B2 (TXB2). This decrease in AA is associated with decreased inflammatory responses (Nomura, et al., 2011).



*Figure 1.* Depiction of a synapse with endocannabinoid system components including CB<sub>1</sub> receptors, ligands, and biosynthetic and degradative pathways. Figure adapted from (Ahn, et al., 2008).



## **AEA and 2-AG: Distinct Endocannabinoids**

Although AEA and 2-AG are structurally similar and have been shown to bind to CB<sub>1</sub> and CB<sub>2</sub> receptors, they are pharmacologically distinct ligands. There are a few important observed differences between these two endocannabinoids that set them apart from each other. 2-AG is found at 170-1000 fold greater concentrations in the central nervous system than AEA (Sugiura et al., 2002). This relationship was confirmed at the synaptic level with a study that measured AEA and 2-AG in mouse and rat nucleus accumbens shell after FAAH and MAGL inhibitor administration using microdialysis (Wiskerke et al., 2012). Wiskerke and colleagues consistently observed basal levels of 2-AG at 9-16 fold those of AEA in the interstitial space in the nucleus accumbens shell of mice and 2-8 fold higher in rats (Wiskerke, et al., 2012), which suggests that 2-AG may be more relevant for CB<sub>1</sub> receptor signaling. Although the ratio between the amounts of AEA and 2-AG in whole brain tissue is less than when measured in the interstitial space, 2-AG is still consistently found in greater quantities there also.

AEA is a partial agonist at cannabinoid receptors *in vitro*, while 2-AG is a full agonist in stimulating GTPgammaS binding, as AEA reduced the functional activity of 2-AG, which suggests differential efficacy of the two endocannabinoids at cannabinoid receptors (Gonsiorek et al., 2000). Related to this fact 2-AG, but not AEA, has been shown to prolong depolarization-induced suppression of inhibition/excitation (DSI, DSE). Suppression of inhibition/excitation refers to suppression of inhibitory or excitatory neuronal transmission via hyperpolarization of a depolarized neuron, which suppresses the release of a neurotransmitter. 2-AG can cause a suppression of stimulatory or excitatory neurotransmitters mediated by CB<sub>1</sub> receptors, while AEA does not, indicating that 2-AG may be more relevant for neuronal retrograde signaling (Pan et al., 2009). Furthermore, in addition to binding to CB<sub>1</sub> and CB<sub>2</sub> receptors, AEA has also been

shown to activate the transient receptor potential cation channel subfamily V member 1 (TrpV1), and other substrates metabolized by FAAH including OEA and PEA have been shown to bind to peroxisome proliferator-activated alpha receptors (PPAR $\alpha$ ) receptors; while 2-AG and targets of MAGL inhibition lack affinity for those receptors (Jhaveri et al., 2008; Ross, 2003; Zygmunt et al., 1999).

The availability of each endocannabinoid is regulated independently by distinct enzymatic pathways. As mentioned above, AEA is broken down by FAAH in the postsynaptic neuron, and other enzymes which do not affect 2-AG including FAAH-2 and NAAA, while 2-AG primarily is broken down by MAGL in the presynaptic neuron, and also can be metabolized by ABHD6, ABHD12, HSL, NTE, or FAAH. Additionally, FAAH metabolism of AEA does not contribute substantially to available arachidonic acid pools in the brain, while disruption of 2-AG metabolism leads to significant reductions of available AA (Nomura, et al., 2011). In addition to differences in the metabolic inactivation of AEA and 2-AG, their biosynthetic precursors also vary, as AEA is synthesized primarily through phosphodiesterase activity or through cleavage of phospholipase C and a phosphatase, while 2-AG in the brain is synthesized primarily through the cleavage of diacylglycerol (DAG) by the enzyme DAG lipase-alpha (DAGL $\alpha$ ) (Gao, et al., 2010; Tanimura, et al., 2010). These differences between biosynthetic and degradative pathways suggest AEA and 2-AG have distinct physiological functions.

Tolerance to cannabinoid tetrad effects and regulation of cannabinoid receptors is differentially modulated when MAGL or FAAH are inhibited repeatedly. Previous studies have shown that potentially negative outcomes such as CB<sub>1</sub> receptor down-regulation, desensitization, and cannabinoid somatic withdrawal symptoms occur after repeated administration with a high dose of the MAGL inhibitor JZL184 (40.0 mg/kg) and in MAGL knock-out mice. However, it

should be noted that follow-up studies to the initial reports have demonstrated that repeated partial inhibition of MAGL (low doses of JZL184; 4.0 mg/kg) does not affect CB<sub>1</sub> receptor function, and can still produce therapeutic effects in preclinical assays (Kinsey et al., 2013). Repeated FAAH inhibition using genetic or pharmacological means fails to produce either of these undesirable effects (Falenski et al., 2010; Schlosburg et al., 2010).

Endocannabinoids are synthesized locally and are produced “on-demand”, meaning that unlike many neurotransmitters, endocannabinoids are produced immediately before being released, rather than being stored in vesicles for later use. The heterogeneous distribution of AEA and 2-AG as well as the ability of endocannabinoids to be inactivated by rapid synthesis and degradation makes specific endocannabinoid enzymes intriguing targets for pharmaceutical development. For example, MAGL inhibition leads to increased 2-AG accumulation in brain, but only in a regionally dependent manner, and will presumably only activate a subset of CB<sub>1</sub> receptors near where it is produced and released. This is in contrast to exogenous administration of direct-acting cannabinoid receptor agonists, which activate cannabinoid receptors throughout the brain and periphery. In addition to the therapeutic potential of endocannabinoid deactivation inhibitors, they also represent an excellent tool to probe the cannabinoid system. The endogenous cannabinoid system is involved intricately in many distinct physiological and psychological processes including cognition, emotion, pain, and inflammation, and reward (Pertwee, 2012). FAAH and MAGL inhibitors are particularly useful tools for the investigation of endocannabinoid-mediated effects.

### **Behavioral Effects of MAGL Inhibition**

MAGL inhibitors have been shown to produce a variety of behavioral effects. JZL184 was the first marginally selective irreversible MAGL inhibitor developed, which produced up to

10-fold elevations of whole brain 2-AG compared to basal levels after acute administration of a high dose (40.0 mg/kg) (J. Z. Long, Li, et al., 2009) without affecting AEA levels. JZL184 has some activity at FAAH also, as repeated administration produces a small, but significant increase in brain AEA concentrations (Schlosburg, et al., 2010). Recently, novel MAGL inhibitors KML29 and MJN110 have been produced and have been reported to be more selective for MAGL over FAAH than JZL184, with observed efficacy in both rats and mice (Ignatowska-Jankowska et al., 2013; Niphakis et al., 2013).

JZL184 has been used as a pharmacological tool to investigate the role of 2-AG in treating several different conditions/symptoms including anxiety, pain, and ulcers. It has been demonstrated that JZL184 decreases anxiety-like behavior in both rats and mice in the elevated plus maze test (Aliczki, Balogh, Tulogdi, & Haller, 2012; Sciolino, Zhou, & Hohmann, 2011) and mice in the marble burying assay (Kinsey, O'Neal, Long, Cravatt, & Lichtman, 2011). JZL184 produces antinociception in a mouse model of neuropathic pain in the chronic constriction injury of the sciatic nerve (CCI) assay (Kinsey et al., 2009), a model of inflammatory pain using the carrageenan inflammatory assay (Ghosh et al., 2012), formalin-induced pain (Guindon, Guijarro, Piomelli, & Hohmann, 2011) and a rat model of sodium monoiodoacetate induced osteoarthritis pain (Sagar et al., 2010). Further, JZL184 has been shown to decrease gastric hemorrhaging produced by gavage of the non-selective cyclooxygenase inhibitor diclofenac (Kinsey et al., 2011) as well as reduce alterations and inflammation of the colon induced by the trinitrobenzene sulfonic acid (TNBS) model of colitis in mice (Alhouayek, Lambert, Delzenne, Cani, & Muccioli, 2011).

## **Behavioral Effects of FAAH Inhibition**

Similar to MAGL inhibitors, there have been a few FAAH inhibitors developed over the past years, with selectivity for FAAH increasing over time. FAAH inhibitors, to name a few, include URB-597 (Kathuria et al., 2003; Piomelli et al., 2006), OL-135 (Boger et al., 2005), PF-622, PF-750 (Ahn et al., 2007), PF-3845 (Ahn et al., 2009) and more recently, JNJ5003 (Hill et al., 2012). FAAH inhibitors have been utilized to examine the effects of increased AEA in various models of behavior and disease states. Most notably, there has been interest in developing them for the treatment of pain. There has only been one clinical trial to date using a FAAH inhibitor, PF-04457845 (PF-7845), which is structurally similar to PF-3845, but optimized for human FAAH (Huggins, Smart, Langman, Taylor, & Young, 2012; Li et al., 2012). PF-7845 did not show any analgesic efficacy in treating osteoarthritis; however it has not been tested to treat other types of pain or conditions in the clinic. In preclinical assays, FAAH inhibitors have been shown to be effective in collagen-induced models of arthritis (Kinsey, Naidu, Cravatt, Dudley, & Lichtman, 2011), LPS-induced allodynia (Booker et al., 2012), neuropathic pain (Caprioli et al., 2012), and multiple sclerosis pain models (Pryce et al., 2013), among others pain assays. In addition to antinociception, FAAH inhibitors have shown positive effects in various preclinical assays including enhancing active stress-coping strategies measured using the tail suspension test with URB597 (Gobbi et al., 2005; Naidu et al., 2007), tail pinch test (Haller, Goldberg, Pelczer, Aliczki, & Panlilio, 2013) and preventing restraint stress-induced biochemical markers in the amygdala with JNJ5003 (Hill, et al., 2012). Anxiolytic effects of FAAH inhibitors have been reported in studies evaluating animals using the elevated-zero maze (Kathuria, et al., 2003) and elevated-plus maze (Haller, et al., 2013; Patel & Hillard, 2006). FAAH inhibitors have also been shown to enhance both the acquisition (Varvel, Wise, Niyuhire,

Cravatt, & Lichtman, 2007; L. E. Wise, Harloe, & Lichtman, 2009) and extinction (Varvel, et al., 2007) of aversive spatial memory tasks.

### **Behavioral Effects of Combined FAAH and MAGL Inhibition**

FAAH or MAGL inhibition alone do not produce all of the disruptive effects commonly associated with cannabinoid agonists including catalepsy and THC-like discriminative stimulus effects (J. Z. Long, Nomura, et al., 2009). Combined inhibition of FAAH and MAGL, however, has been shown to produce more THC-like effects than either enzyme inhibited alone (J. Z. Long, Nomura, et al., 2009; Niphakis, Johnson, Ballard, Stiff, & Cravatt, 2012; L. E. Wise et al., 2012). Dual FAAH/MAGL inhibition has also been shown to substitute for THC in drug discrimination studies, produce catalepsy in mice, as well as produce THC-like memory deficits in Morris water maze learning, all of which were shown to be mediated through CB<sub>1</sub> receptors (J. Z. Long, Nomura, et al., 2009; L. E. Wise, et al., 2012). The effects produced by combined FAAH and MAGL inhibition are reflective of the fact that AEA and 2-AG have distinct signaling pathways. Specifically, it seems that 2-AG signaling produces more cannabimimetic effects than AEA (J. Z. Long, Nomura, et al., 2009).

Although combined inhibition of FAAH and MAGL may produce undesirable effects, it has also shown some promising effects in some preclinical assays, as it has been shown to reduce somatic withdrawal signs in mice during spontaneous morphine withdrawal (Ramesh et al., 2013); as well as produce enhanced reduction of inflammation and hypersensitivity to non-noxious stimuli in the preclinical assay of carrageenan inflammation in mice (Ghosh and Lichtman, unpublished).

## **Cannabinoids and Reward**

Abuse-related effects of cannabinoids have been demonstrated in humans through positive subjective effect ratings associated with marijuana use (Hart et al., 2010; Hart, van Gorp, Haney, Foltin, & Fischman, 2001), as well as through human brain imaging studies implicating reward-relevant brain responses in the bilateral amygdala and hippocampus during marijuana cue presentations and voluntary self-administration in marijuana-dependent individuals (Goldman et al., 2013). In recent years the use of synthetic cannabinoid agonists by humans has risen dramatically, as “herbal incense blend” products containing synthetic cannabinoids have become commercially available (Brents & Prather, 2013).

Of particular importance, the majority of human reports of rewarding effects of cannabinoids refer to inhaled marijuana or synthetic cannabinoid blends (Calhoun, Galloway, & Smith, 1998). THC is not commonly used for recreational purposes by humans. In fact, dronabinol (Marinol), which is synthetic THC delivered in capsules with sesame oil, is classified as a schedule III compound by the FDA, while marijuana schedule I, meaning it has less abuse and dependence liability than marijuana and certain synthetic cannabinoid agonists. There has been no evidence of dronabinol prescriptions being diverted to non-medical use, and marijuana users have not reported any interest in the drug (Calhoun, et al., 1998). Furthermore, the effects of oral dronabinol have a slow onset, which reduces its potential for abuse, and the majority of people who try it for recreational purposes report it produces dysphoric effects (Calhoun, et al., 1998), or at most modest reinforcing effects (Hart, Haney, Vosburg, Comer, & Foltin, 2005). Interestingly, dronabinol has been found to reduce symptoms of marijuana withdrawal without producing many negative side-effects, or affecting the subjective effects of smoked marijuana (Vandrey et al., 2013). Although THC is the main active constituent in marijuana, there are

hundreds of other chemicals present the plant material typically smoked by humans. Thus, preclinical research with THC may not be completely applicable to human use of marijuana. In addition to marijuana containing other chemicals, the route of administration also differs, as few preclinical studies have delivered cannabinoids via inhalation as they are typically consumed by humans (Niyuhire, Varvel, Martin, & Lichtman, 2007; Wiebelhaus et al., 2012).

### **Preclinical Models of Reinforcement and Reward-Associated Behavior**

Reinforcing and rewarding properties of cocaine and other drugs of abuse are assessed in animals using various behavioral measures. Three of the most used preclinical assays to assess abuse liability of drugs are drug self-administration, conditioned place preference (CPP), and intracranial self-stimulation (ICSS). Descriptions of these assays and the respective effects of cannabinoids are detailed below.

#### **Self-Administration**

A preclinical measure of drug reinforcement that has high face validity is drug self-administration. In this paradigm, subjects learn to perform a specific operant task, such as pressing a lever, spinning a wheel, or nose-poking, to obtain a specific drug reinforcer. Studies have shown that animals can learn to self-administer many substances that humans are known to abuse including cocaine (Panlilio & Goldberg, 2007), heroin (S. A. Chen et al., 2006), and ethanol (Mittleman, Van Brunt, & Matthews, 2003). While cannabinoids have been demonstrated to be self-administered by laboratory animals, historically researchers have had difficulty demonstrating consistent cannabinoid self-administration in any species of animals (Tanda & Goldberg, 2003). However, some studies have demonstrated self-administration of cannabinoid full agonists in mice and rats (Fattore, Cossu, Martellotta, & Fratta, 2001; Flores, Maldonado, & Berrendero, 2013; Martellotta, Cossu, Fattore, Gessa, & Fratta, 1998; Takahashi



& Singer, 1979, 1980). The rodent studies that have demonstrated self-administration of cannabinoids have employed specific methodological techniques that may have increased the likelihood to train self-administration of a cannabinoid agonist including food-deprivation, restraint-stress, short time-course of studies, and co-administration of food or water with cannabinoids, which can make interpretation of their results difficult. Squirrel monkeys have been demonstrated to self-administer cannabinoids (Justinova, Solinas, Tanda, Redhi, & Goldberg, 2005; Justinova, Tanda, Redhi, & Goldberg, 2003; Justinova, Yasar, Redhi, & Goldberg, 2011; Tanda, Munzar, & Goldberg, 2000). Initial studies utilized squirrel monkeys that had previously learned to self-administer cocaine, but follow-up studies demonstrated that naïve squirrel monkeys can also be trained to administer THC (Justinova, et al., 2003) as well as the endogenous cannabinoid AEA and the longer lived methanandamide (Justinova, et al., 2005) and 2-AG (Justinova, et al., 2011). Overall, cannabinoid self-administration is far less robust compared to other abused drugs and relies heavily upon non-traditional manipulations, making it difficult to interpret results.

### **Conditioned Place Preference**

The conditioned place preference (CPP) model is based on the Pavlovian principle of classical conditioning. Presumed drugs of abuse are assessed by pairing a given drug to an experimental chamber with specific contextual cues, and then allowing an animal to choose whether or not to spend time in that environment while in a drug-free state. The drug serves as a unconditioned stimulus (US) and the specific environment it is paired to serves as a conditioned stimulus (CS). If the subjects choose to spend more time in the drug-paired environment than a similar, but distinct environment, it is inferred that there is a positive association between the drug and the context in which it was administered (Tanda & Goldberg, 2003).

While most drugs of abuse including psychomotor stimulants and opiates produce CPP, cannabinoids often fail to do so, or only do so within an extremely limited range of doses, with a large degree of inconsistency between studies (Cheer, Kendall, & Marsden, 2000; McGregor, Issakidis, & Prior, 1996; Sanudo-Pena et al., 1997). Even when cannabinoids are found to produce CPP, this effect can be dependent on timing of injections, and other procedural manipulations. Additionally, conditioned place aversion is often observed with higher doses (Braida, Pozzi, Cavallini, & Sala, 2001; Ghozland et al., 2002; Lepore, Vorel, Lowinson, & Gardner, 1995).

### **Intracranial Self-Stimulation**

Intracranial self-stimulation (ICSS) is a methodology in which a subject performs an operant response in order to receive electrical stimulation of a brain region implicated in reward function. ICSS has most commonly been used to assess the motivational effects and abuse liability of drugs (Carlezon & Chartoff, 2007), but has also been used to assess affective signs of drug withdrawal and other aversive states (Cryan, Hoyer, & Markou, 2003; Markou & Koob, 1991; Schulteis, Markou, Cole, & Koob, 1995). ICSS was first discovered serendipitously by Olds and Milner in 1954 while attempting to stimulate the reticular formation (Milner, 1989; Olds & Milner, 1954). James Olds noticed that a particular rat in which he had implanted an electrode seemed to “seek out” the electrical stimulation that he applied, and he confirmed that the stimulation served as a reinforcer after constructing a crude operant chamber and allowing the rat to lever press for stimulation. He later realized that the electrode was bent when it was inserted into the brain, and had actually terminated in the septum, and concluded that this brain area was involved in reward (Milner, 1989). Gradually, various methodologies for studying reward substrates and effects of drugs grew out of his early research. Today, these methodologies

are referred to collectively as ICSS or brain stimulation reward. ICSS is one of the most salient forms of reinforcement, and it is based on electrical activation of the mesolimbic dopamine system (Kornetsky, 2004; Roy A. Wise, 2002). A stimulating electrode is implanted into one of several possible sites including the various parts of the hypothalamus including the medial forebrain bundle, or other areas including the septum, which have projections to the ventral tegmental area (VTA). A large group of dopaminergic neurons in the VTA known as A10 neurons project to forebrain areas including the nucleus accumbens and prefrontal cortex. ICSS is mediated mainly through activation of glutamatergic projections to dopaminergic neurons in the VTA, which stimulates dopamine release originating from the VTA to the nucleus accumbens (Shizgal, Schindler, & Rompre, 1989), where it activates GABAergic medium spiny neurons in the nucleus accumbens. Dopamine release in the nucleus accumbens is associated with natural rewards, including palatable feeding and increases in spontaneous locomotor activity, as well as drug reward (Cheer, Aragona, et al., 2007; Neill, Fenton, & Justice, 2002; R. A. Wise, 2005). This indirect stimulation of dopamine neurons in the nucleus accumbens is thought to maintain ICSS behavior.

There are several different methodologies of ICSS, including discrete trial, auto-titration, rate-based studies, and rate-frequency. All of the models except rate-based vary the electrical current during session, which allows for analysis of drug effects across a range of response rates, and reinforcer values. This dissertation will mainly focus on rate-frequency procedures. During baseline conditions subjects typically respond at low rates for lower frequencies of stimulation, and at increasingly higher rates as frequency is increased. Many drugs of abuse, and specifically psychomotor stimulants such as cocaine, produce an increase in total responses, and manifests as a leftward shift from baseline on the rate-frequency response curve (Corbett, 1991; Fish et al.,

2012; Robinson, Agoglia, Fish, Krouse, & Malanga, 2012). This profile of effects also is referred to as a decrease in the reward threshold because the frequency, or number of pulses per train in the medial forebrain bundle necessary to maintain responding is reduced. ICSS is also used to assess affective signs of drug withdrawal and other aversive states (Cryan, et al., 2003; Markou & Koob, 1991; Schulteis, et al., 1995), as these manipulations can produce a reduction of ICSS, or an increase in reward threshold, reflected by a rightward shift of the rate-frequency curve. Many animal species will respond for brain stimulation including mice, rats, monkeys, and humans (Bewernick, Kayser, Sturm, & Schlaepfer, 2012; Olds & Milner, 1954; Randt & Quartermain, 1972; Rolls, Burton, & Mora, 1980). The first procedure in mice was established by Randt and Quartermain in 1972 (Randt & Quartermain, 1972) and was followed by several studies in mice by Cazala and Cardo (Cazala & Cardo, 1972).

Cannabinoid facilitation of ICSS has been difficult to observe and studies have resulted in inconsistent observations similar to the effects of cannabinoids on reward or reinforcement in other preclinical models. Some studies have shown that THC reduces reward thresholds of ICSS in rats (Gardner et al., 1988; Katsidoni, Kastellakis, & Panagis, 2013; Lepore, Liu, Savage, Matalon, & Gardner, 1996), while others have demonstrated that THC (Fokos & Panagis, 2010; Stark & Dews, 1980) and synthetic full agonists WIN 55,212-2 (Vlachou, Nomikos, & Panagis, 2003) and CP 55,940 (Arnold, Hunt, & McGregor, 2001) fail to affect ICSS responding. The majority of studies with cannabinoids and ICSS have found threshold increasing, or response-attenuating effects of various cannabinoid drugs including THC (Kwilas & Negus, 2012; Vlachou, Nomikos, Stephens, & Panagis, 2007), synthetic agonists WIN 55,212-2 (Mavrikaki, Markaki, Nomikos, & Panagis, 2010; Vlachou, Nomikos, & Panagis, 2005), levonantradol (Kucharski, Williams, & Kornetsky, 1983), nabilone and canbisol (Stark & Dews, 1980), CP

55,940 (Kwilasz & Negus, 2012; Vlachou, et al., 2005), AMG-3 (Antoniou et al., 2005), and HU-210 (Vlachou, et al., 2005), and endocannabinoid enhancing (and purportedly enhancing) drugs URB-597, PMSF, and OMDM-2 (Vlachou, Nomikos, & Panagis, 2006) and the supposed AEA transport inhibitor AM-404 (Vlachou, Stamatopoulou, Nomikos, & Panagis, 2008). For a complete summary of cannabinoid agonist and endocannabinoid modulator effects on ICSS, see Table 1. A common trend among ICSS studies with THC was that low doses produced facilitation, or leftward shifts of the rate-frequency curves, or did not affect responding, and higher doses reduced responding, or produced a rightward shift in the curve (Katsidoni, et al., 2013; Lepore, et al., 1996). Furthermore, three of the four studies that found a facilitating/threshold decreasing effect of THC utilized Lewis rats (Gardner, et al., 1988; Lepore, et al., 1996); evidence that THC-induced facilitation of ICSS is a strain-dependent effect. Of particular importance to the present studies is that no cannabinoid compounds have been assessed in mouse ICSS.

The effects of CB<sub>1</sub> receptor antagonists on ICSS are also somewhat inconsistent between studies. The CB<sub>1</sub> antagonist, rimonabant, has been shown to attenuate ICSS responding or reduce reinforcement in some studies, usually at higher doses (De Vry, Schreiber, Eckel, & Jentsch, 2004; Deroche-Gamonet, Le Moal, Piazza, & Soubrie, 2001; Trujillo-Pisanty et al., 2011; Xi, et al., 2011), while it produces no change on ICSS responding in others (Antoniou, et al., 2005; Arnold, et al., 2001; Kwilasz & Negus, 2012; Vlachou, et al., 2003; Vlachou, et al., 2005, 2006; Vlachou, et al., 2007; Vlachou, et al., 2008). For a complete summary of cannabinoid antagonist effects in ICSS, see Table 1 on pages 18 - 21. Certainly dose is a factor, as higher doses tend to disrupt responding, and lower doses tend to have no effect on ICSS. As shown in Table 1, doses of 3.0 mg/kg rimonabant or higher tend to produce rightward shifts in ICSS, or attenuate

responding. These discrepancies may also be due to differences in endocannabinoid tone produced by distinct experimental procedures. For example, procedural variations that produce more stress than others may alter the endocannabinoid tone. The differences in amount of AEA or 2-AG available to bind to CB<sub>1</sub> receptors may alter the way that CB<sub>1</sub> antagonists affect ICSS behavior. If there is an abundance of endocannabinoids available and binding to CB<sub>1</sub> receptors, CB<sub>1</sub> antagonists could conceivably produce more aversive effects than when an antagonist is administered under less stressful conditions. Also, similar to results with agonists, all assessments of CB<sub>1</sub> antagonists have been conducted in rats. Certainly, there may be a species difference in response to CB<sub>1</sub> antagonists, as the facilitation caused by agonists seems to be strain-dependent in rats. For a summary of all cannabinoid ICSS studies ICSS, see Table 1.

Table 1

*Summary of Cannabinoids Agonist, Antagonist, and Endocannabinoid Modulating Drug Effects in ICSS*  
*Cannabinoid Agonists*

<b>Drug</b>	<b>Dose/R.O.A.</b>	<b>Rat Strain</b>	<b>ICSS Test</b>	<b>Effect</b>	<b>Reference</b>
THC	0.1 & 1.0 mg/kg i.p.	S-D	R-F	* decreased thresholds w/0.1 mg/kg (n=8) * increased thresholds w/1.0 mg/kg (n=8)	(Katsidoni, et al., 2013)
THC CP	0.1 – 10.0 mg/kg i.p. 10.0 - 320 µg/kg i.p.	S-D	R-F	* attenuated responding w/3.2 & 10 mg/kg (n=6) attenuated responding w/100 & 320 µg/kg (n=5)	(Kwilasz & Negus, 2012)
THC	0.5 & 1.0 mg/kg i.p.	S-D	R-F	n.s. trend of increased thresholds w/1 mg/kg (note: showed same trend in rats exposed to chronic unpredictable stress as control rats) (n=7)	(Fokos & Panagis, 2010)
THC	0.5 – 2.0 mg/kg i.p.	S-D	R-F	* increased thresholds w/1.0 & 2.0 mg/kg (n=6-9)	(Vlachou, et al., 2007)
THC	1.0 mg/kg i.p.	Lewis	R-F	decreased thresholds	(Lepore, et al., 1996)
THC	1.0 mg/kg i.p.	S-D		no change in thresholds	
THC	1.0 mg/kg i.p.	Fischer-344		no change in thresholds (n=9? not reported, but Determined from degrees of freedom reported)	
THC	1.0 & 1.5 mg/kg i.p.	Lewis	???	decreased thresholds (n not reported)	(Gardner et al., 1989)
THC	1.5 mg/kg i.p.	Lewis	A-T	decreased thresholds (n=10? not reported, but determined from degrees of freedom reported)	(Gardner, et al., 1988)
THC nabilone canbisol	10.0 mg/kg; oral 0.12 - 4.0 mg/kg; oral 0.25 & 0.32 mg/kg; oral	L-E	Rate	n.s. decreased response rates by 50% (n=3) decreased response rates w/1 & 4 mg/kg (n=6) decreased response rates w/0.32 mg/kg (n=6)	(Stark & Dews, 1980)

<b>Drug</b>	<b>Dose/R.O.A.</b>	<b>Rat Strain</b>	<b>ICSS Test</b>	<b>Effect</b>	<b>Reference</b>
WIN	0.1 – 3.0 mg/kg i.p.	S-D	R-F	increased thresholds w/1 mg/kg (n=5-6)	(Mavrikaki, et al., 2010)
WIN	0.1 – 3.0 mg/kg i.p.	S-D	R-F	*increased thresholds w/3 mg/kg (n=7)	(Vlachou, et al., 2005)
CP	10.0 – 100.0 µg/kg i.p.			*increased thresholds w/100 µg/kg (n=8)	
HU-210	10.0 – 100.0 µg/kg i.p.			*increased thresholds w/30 & 100 µg/kg (n=7)	
WIN	0.1 – 1.0 mg/kg i.p.	S-D	R-F	no change in thresholds (n=4-9)	(Vlachou, et al., 2003)
CP	10.0 – 50.0 µg/kg i.p.	Lewis	R-F	no change in thresholds (n=7)	(Arnold, et al., 2001)
^AMG-3	1.0 – 8.0 mg/kg i.p.	S-D	R-F	*increased thresholds w/8.0 mg/kg (n=14)	(Antoniou, et al., 2005)
levonantradol (0.0125 - 0.3 mg/kg; s.c.)		CDF	D-T	increased thresholds w/0.2 & 0.3 mg/kg (n=4)	(Kucharski, et al., 1983)

#### **Endocannabinoid Enzyme/Transport Inhibitors**

#AM-404	1.0 – 30.0 mg/kg i.p.	S-D	R-F	*increased thresholds w/30 mg/kg (n=11)	(Vlachou, et al., 2008)
\$URB-597	0.3 – 3.0 mg/kg i.p.	S-D	R-F	*increased thresholds w/1 and 3 mg/kg (n=7)	(Vlachou, et al., 2006)
%PMSF	15.0 – 60.0 mg/kg i.p.			increased thresholds w/15, 30, & 60 mg/kg (n=7)	
#OMDM-2	3.0 – 30.0 mg/kg i.p.			*increased thresholds w/30 mg/kg (n=5)	

#### **Cannabinoid Antagonists**

RIM	0.02 mg/kg	S-D	R-F	no change in responding; 0.02 mg/kg blocked facilitation produced by 0.1 mg/kg and attenuation by 1.0 mg/kg THC (listed above)	(Katsidoni, et al., 2013)
-----	------------	-----	-----	--	---------------------------



Drug	Dose/R.O.A.		Rat Strain	ICSS Test	Effect	Reference
RIM	1.0 mg/kg i.p.	S-D	R-F		no change in responding; 1 mg/kg blocked (n=5-6) attenuated responding by 3.2 mg/kg THC (listed above)	(Kwilasz & Negus, 2012)
RIM	0.1 – 10.0 mg/kg i.p.	L-E	R-F		!increased thresholds w/10 mg/kg (n=6-7)	(Xi et al., 2008)
AM-251	0.1 – 10.0 mg/kg i.p.				!no change in thresholds (n=7-9)	
RIM	0.02 mg/kg i.p.	S-D	R-F		no change in thresholds (n=6), blocked threshold increase by 30 mg/kg AM-404 (n=6) (listed above)	(Vlachou, et al., 2008)
RIM	0.02 mg/kg i.p.	S-D	R-F		no change in thresholds, blocked threshold increase by 2 mg/kg THC (n=6) (listed above)	(Vlachou, et al., 2007)
RIM	0.02 – 1.0 mg/kg i.p.	S-D	R-F		no change in thresholds; 0.02 mg/kg blocked threshold increase by URB-597 and OMDM-2 (n=5-7) (listed above)	(Vlachou, et al., 2006)
RIM	0.02 – 3.0 mg/kg i.p.	S-D	R-F		no change in thresholds (n=9), blocked threshold increase by 100 µg/kg CP (n=9) and 3 mg/kg WIN (n=11) with 0.02 mg/kg SR	(Vlachou, et al., 2005)
AM-251	1.0 & 3.0 mg/kg i.p.	S-D	R-F		no change in thresholds (n=6), blocked threshold increase by 100 µg/kg HU-210 (n=6) with 1 & 3 mg/kg AM251 (listed above)	
RIM	1.0 – 10.0 mg/kg i.p.	W-f	R-F		decreased responses on FR10 schedule of ICSS w/3 & 10 mg/kg SR (n=3-12) (note: also reduced operant responding for food in female and male rats w/1, 3, & 10 SR)	(De Vry, et al., 2004)

Drug	Dose/R.O.A.	Rat Strain	ICSS Test	Effect	Reference
RIM	0.02 & 0.3 mg/kg i.p.	S-D	R-F	no change in thresholds (n=9) (note: reversed the effects of WIN, which blocked cocaine-induced threshold decreases) (listed above)	(Vlachou, et al., 2003)
RIM	1.0 – 20.0 mg/kg i.p.	S-D	R-F	increased thresholds w/20 mg/kg SR (n=6) (listed above)	(Arnold, et al., 2001)
RIM	0.3 – 10.0 mg/kg i.p.	S-D	R-F	increased thresholds w/1, 3, and 10 mg/kg (n=8)	(Deroche-Gamonet, et al., 2001)
AM-251	3.0 mg/kg i.p.	L-E	MT	decreased the price (lever press duration) able to sustain ICSS responding without significantly affecting frequency threshold (n=8)	(Trujillo-Pisanty, et al., 2011)
AM-251	1.0 mg/kg i.p.	S-D	R-F	no change in thresholds (n=14), blocked threshold increase by 8 mg/kg <sup>^</sup> AMG-3 (n=14) (listed above)	(Antoniou, et al., 2005)

Table 1 was adapted from (Panagis, Vlachou, & Nomikos, 2008) and expanded upon.

Symbol definitions: <sup>\*</sup> effect was also blocked by a CB<sub>1</sub> antagonist, <sup>^</sup>Δ(8)-THC analogue with cannabimimetic properties, <sup>\$</sup>FAAH inhibitor, <sup>#</sup>purported AEA uptake/transport inhibitor, <sup>%</sup>nonselective serine protease inhibitor (non-selectively inhibits FAAH), <sup>!</sup>produced a reduction in cocaine-facilitated ICSS.

Abbreviations: R.O.A. = route of administration, S-D = Sprague-Dawley, L-E = Long-Evans, CDF = albino CDF, W-f = Wister (female), R-F = rate frequency, A-T = auto-titration, Rate = stimulation parameters were not varied and rate was measured, DT = discrete trials, MT = reinforcement mountain, THC = tetrahydrocannabinol, RIM = rimonabant, WIN = WIN 55212-2 a synthetic full agonist at the CB<sub>1</sub> receptor, AEA = anandamide, CP = CP 55,940 a synthetic full agonist at the CB<sub>1</sub> receptor, n.s. = not statistically significant, levonantradol = CP 50,556-1

The results with CB<sub>1</sub> antagonists in ICSS provide evidence that the endocannabinoid system is involved in mesolimbic reward. Since high doses of CB<sub>1</sub> antagonists can reduce ICSS, it may indicate that endocannabinoid tone is necessary to produce normal mesolimbic reward functioning. If blockade of endocannabinoid tone reduces responding for mesolimbic activation, 2-AG and/or AEA may be necessary for normal functioning in that system. It is important to note however, that rimonabant has also been shown to disrupt other behaviors, including operant responding for food, so the effect may not be specific to brain stimulation reward (De Vry, et al., 2004; Marusich & Wiley, 2012).

### **Cannabinoid Activity in the Mesolimbic System**

As briefly discussed earlier, the mesolimbic system is a dopaminergic signaling pathway that contains projections and interconnections to and from the ventral tegmental area (VTA), amygdala, hippocampus, and prefrontal cortex, and to the nucleus accumbens. It is largely comprised of dopaminergic projections from the VTA, and is innervated by many excitatory and inhibitory inputs including glutamatergic and GABAergic inputs (Bielajew & Shizgal, 1986). This system has been studied exhaustively as a potential common pathway of drug and non-drug reward and reward-motivated behavior, and it has been reported that ICSS activates this system and increases dopamine in this system (Cheer, Wassum, et al., 2007; Neill, et al., 2002; R. A. Wise, 2005). Many psychomotor stimulants including cocaine and amphetamine interact with this pathway due to their direct activity at dopaminergic neurons.

Cannabinoid receptors and endocannabinoids are located within the mesolimbic system. CB<sub>1</sub> receptors are located in the nucleus accumbens, VTA, PFC, as well as the amygdala and many other regions (Hrabovszky et al., 2012; Yoshida et al., 2006). Additionally, AEA and 2-AG and their precursors have been found throughout these regions (Matyas et al., 2008; Melis et

al., 2006; Yoshida, et al., 2006). Also, of interest to this study, the biosynthetic enzyme of 2-AG, diacylglycerol lipase (DAGL) is found in the membranes of dopaminergic neurons in the VTA (Matyas, et al., 2008; Yoshida, et al., 2006), though CB<sub>1</sub> receptors are not present on those neurons. Specifically, CB<sub>1</sub> receptors have been found within GABAergic and glutamatergic terminals in the VTA and nucleus accumbens (Kortleven, Fasano, Thibault, Lacaille, & Trudeau, 2011; Matyas, et al., 2008). The presence of cannabinoid receptors and endocannabinoids in these regions suggests a role for cannabinoid modulation of dopamine release.

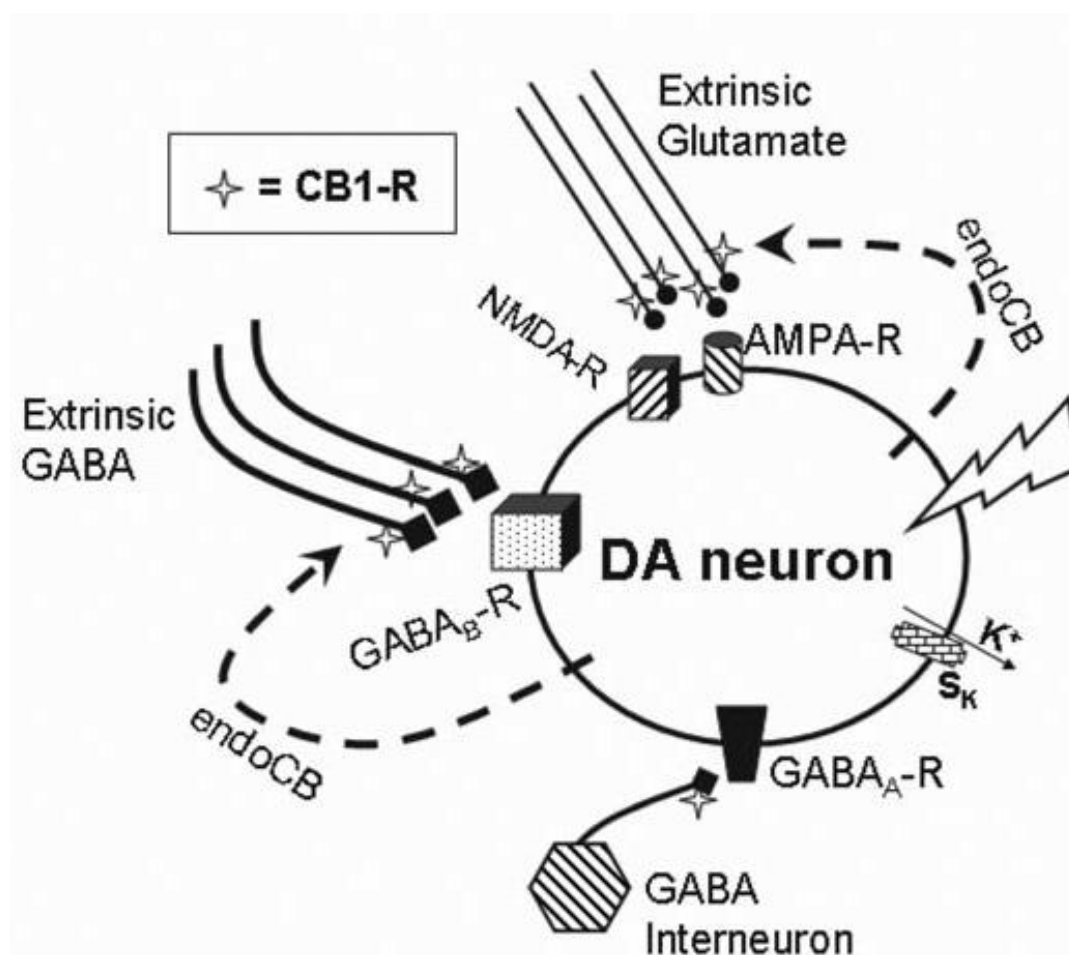
Interestingly, rat studies with exogenous cannabinoids have been shown to increase dopamine release in brain regions in the mesolimbic system including the nucleus accumbens (NAc) and prefrontal cortex. For example, i.v. administration of the CB<sub>1</sub> agonist WIN55,212-2 increases dopamine release in the NAc core and shell (Cheer, Wassum, Heien, Phillips, & Wightman, 2004; Tanda, Pontieri, & Di Chiara, 1997), i.v. THC mimics those effects (Tanda, et al., 1997) Conversely, i.v. administration of the CB<sub>1</sub> antagonist, RIM, blocks enhanced dopamine release in the NAc shell produced by drugs of abuse such as cocaine, ethanol, nicotine, and THC measured with *in vivo* voltammetry (Cheer, Wassum, et al., 2007) and microdialysis (Tanda, et al., 1997). Furthermore, THC has been shown to strain-dependently enhance dopamine release in the nucleus accumbens and medial prefrontal cortex (J. Chen, Paredes, Lowinson, & Gardner, 1990; J. P. Chen et al., 1990; J. P. Chen, Paredes, Lowinson, & Gardner, 1991). In another study, i.v. administration of THC was shown to produce increases in striatal dopamine release measured with microdialysis (Malone & Taylor, 1999). Further evidence of cannabinoid involvement in the mesolimbic system shows that micro-injection of THC into the VTA also enhances dopamine release (J. Chen, Marmur, Pulles, Paredes, & Gardner, 1993). In addition to these data, cannabinoid agonists have been shown to increase dopaminergic neuron firing in the

rat VTA, which has projections to the nucleus accumbens. This effect has been shown with i.v. administration of the cannabinoid agonists THC, WIN 55,212-2, and CP 55,940 measured by whole cell electrophysiology (French, 1997; French, Dillon, & Wu, 1997; Gessa, Melis, Muntoni, & Diana, 1998). These studies suggest that cannabinoids may produce rewarding effects through a similar mechanism to other drugs of abuse including psychomotor stimulants and opiates.

A generally accepted theory of how cannabinoids may modulate activity in the mesolimbic system was proposed by Carl Lupica's group in 2005. As mentioned earlier, CB<sub>1</sub> receptors are generally located on pre-synaptic neuron terminals where their activation reduces the release of a specific neurotransmitter from that terminal (Cadogan, et al., 1997; Katona, et al., 2000; Robbe, et al., 2001). Additionally, GABAergic projections are responsible for tonic inhibition of spontaneous dopamine firing in the VTA, while glutamatergic projections excite dopaminergic neurons, as depicted on Fig. 2. Lupica and colleagues propose that CB<sub>1</sub> stimulated dopamine release is maintained through CB<sub>1</sub> receptors on GABAergic primary and interneurons projecting onto dopaminergic neurons in the VTA (Lupica & Riegel, 2005). By reducing GABAergic signaling, which normally inhibits dopaminergic activity, it could result in a functional increase in dopamine. It is important to note though, that CB<sub>1</sub> receptors are located on GABAergic neuron terminals, (Fernandez-Ruiz, Hernandez, & Ramos, 2010; Katona, et al., 2000; Ong & Mackie, 1999) as well as on glutamatergic and cholinergic neuron terminals (Fernandez-Ruiz, et al., 2010; Lu, Ong, & Mackie, 1999; Ong & Mackie, 1999). In contrast to GABAergic CB<sub>1</sub> receptor activation, CB<sub>1</sub> receptors on glutamate neurons can reduce spontaneous dopaminergic neuron firing (Fig. 2). There is evidence however, that CB<sub>1</sub> receptors are found in higher levels in GABAergic interneurons than on cholinergic or glutamatergic

projecting neurons, which indicates they may be more sensitive to cannabinoid activation (Marsicano & Lutz, 1999). Additionally, electrophysiology data shows that the cannabinoid agonist, WIN55,212-2 is more potent at reducing neuronal transmission in GABAergic neurons than glutamatergic neurons (Hajos & Freund, 2002a, 2002b; Ohno-Shosaku et al., 2002).

This evidence supports observations that cannabinoids can produce biphasic effects in some behavioral tests measuring reward or reinforcement, and may only do so within a limited dose-range. High doses of CB<sub>1</sub> agonists produce different effects than low doses, which may indicate the balance of CB<sub>1</sub> receptor activation shifts from GABAergic to glutamatergic/cholinergic in the VTA.



*Figure 2.* A depiction of cannabinoid receptors and endocannabinoid activity on GABAergic and glutamatergic axons projecting to dopaminergic neurons in the ventral tegmental area (VTA). Cannabinoid receptor activation on GABAergic neurons increases dopaminergic firing, while activation of cannabinoid receptors on glutamatergic neurons produces the opposite effect. Figure adapted from (Lupica & Riegel, 2005).

## Rationale

Although reward-associated effects of cannabinoids are difficult to assess using preclinical measures, there is much evidence that marijuana produces positive subjective effects in humans, including positive subjective effect ratings associated with marijuana use (Hart, et al., 2010; Hart, et al., 2001), and brain imaging studies implicating reward-relevant brain areas during marijuana cue presentations in marijuana-dependent individuals (Goldman, et al., 2013). Several studies have demonstrated that THC facilitates ICSS (Gardner, et al., 1988; Lepore, et

al., 1996), which suggests that ICSS may allow for evaluation of reinforcement-potentiating effects associated with cannabinoids. Additionally, blockade of CB<sub>1</sub> receptors has been shown to reduce ICSS behavior, which provides further evidence that the endocannabinoids and cannabinoid receptors are involved in mesolimbic brain functioning.

Neurochemical correlates associated with other drugs of abuse in the mesolimbic system are also observed during cannabinoid administration. For example, exogenous cannabinoids increase dopamine release in brain areas including the nucleus accumbens and prefrontal cortex as measured with *in vivo* microdialysis or voltammetry (Cheer, et al., 2004; Cheer, Wassum, et al., 2007; J. Chen, et al., 1993; J. Chen, et al., 1990; J. P. Chen, et al., 1991; Malone & Taylor, 1999; Ng Cheong Ton et al., 1988; Tanda, et al., 1997). Additionally, cannabinoid agonists have been shown to increase dopaminergic neuron firing in the rat VTA (French, 1997; French, et al., 1997; Gessa, et al., 1998), suggesting they activate the mesolimbic system, and may in fact potentiate reinforcement produced by stimulation of the medial forebrain bundle.

Almost all of the findings with THC and reward-related behaviors discussed above have been demonstrated in rats. However, there are far fewer studies investigating these effects in mice. As shown on table 1, there have been no studies involving cannabinoid agonists or antagonists with ICSS in mice. C57BL/6 mice are known to engage in certain drug-taking behaviors that other strains and species do not, such as freely drinking unsweetened ethanol diluted with water (Elmer, Meisch, & George, 1987). Furthermore, alcohol has been shown to facilitate ICSS in mice (Fish et al., 2010), while producing mixed effects in rats depending on strain and ICSS assay used (Carlson & Lydic, 1976; Schaefer & Michael, 1992; Schaefer, Richardson, Bonsall, & Michael, 1988). Additionally, there have been three studies demonstrating self-administration of i.v. WIN55,212-2 in mice (Flores, et al., 2013; Martellotta,



et al., 1998; Mendizabal, Zimmer, & Maldonado, 2006), and reports of increases in dopamine in the nucleus accumbens with systemic administration of the synthetic cannabinoid agonist WIN55,212-2, which suggests that this strain may be a good candidate to observe ICSS facilitation with cannabinoids, and demonstrate cannabinoid rewarding effects in a preclinical mouse model (Robledo, Trigo, Panayi, de la Torre, & Maldonado, 2007).

The effects of THC and selective FAAH, MAGL and combined FAAH/MAGL inhibitors have not been investigated in mouse ICSS assays. Furthermore, no published studies to date have evaluated MAGL inhibitors, or the FAAH inhibitor PF-3845 in any species with ICSS. In this study, we assessed THC and selective inhibitors of FAAH, MAGL, and a novel dual FAAH/MAGL using ICSS to infer the consequences of increased endocannabinoid levels on operant responding for electrical stimulation of the medial forebrain bundle. Mechanism of action for any observed effects were evaluated with CB<sub>1</sub> and CB<sub>2</sub> antagonism tests. We also investigated spontaneous locomotor behavior and operant responding for food pellets in order to ascertain whether these pharmacological treatments affected other behavioral measures in a similar fashion as ICSS. Additionally, we measured brain endocannabinoid levels in regions associated with the mesolimbic system including the prefrontal cortex, ventral mid-brain (approximation of VTA), nucleus accumbens and amygdala after specific enzyme inhibitor treatments to determine changes after drug treatment. The cerebellum was assessed as a non-mesolimbic region.

### **Overall Hypothesis**

Cannabinoid agonists and endocannabinoid enzyme inhibitors will potentiate reinforcement maintained by self-stimulation of the medial forebrain bundle in C57BL/6 mice.

## **Selection of Pharmacological Agents**

The CB<sub>1</sub> agonist THC was assessed in ICSS to determine the effect of the primary active constituent in marijuana. The MAGL inhibitor JZL184 was used to assess elevation of 2-AG and other effects attributable to MAGL inhibition. JZL184 is a selective and readily available MAGL inhibitor that is irreversible, and has been shown to produce 10-fold elevations in whole mouse brain 2-AG that persist over 12 hours after administration (J. Z. Long, Li, et al., 2009). JZL184 also produces increases of interstitial 2-AG in the mouse nucleus accumbens, and although it has been shown to inhibit FAAH when given repeatedly, it does not affect interstitial AEA levels when give acutely (Wiskerke, et al., 2012). The FAAH inhibitor PF-3845 was used to assess consequences of preventing AEA degradation, and other effects attributable to FAAH inhibition. PF-3845 is highly selective for FAAH inhibition and also produces up to 10-fold increases of AEA that persist over a similar time-course as JZL184 increasing levels of 2-AG (Ahn, et al., 2009). The dual (FAAH/MAGL) inhibitor SA-57 was used to assess the effects of combined inhibition of both endocannabinoid degrading enzymes on ICSS responding. SA-57 produces dose-related elevations in AEA and 2-AG, and has a higher affinity for FAAH than MAGL (Niphakis, et al., 2012). Cocaine HCl was used as a positive control, as it reliably facilitates ICSS in a robust fashion (Corbett, 1991; Fish, et al., 2012; Robinson, et al., 2012).

## **Methods**

### **Subjects**

A total of 245 male C57Bl/6 mice aged 8 – 10 weeks (22-30g), obtained from Jackson Laboratories (Bar Harbor, Maine) were used for the experiments described (78 for ICSS studies, 13 for operant studies, 90 for endocannabinoid measurement, and 64 for locomotor activity studies). Mice were between 8 and 12 weeks of age at the time of either surgery, brain dissection

for endocannabinoid quantification, operant training, or locomotor activity testing. Mice were maintained on a 12:12 hour light/dark cycle beginning at 6:00 AM, individually housed, and given free access to food and water. All experiments were conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals, and have been approved by the Virginia Commonwealth University Institutional Animal Care and Use Committee (IACUC).

## **Drugs**

Cocaine HCl (obtained from NIDA drug supply) was dissolved in 0.9% saline. JZL184, rimonabant, SR144528, and THC (obtained from NIDA drug supply) SA-57 (obtained from Micah Niphakis, TSRT, La Jolle, CA), and PF-3845 (obtained from Organix, Woburn, MA) was dissolved in a vehicle solution (VEH) of 1 part ethanol, 1 part Emulphor-620 (Rhone-Poulenc, Princeton, NJ), and 18 parts 0.9% saline (1:1:18).

## **Apparatus**

ICSS testing was conducted using eight standard (18 X 18 X 18 cm) mouse operant conditioning chambers (Med Associates Inc., St. Albans, VT, USA). 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 is fed through a hole in the top of the chamber, in order to be connected to the electrode attached to a mouse. 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 record data during training and testing sessions. Operant responding for food tests were conducted in mouse operant conditioning chambers (Med Associates Inc.) similar to those described above. Each chamber was equipped with two nose

poke apertures (instead of levers), a food hopper to deliver sweetened food pellets (Bio-Serv) to a recessed well within the chamber. All other aspects of the chamber were identical to those used for ICSS testing, except they lack tone generators, and all equipment related to electrical stimulation, including commutators, tethers, etc. Locomotor activity testing was conducted in Plexiglas boxes (17.5 X 8.5 X 8 in) with plastic floor inserts, capped with clear Plexiglas lids, and enclosed within light and sound attenuating chambers. Locomotor activity was recorded during the tests, using Unibrain Fire-I digital cameras and analyzed using ANY-maze software (Stoelting, Kiel, WI) track and quantify movements.

### **Stereotaxic Surgery**

Surgical procedures for implanting electrodes in mice for ICSS studies were similar to those previously reported (Carlezon & Chartoff, 2007). Mice were anesthetized with isoflurane and received constant delivery during surgical procedures. Bipolar twisted stainless steel electrodes (0.125 mm single wire diameter without insulation; Plastics One, Roanoke, VA) were implanted into the right medial forebrain bundle of the mice using coordinates similar to those reported by Straub and colleagues: 2.0 mm posterior of bregma, 0.8 mm lateral from midline, and 4.8 mm below dura (Straub, Carlezon, & Rudolph, 2010). After the hole for the electrode was drilled, three holes were bored into the skull surrounding the area the electrode would be placed, anchoring screws were secured in those holes. Finally, an electrode was inserted and fixed to the skull 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**

**Locomotor activity.** Mice were acclimated to testing room 24 hours before testing began. After mice were given selected drugs/doses, they were returned to their home cages for two hours. Following the two hour pre-treatment time mice were immediately placed in the Plexiglas activity observation boxes and recording using Any-maze software (Stoelting, Kiel, WI) began. Activity was assessed for 25 minutes and mice were returned to home cages at the conclusion of the test. All tests were recorded, and distance travelled, time-immobile, and adjusted speed (distance/time – time immobile) was calculated. Box assignment and time-of test were counter-balanced between the different drug/dose conditions. Separate groups of mice were used for each treatment condition. Mice were exposed to the Plexiglas observation boxes once under a single experimental condition to prevent habituation from altering results.

**Operant responding for food.** Mice were initially food deprived to 85% of their free feeding weight, before being exposed to operant chambers. During 15 minute operant sessions, mice were assigned one nose-poke aperture, while the 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 reinforcers (Bio-Serv), and FR value was gradually increased to a FR-10 schedule of reinforcement. A computer with logic interface and MED-PC software (Med Associates) was used to manipulate rate of reinforcement and record data from training and test sessions. Mice began testing when they maintained at least 20 responses per minute, and total responses remained within 10% of the prior 2 training days for at least 3 consecutive training days. These criteria were evaluated before every operant test was conducted, also ensuring that mice had a minimum of 96 hours between testing conditions. Subjects were given a minimum 1 week wash-

out period before beginning testing a new compound. On test days, mice were injected in the testing room, and allowed to remain in home cages during the given pre-treatment time. Vehicle (VEH) solution 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.

**ICSS.** Seventy-eight mice were implanted with electrodes, twenty-one mice were able to complete at least one experiment, while fifty-seven mice either didn't reach testing criteria, or lost electrode viability before completing a single test series. After recovery, mice began lever-press training. Mice were trained to lever press on a fixed-ratio 1 (FR1) schedule of reinforcement, where 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, and 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 a reasonable response rate (i.e.  $\geq 35$  responses per minute). Stimulation amplitudes were adjusted individually between 20 and 300  $\mu$ A in order to maintain maximal rates before testing began.

After stable responding was maintained, mice were moved to rate-frequency training. During rate-frequency training mice were presented with and allowed to respond for a series of 10 descending stimulation frequencies at 0.05 log unit increments (56-158 Hz), in contrast to only being exposed to one frequency (141 Hz) during FR training. During each frequency presentation within each series, mice were first given a 5 s time-out in which levers were not present in the boxes, followed by 5 s during which the lever was extended and 5 non-contingent stimulations at a given frequency were delivered, followed by a 60 s period when they were

allowed to respond for stimulations at that specific frequency. The lever was then retracted, the stimulation frequency decreases by 0.05 log units, and the next frequency presentation begins. For example the first frequency presentation is 158 Hz, after the 60 s response period has concluded, and mice were allowed to respond for 141 Hz for the next 60 s, followed by 126, and so on until they are presented with the tenth and last frequency (56 Hz), which concluded the series. These frequency presentation series' were repeated between 3 and 5 times per training session. Current amplitudes were further individually adjusted if needed for each subject to maintain  $\geq 50\%$  baseline maximal responding in at least three and fewer than eight frequencies. The first series of frequency presentations each day was considered a daily "warm-up" period and data is excluded from all analyses. The next two series were averaged together to create the baseline response curve for that day. Following a given treatment, mice were again allowed to respond for two series of frequencies, which were averaged together for analysis. When the total responses during baseline varied by less than 20% within each mouse for 3 consecutive days of assessment, and VEH/saline tests produced between 80 and 120% of baseline responding for at least two consecutive assessments, and visual analysis of rate-frequency curves confirmed they were curvilinear, mice were deemed able to begin testing.

On test days, mice were placed in the chambers and allowed to respond during the "warm-up" and baseline phases. Immediately after baseline responding, mice were removed from operant chambers, placed in their home cages, injected with drug, saline, or VEH, and returned to home cages for pre-treatment time before beginning testing. Mice were then returned to the operant chambers to respond during two additional series of frequency presentations, which were considered the test series. Mice first received injections of vehicle (VEH) and/or

saline to ensure that neither solution affected the frequency response curves before beginning dose response curves.

Drugs were tested with a minimum of 72 hours between tests, and at least 1 week between drugs, to ensure drug clearance and baseline assessments were made before all tests, to ensure the subjects were responding normally before drugs are administered. VEH or saline tests were conducted before and after determining dose-effect curves to ensure that conditioned effects did not develop over time, and that baselines remained stable throughout the course of the experiment. Mice were exposed to doses in ascending or descending order in a counter-balanced design. Antagonist test conditions were tested in a quasi-randomized order. This study used a mixed design wherein some subjects completed only one drug curve, and other subjects completed all drug tests. All drugs tested included some drug-naïve subjects. Cannabinoid effects on ICSS were reproducible over time during time-course and antagonist testing.

### **Analytical Procedures**

**Tissue extraction and quantification of AEA, 2-AG and AA.** Drug naïve subjects were injected with VEH, JZL184 (4.0 and 40.0 mg/kg) or SA-57 (1.0 or 10.0 mg/kg) i.p. two hours prior to sacrificing. Immediately following cervical dislocation and decapitation, brains of C57 male mice were removed, dissected, and the prefrontal cortex, nucleus accumbens, ventral mid-brain, amygdala, and cerebellum were removed. The selected brain regions of interest were dissected from whole fresh brains. The prefrontal cortex was dissected by making a cut at the posterior extent of the anterior olfactory nucleus after which the olfactory nuclei were removed. This sample included frontal association, primary and secondary motor, anterior cingulate, prelimbic and orbital frontal cortices. The next cut was made anterior to the optic chiasm to produce a thick coronal section. The nucleus accumbens was dissected by removing the cortex



ventrally and the septum and nucleus of the horizontal limb of the diagonal band medially and then collecting the tissue surrounding the anterior commissure. The amygdala (including the central nucleus, basolateral and basomedial nuclei) dissection was made by first cutting caudal to the optic chiasm, and then making a second cut directly caudal to the median eminence. The amygdala was isolated by removing piriform cortex lateral of the amygdala and taking the region included in the bifurcated corpus collosum and all regions ventral to that. The ventral midbrain dissection was made by making a cut rostral to the cerebellar peduncles, then removing the remaining hypothalamic subregions ventrally and substantia nigra laterally. Isolation of ventral midbrain was made by removing the remaining regions below the red nucleus and includes the ventral tegmental area and interfascicular nucleus. The cerebellum was then removed from the brain stem.

On the day of processing, the pre-weighed tissues were homogenized with 1.4 ml chloroform: methanol (2:1 v/v containing 0.0348g PMFS/ml) after the addition of internal standards to each sample (2 pmole AEA -d8, 1 nmole 2-AG-d8 and 1 nmole AA-d8). Homogenates were then mixed with 0.3 ml of 0.73% w/v NaCl, vortexed and then centrifuged for 10 min at 2054g (4° C). The aqueous phase plus debris were collected and extracted again twice with 0.8 ml chloroform. The organic phases from the three extractions were pooled and the organic solvents were evaporated under nitrogen gas. Dried samples were reconstituted with 0.1 ml chloroform and mixed with 1 ml cold acetone. The mixtures were then centrifuged for 5 min at 2054g and 4 °C to precipitate the proteins. The upper layer of each sample was collected and evaporated under nitrogen. Dried samples were reconstituted with 0.1 ml methanol and placed in autosample vials for analysis.

LC/MS/MS was used to quantify AEA, 2-AG and AA. The mobile phase consisted of (10:90) water: methanol with 0.1% ammonium acetate and 0.1% formic acid. The column used was a Discovery® HS C18, 2.1\* 150 mm, 3 micron (Supelco, USA). Ions were analyzed in multiple reaction monitoring mode and the following transitions were monitored in positive mode: (348>62) and (348>91) for AEA; (356>62) for AEA-d8; (379>287) and (379>269) for 2-AG; (387>96) for 2AG-d8; (300>62) and in negative mode: (303>259) and (303>59) for AA and (311>267) for AA-d8. A calibration curve was constructed for each assay based on linear regression using the peak area ratios of the calibrators. The extracted standard curves ranged from 0.039 pm to 40 pm for AEA, from 0.0625nm to 64 nm for 2-AG and from 1 nm to 32 nm for AA.

### **Data Analysis**

The independent variable measured in the ICSS studies was stimulations per min during each frequency trial. ICSS response data were evaluated using two separate methods. In the first method, data were normalized to the highest response rate recorded during the baseline rate-frequency curve generated for each individual mouse. This transformation allowed us to display the data as the percent maximum control rate (% MCR) and normalized individual response rates between subjects. These data were used to generate the rate-frequency curves which were assessed using repeated-measures, two-way ANOVAs (treatment x frequency) between baseline and treatment curves for each drug/dose tested. Holm-Sidak tests were used for post-hoc analyses of rate-frequency results which allowed multiple comparisons at all frequencies.

A second method to analyze the data was also used in order to perform between-dose comparisons, and provide summary data. The total number of stimulations during the test series was divided by the total number of responses during the baseline series and multiplied by 100 to

produce percent baseline stimulations (% Baseline Stimulations) (Negus, Morrissey, Rosenberg, Cheng, & Rice, 2010). This effectively collapsed all the separate frequency stimulation rates into an overall stimulation count and allowed for comparison of data between days/treatments. This calculation was performed for all doses of each drug and VEH/saline and those values were analyzed using repeated-measures one-way ANOVAs. Dunnett's post-hoc test were used following significant ANOVAs to compare treatment groups to VEH controls.

Distance travelled, time-immobile, and adjusted mean speed (distance/(time – time immobile)) were observed during locomotor activity studies and analyzed using independent sample 1-way analysis of variance (ANOVA), and followed up with Dunnett's post-hoc tests. In the operant responding for food studies response rate was the independent variable of interest, and was analyzed using repeated-measures 1-way ANOVAs, post-hoc analyses were conducted using Dunnett's post-hoc test. Brain endocannabinoid and AA levels (pm/g or nm/g) were analyzed using individual one-way ANOVA for each brain region and substrate measured. Significant ANOVAs were assessed using Dunnett's post-hoc analysis.

## **Results**

### **Cocaine ICSS Dose-Response Assessment**

This study evaluated the ability of cocaine to facilitate ICSS in C57Bl/6 mice. Seven mice were assessed with ICSS after saline and cocaine (1.0, 5.0 and 10.0 mg/kg), and data were analyzed using repeated-measures two-way ANOVAs (treatment x frequency) for each test conducted. Saline and 1.0 mg/kg cocaine did not affect ICSS responding, while 5.0 and 10.0 mg/kg cocaine significantly facilitated self-stimulations. Saline produced a main effect of frequency ( $F_{9, 54} = 47.16, p < 0.0001$ ), but no main effect of treatment ( $F_{1, 6} = 0.005, p > 0.05$ ), and no interaction ( $F_{9, 54} = 1.83, p > 0.05$ ) (Fig. 3a); similarly, 1.0 mg/kg cocaine showed a main

effect of frequency ( $F_{9,36} = 48.23, p < 0.0001$ ), but no main effect of treatment ( $F_{1,4} = 0.09, p > 0.05$ ), and no interaction ( $F_{9,36} = 1.83, p > 0.05$ ) (Fig. 3b). Higher doses of cocaine (5.0 and 10.0 mg/kg) significantly and robustly facilitated ICSS. Cocaine (5.0 mg/kg) had a main effect of frequency ( $F_{9,54} = 33.27, p < 0.0001$ ), treatment ( $F_{1,6} = 76.72, p < 0.001$ ) and an interaction ( $F_{9,54} = 11.52, p < 0.0001$ ) (Fig. 3c); 10.0 mg/kg cocaine also produced a main effect of frequency ( $F_{9,54} = 63.04, p < 0.0001$ ), treatment ( $F_{1,6} = 37.69, p < 0.001$ ) and an interaction between frequency and treatment ( $F_{9,54} = 4.15, p < 0.001$ ) (Fig. 3d), indicating that 5.0 and 10.0 mg/kg cocaine facilitated ICSS responding. Post-hoc analysis revealed increases at five and seven frequencies respectively as shown on Fig. 3c and d. Baseline rate-frequency stimulation rates did not differ over the course of testing; there was a main effect of frequency ( $F_{9,36} = 81.22, p < 0.0001$ ), but no main effect of baseline day ( $F_{3,12} = 0.95, p > 0.05$ ), and no interaction ( $F_{27,108} = 0.82, p > 0.05$ ) (Fig. 3e).

The percent baseline stimulations data were analyzed using a repeated-measures one-way ANOVA. Cocaine facilitated ICSS ( $F_{4,12} = 18.56, p < 0.001$ ) (Fig. 3f); post-hoc analysis showed that 5.0 and 10.0 mg/kg cocaine produced significant facilitation of ICSS, while 1.0 mg/kg did not differ from saline treatment. These results are in accordance with the rate-frequency analysis. Cocaine produced a dose-related facilitation of ICSS, as has been shown in the literature.

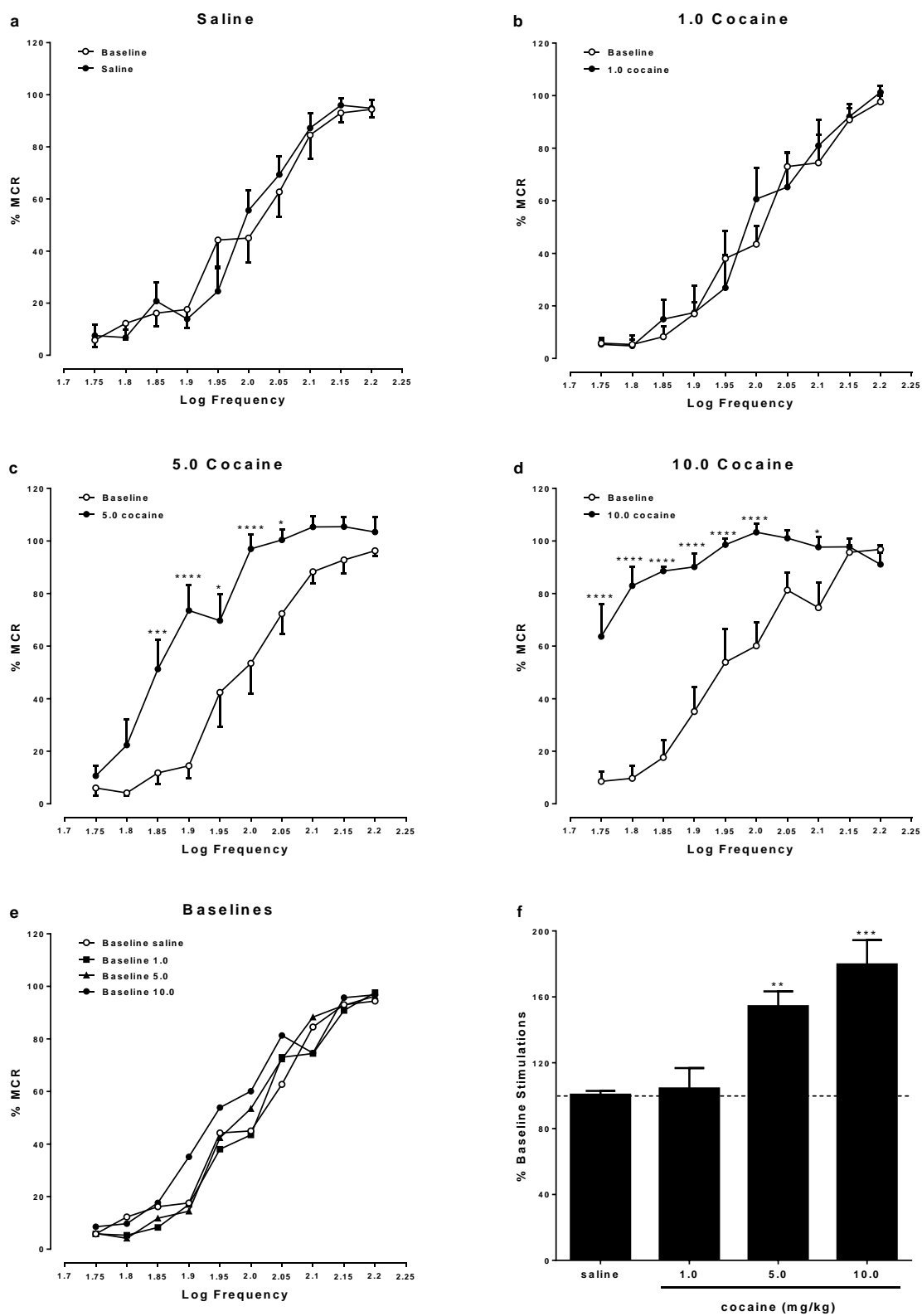


Figure Continues

## Figure Continued

*Figure 3.* Cocaine ICSS assessment. Panels a – e show rate-frequency stimulation values ( $\pm$  SEM) after saline or cocaine (1.0, 5.0, and 10.0 mg/kg); percent maximum control rate is displayed on the Y-axis, log-frequency values are shown on the x-axis. Panel e shows all baselines during testing. Panel f shows percent baseline stimulations ( $\pm$  SEM) from all tests. With rate-frequency tests, interactions that achieved significance and were accompanied by significant post-hoc analyses are denoted by asterisks at values that are significantly different from baseline, \*\*\*\* $p < 0.0001$ , \*\*\* $p < 0.001$ , \*\* $p < 0.01$ , \* $p < 0.05$ . Raw overall response rates (stimulations/min; mean  $\pm$  SEM) during baseline (B) and test (T) sessions were as follows: (a) saline: B ( $31.98 \pm 3.14$ ) T ( $32.09 \pm 3.06$ ); (b) 1.0 mg/kg cocaine: B ( $27.83 \pm 2.76$ ) T ( $29.61 \pm 5.40$ ); (c) 5.0 mg/kg cocaine: B ( $31.25 \pm 2.10$ ) T ( $47.51 \pm 2.75$ ); (d) 10.0 mg/kg cocaine: B ( $36.26 \pm 4.40$ ) T ( $61.44 \pm 2.66$ ).

## THC ICSS Experiments

**THC dose-response assessment.** These experiments were conducted to determine the effect of THC on brain-stimulation reinforcement. ICSS was assessed in seven mice after treatment of vehicle (VEH) and THC (0.3, 1.0, 3.0, 5.6, and 10.0 mg/kg). Data were analyzed using two-way repeated-measures ANOVAs (treatment  $\times$  frequency) for each condition. Analysis of rate frequency data showed that VEH, 0.3 and 1.0 mg/kg THC did not alter responding from baseline while 3.0, 5.6, and 10.0 mg/kg THC reduced ICSS. With VEH, there was a main effect of frequency ( $F_{9,54} = 52.09$ ,  $p < 0.0001$ ), but no main effect of treatment ( $F_{1,6} = 1.21$ ,  $p > 0.05$ ) and no interaction ( $F_{9,54} = 1.03$ ,  $p > 0.05$ ) (Fig. 4a). THC (0.3 mg/kg) produced a main effect of frequency ( $F_{9,54} = 71.93$ ,  $p < 0.0001$ ), but no main effect of treatment ( $F_{1,6} = 4.28$ ,  $p > 0.05$ ) and no interaction ( $F_{9,54} = 1.13$ ,  $p > 0.05$ ) (Fig. 4b). Similarly, 1.0 mg/kg THC produced a main effect of frequency ( $F_{9,54} = 60.20$ ,  $p < 0.0001$ ), but no main effect of treatment ( $F_{1,6} = 3.16$ ,  $p > 0.05$ ) and no interaction ( $F_{9,54} = 0.67$ ,  $p > 0.05$ ) (Fig. 4c). As mentioned above, 3.0 mg/kg THC led to a significant reduction of ICSS; there were significant main effects of frequency ( $F_{9,54} = 53.27$ ,  $p < 0.0001$ ) and drug treatment ( $F_{1,6} = 8.15$ ,  $p < 0.05$ ). However, the interaction between treatment and frequency did not achieve significance ( $F_{9,54} = 1.83$ ,  $p > 0.05$ ).

(Fig. 4d). Higher doses of THC (i.e., 5.6 and 10 mg/kg) also produced significant decreases in ICSS. THC (5.6 mg/kg) produced main effects of frequency ( $F_{9, 54} = 44.15, p < 0.0001$ ) and treatment ( $F_{1, 6} = 13.54, p < 0.05$ ), but no interaction ( $F_{9, 54} = 1.05, p > 0.05$ ) (Fig. 4e). THC (10.0 mg/kg) produced main effects of frequency ( $F_{9, 54} = 46.58, p < 0.0001$ ) and treatment ( $F_{1, 6} = 60.55, p < 0.001$ ) as well as an interaction ( $F_{9, 54} = 5.83, p < 0.0001$ ) (Fig. 4f). Post-hoc analysis showed that stimulations were significantly reduced at eight frequencies, which are denoted on Fig. 4f. These findings indicated that THC (3.0, 5.6, and 10.0 mg/kg) reduced ICSS.

All baseline rate-frequency curves were analyzed after all doses were assessed in order to ascertain if ICSS behavior differed over time (Fig. 5a). Two-way ANOVA revealed a main effect of frequency ( $F_{9, 54} = 46.58, p < 0.0001$ ), but no main effect of baseline day ( $F_{6, 36} = 0.70, p > 0.05$ ) and no interaction between treatment and baseline day ( $F_{54, 324} = 1.2, p > 0.05$ ).

Additionally, a second VEH test was conducted and compared to the initial VEH assessment and showed VEH tests did not differ (Fig. 5b). There was a main effect of frequency ( $F_{9, 54} = 68.82, p < 0.0001$ ), but no main effect of treatment ( $F_{1, 6} = 0.24, p > 0.05$ ) and no interaction ( $F_{9, 54} = 0.91, p > 0.05$ ). These findings indicate that ICSS remained stable throughout the course of this experiment.

Percent baseline stimulations were calculated and evaluated using one-way repeated measures ANOVA. VEH percent baseline stimulations were used for comparison. THC reduced percent baseline stimulations ( $F_{6, 30} = 24.14, p < 0.0001$ ). The three lowest doses of THC (0.3, 1.0, and 3.0 mg/kg) did not differ from VEH (Fig. 6). In contrast, the higher doses of THC (i.e., 5.6 and 10.0 mg/kg) did significantly reduce percent baseline stimulations. Post-hoc analysis showed that the respective doses of THC produced 73.56 % and 30.71% baseline stimulations and were significantly lower ( $p < 0.05$ ;  $p < 0.01$ ) than VEH percent baseline stimulations (Fig.

6). These results confirm rate-frequency analysis that 5.6 and 10.0 mg/kg THC produced a significant reduction of ICSS. Interestingly, rate-frequency analysis showed 3.0 mg/kg THC produced a small reduction in ICSS, but that effect was not detected when analyzing percent baseline stimulations.



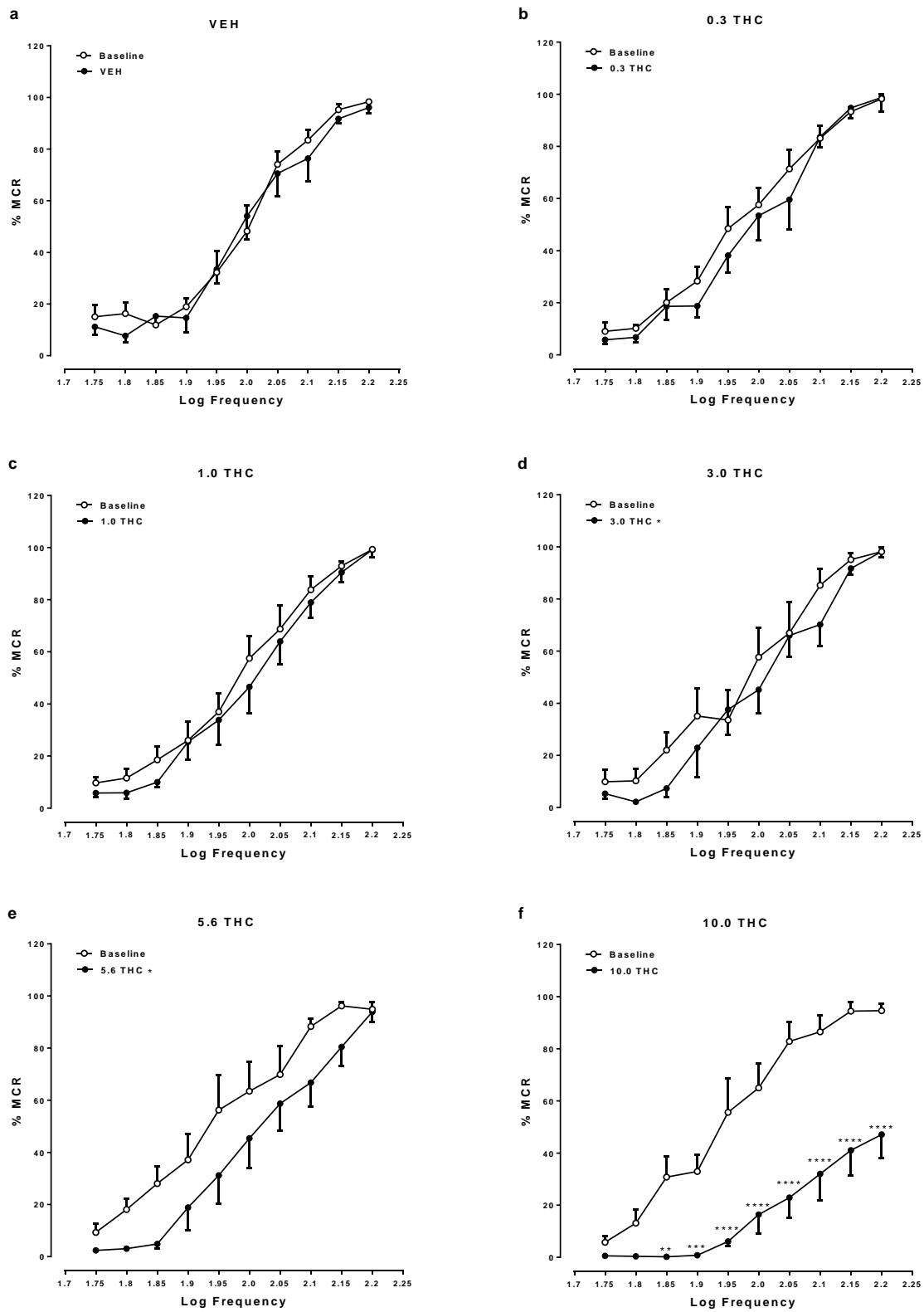
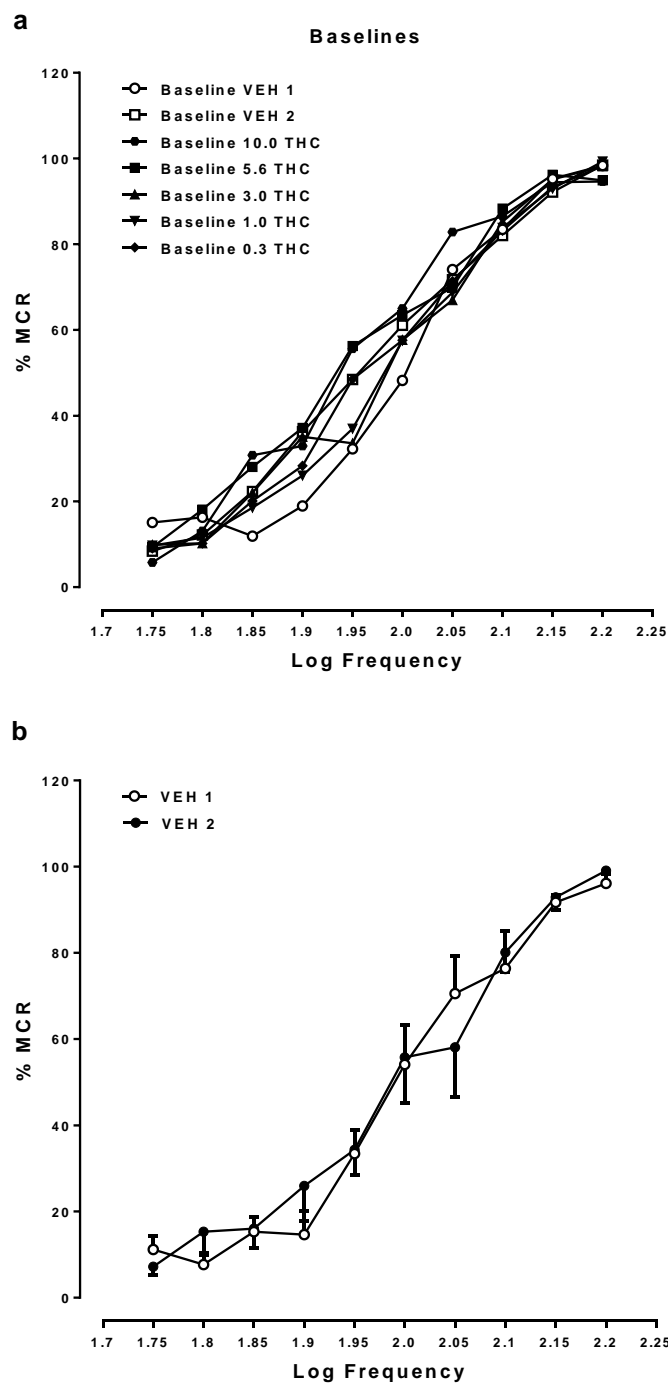


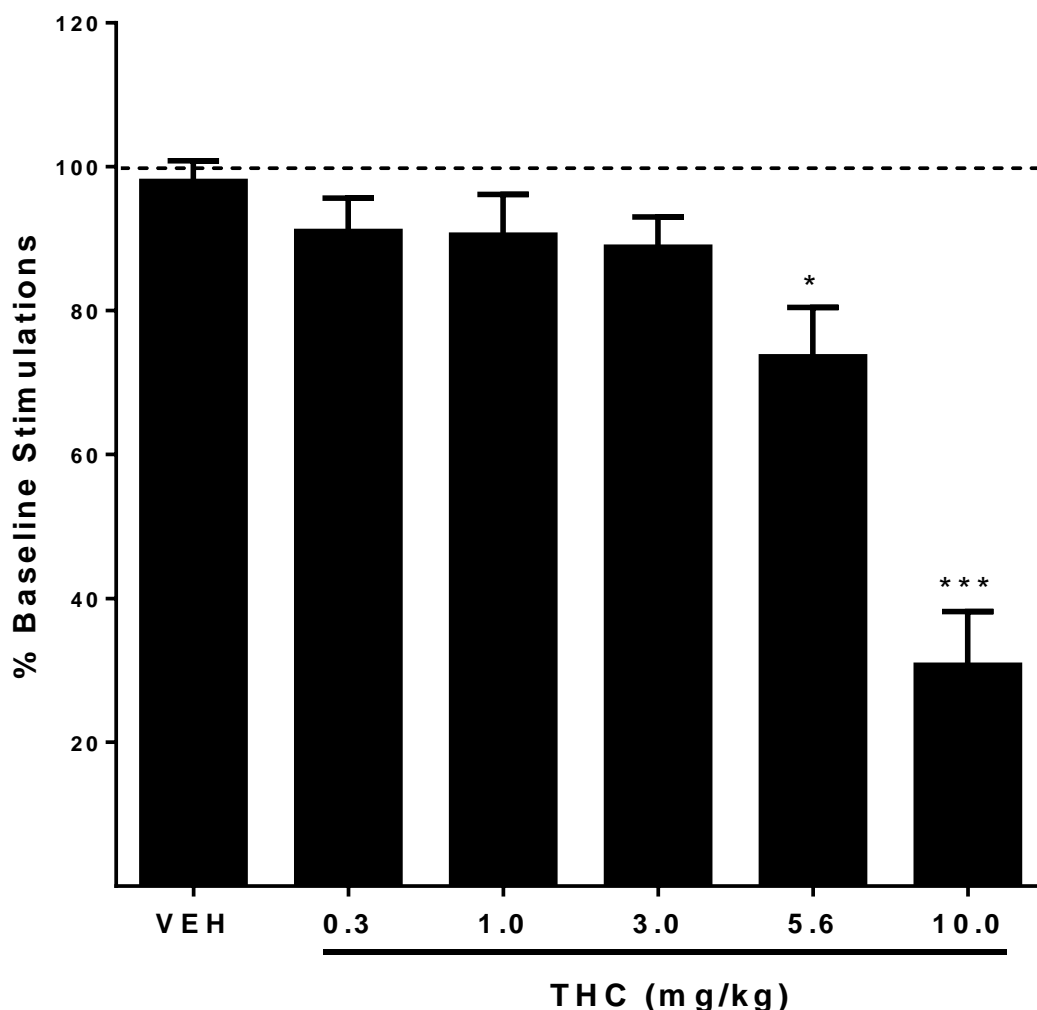
Figure Continues

## Figure Continued

*Figure 4.* THC ICSS dose-response assessment. Panels a – f show rate-frequency stimulation values ( $\pm$  SEM) after VEH or THC (0.3, 1.0, 3.0, 5.6, and 10.0 mg/kg); percent maximum control rate is displayed on the Y-axis, log-frequency values are shown on the x-axis. Significant main effects of treatment are denoted by asterisks by the figure legend; interactions that achieved significance and were accompanied by significant post-hoc results and are denoted by asterisks at values that are significantly different from baseline. \*\*\*\* $p < 0.0001$ , \*\*\* $p < 0.001$ , \*\* $p < 0.01$ , \* $p < 0.05$ . Raw overall response rates (stimulations/min; mean  $\pm$  SEM) during baseline (B) and test (T) sessions were as follows: (a) VEH: B ( $27.67 \pm 2.98$ ) T ( $27.25 \pm 3.23$ ); (b) 0.3 mg/kg THC: B ( $30.94 \pm 3.72$ ) T ( $28.93 \pm 4.15$ ); (c) 1.0 mg/kg THC: B ( $29.49 \pm 3.52$ ) T ( $27.16 \pm 3.85$ ); (d) 3.0 mg/kg THC: B ( $30.61 \pm 5.21$ ) T ( $26.72 \pm 4.39$ ); (e) 5.6 mg/kg THC: B ( $35.06 \pm 4.84$ ) T ( $25.22 \pm 4.17$ ); (f) 10.0 mg/kg THC: B ( $33.47 \pm 4.60$ ) T ( $10.85 \pm 2.77$ ).



*Figure 4.* Baseline ICSS tests. Mean rate-frequency stimulation values ( $\pm$  SEM) during (a) baselines of THC tests and (b) comparing initial VEH tests to VEH tests after THC testing was completed. Percent maximum control rate is displayed on the Y-axis, log-frequency values are shown on the x-axis. Raw overall response rate (stimulations/min; mean  $\pm$  SEM) during VEH 1 was  $(27.25 \pm 3.23)$  and VEH 2 was  $(28.81 \pm 3.53)$  (Panel b).



*Figure. 6.* THC ICSS percent baseline stimulations. Fig. 6 shows percent baseline stimulations ( $\pm$  SEM) from all THC tests. The one-way ANOVA was significant and post-hoc analysis showed significant differences from VEH, which are denoted with asterisks. \*\*\*\* $p < 0.0001$ , \*\*\* $p < 0.001$ , \*\* $p < 0.01$ , \* $p < 0.05$

**THC time-course assessment.** This study was conducted to assess the time-course of the ICSS rate-reduction produced by 10.0 mg/kg THC. Seven mice completed THC time-course testing, in which ICSS was assessed at 5 min, as well as 0.5, 2, 4, 8, 24, and 48 h after administration of VEH or 10.0 mg/kg THC. Two-way repeated measures ANOVAs (Treatment x Frequency) were used to compare rate-frequency curves of VEH and THC at each time-point. Stimulations after 10.0 mg/kg THC were significantly reduced compared with VEH at 5 min,

0.5, 2, 4, and 8 h after injection. Baseline rate-frequency curves between the two conditions did not differ from each other, as indicated by a main effect of frequency ( $F_{9,54} = 42.73, p < 0.0001$ ), but no main effect of treatment ( $F_{1,6} = 0.12, p > 0.05$ ) and no interaction ( $F_{9,54} = 0.41, p > 0.05$ ) (Fig. 7a). At the five minute time-point, there was a main effect of frequency ( $F_{9,54} = 69.58, p < 0.0001$ ) and treatment ( $F_{1,6} = 6.84, p < 0.05$ ), but no interaction ( $F_{9,54} = 0.71, p > 0.05$ ) (Fig. 7b). At the 30 minute time-point there was a main effect of frequency ( $F_{9,54} = 52.82, p < 0.0001$ ), treatment ( $F_{1,6} = 202.20, p < 0.0001$ ) and an interaction ( $F_{9,54} = 9.05, p < 0.0001$ ) (Fig. 7c). Post-hoc analysis showed reductions at six specific frequencies which are denoted on Fig. 7c. At the two hour time-point there was a main effect of frequency ( $F_{9,54} = 39.26, p < 0.0001$ ), treatment ( $F_{1,6} = 82.48, p < 0.001$ ), and an interaction ( $F_{9,54} = 12.44, p < 0.0001$ ) (Fig. 7d). Post-hoc analysis showed reductions at six specific frequencies which are denoted on Fig. 7d. At the four hour time-point there was a main effect of frequency ( $F_{9,54} = 25.96, p < 0.0001$ ), treatment ( $F_{1,6} = 79.54, p < 0.001$ ) and an interaction ( $F_{9,54} = 10.15, p < 0.0001$ ) (Fig. 7e). Post-hoc analysis revealed reductions at five specific frequencies which are denoted on Fig. 7e. Eight hours after injection, there was a main effect of frequency ( $F_{9,54} = 27.45, p < 0.0001$ ), treatment ( $F_{1,6} = 50.42, p < 0.001$ ) and an interaction ( $F_{9,54} = 4.22, p < 0.001$ ) (Fig. 7f). Post-hoc analysis showed reductions at six frequencies which are denoted on Fig. 7f. ICSS returned to VEH levels at 24 and 48 h after administration; at 24 hours, there was a main effect of frequency ( $F_{9,54} = 39.08, p < 0.0001$ ), but no main effect of treatment ( $F_{1,6} = 0.55, p > 0.05$ ) and no interaction ( $F_{9,54} = 0.87, p > 0.05$ ) (Fig. 7g). Similarly, at 48 hours after injection, there was a main effect of frequency ( $F_{9,54} = 37.13, p < 0.0001$ ), but no main effect of treatment ( $F_{1,6} = 0.35, p > 0.05$ ) and no interaction ( $F_{9,54} = 1.41, p > 0.05$ ) (Fig. 7h). The reduction of ICSS produced by THC occurs as early as five minutes after administration and returned to baseline within 24 h.

A two-way repeated-measures ANOVA (Time-point x Treatment) was used to compare the percent baseline stimulations of VEH and 10.0 mg/kg THC across all time-points (Fig. 8). 10.0 mg/kg THC produced a reduction from VEH percent baseline stimulations, as there was a main effect of time-point ( $F_{6,36} = 44.14, p < 0.0001$ ), treatment ( $F_{1,6} = 128.5, p < 0.0001$ ) and an interaction between time-point and treatment ( $F_{6,36} = 17.09, p < 0.0001$ ). Post-hoc analysis revealed THC reduced ICSS at 0.5, 2, 4, and 8 h, while it did not differ from VEH at 5 min, 24 and 48 h as denoted on Fig. 8. The reduction of ICSS produced by 10.0 mg/kg began 30 min after administration, and returned to baseline levels within 24 h, which is similar to rate-frequency analysis results.

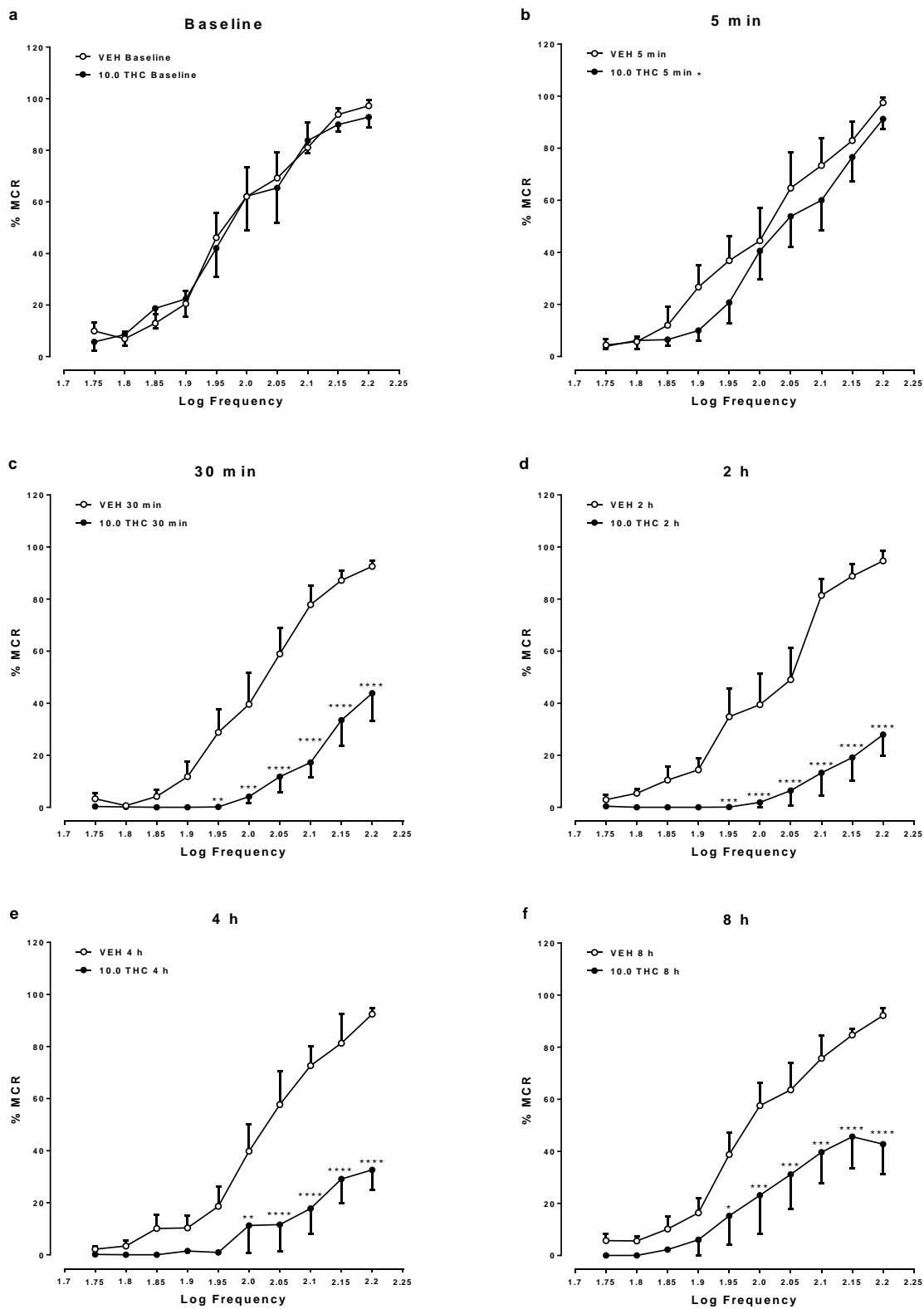
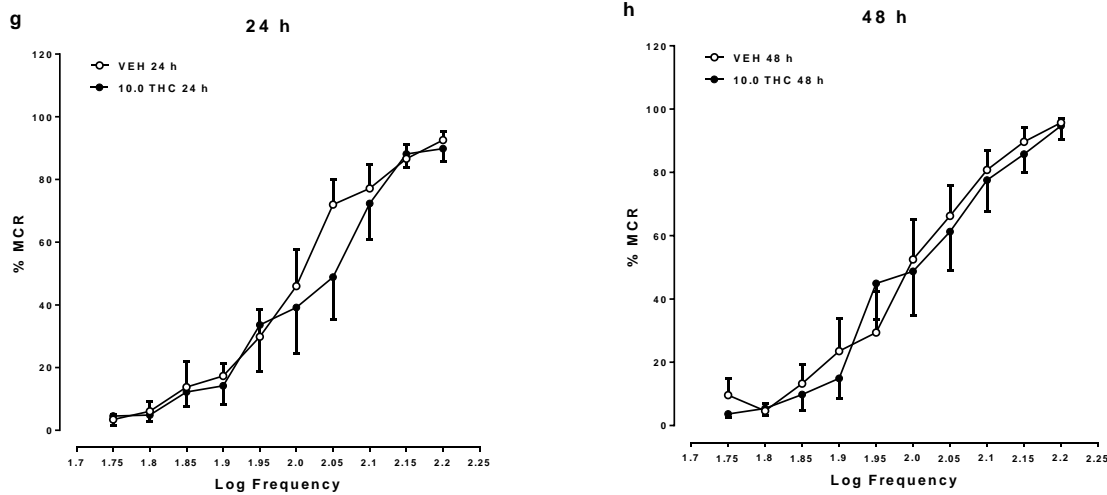


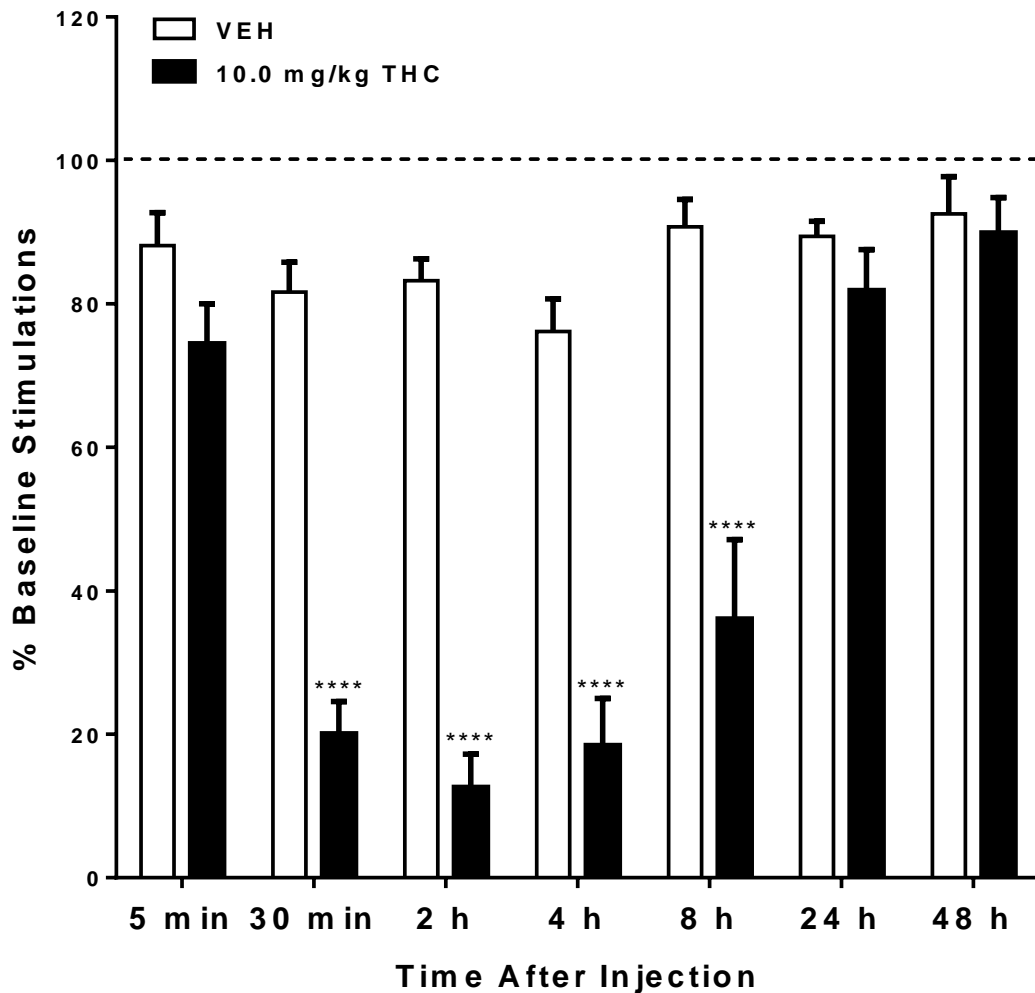
Figure Continues

Figure Continued



**Figure 7.** THC ICSS time-course evaluation. Panels a – h show rate-frequency stimulation values ( $\pm$  SEM) after VEH or THC (10.0 mg/kg) at each time-point (5 min, 0.5, 2, 4, 8, 24 and 48 h); percent maximum control rate is displayed on the Y-axis, log-frequency values are shown on the x-axis. Significant main effect of treatment is denoted by asterisks by the figure legend. Interactions that achieved significance and were accompanied by significant post-hoc analyses are denoted by asterisks at values that are significantly different from VEH. \*\*\*\* $p < 0.0001$ , \*\*\* $p < 0.001$ , \*\* $p < 0.01$ , \* $p < 0.05$ . Raw overall response rates (stimulations/min; mean  $\pm$  SEM) during VEH (V) and 10.0 mg/kg THC (T) conditions were as follows: (a) Baseline: V ( $31.2 \pm 3.90$ ) T ( $30.99 \pm 4.36$ ); (b) 5 min: V ( $28.12 \pm 4.16$ ) T ( $23.24 \pm 3.74$ ); (c) 30 min: V ( $25.34 \pm 3.36$ ) T ( $7.24 \pm 1.90$ ); (d) 2 h: V ( $26.21 \pm 3.61$ ) T ( $4.51 \pm 1.92$ ); (e) 4 h: V ( $24.13 \pm 3.57$ ) T ( $6.89 \pm 2.77$ ); (f) 8 h: V ( $28.10 \pm 3.53$ ) T ( $13.61 \pm 4.69$ ); (g) 24 h: V ( $27.83 \pm 3.50$ ) T ( $25.79 \pm 4.54$ ); (h) 48 h: V ( $29.14 \pm 4.17$ ) T ( $28.34 \pm 4.43$ ).





*Figure 8.* THC ICSS percent baseline stimulations time-course evaluation. Percent baseline stimulations ( $\pm$  SEM) between VEH and THC (10.0 mg/kg) tests were compared across time-points. The two-way ANOVA was significant and post-hoc analysis showed significant differences from VEH, which are denoted with asterisks. \*\*\*\* $p < 0.0001$

**THC: Evaluation of CB<sub>1</sub> receptors.** In this study, we tested whether the CB<sub>1</sub> antagonist/inverse agonist rimonabant (RIM) would block the rate-decreasing effects of THC (10.0 mg/kg) on ICSS. Six mice completed testing and the data were analyzed using a within-subjects two-way ANOVA (treatment x frequency) for each dose combination tested. Administration of VEH with VEH did not produce a change from baseline; there was a main effect of frequency ( $F_{9, 54} = 69.10, p < 0.0001$ ), but no main effect of treatment ( $F_{1, 6} = 0.001, p$

> 0.05) and no interaction ( $F_{9, 54} = 1.21, p > 0.05$ ) (Fig. 9a). Combined treatment of VEH and 10.0 mg/kg THC reduced stimulations from baseline similar to THC alone; there was a main effect of frequency ( $F_{9, 54} = 58.59, p < 0.0001$ ), treatment ( $F_{1, 6} = 251.8, p < 0.0001$ ) and an interaction ( $F_{9, 54} = 6.97, p < 0.0001$ ) (Fig. 9b); post-hoc analysis showed reductions at seven frequencies which are denoted on Fig. 9b. RIM (3.0 mg/kg) and VEH administration did not produce a change from baseline, indicating that CB<sub>1</sub> antagonism does not affect ICSS; there was a main effect of frequency ( $F_{9, 54} = 44.65, p < 0.0001$ ), but no main effect of treatment ( $F_{1, 6} = 0.32, p > 0.05$ ) and no interaction ( $F_{9, 54} = 0.92, p > 0.05$ ) (Fig. 9c). RIM (3.0 mg/kg) blocked the reduction of ICSS produced by THC (10.0 mg/kg), as stimulations did not differ from baseline, indicated by a main effect of frequency ( $F_{9, 54} = 34.02, p < 0.0001$ ), but no main effect of treatment ( $F_{1, 6} = 0.79, p > 0.05$ ), and no interaction ( $F_{9, 54} = 1.78, p > 0.05$ ) (Fig. 9d). Baseline rate-frequency curves did not differ under any condition tested (Fig. 9e). There was a main effect of frequency ( $F_{9, 54} = 46.98, p < 0.0001$ ), but no main effect of baseline day ( $F_{3, 18} = 0.98, p > 0.05$ ), and no interaction between baseline day and frequency ( $F_{27, 162} = 1.10, p > 0.05$ ).

A two-way ANOVA (3.0 mg/kg rimonabant vs. vehicle x 10.0 mg/kg THC vs. vehicle) was conducted using the percent baseline stimulation data. The reduction produced by 10.0 mg/kg THC was blocked by administration of 3.0 mg/kg RIM. There was a main effect of first treatment (VEH vs. RIM) ( $F_{1, 6} = 54.41, p < 0.001$ ), a main effect of the second treatment (VEH vs. THC) ( $F_{1, 6} = 54.41, p < 0.001$ ) and an interaction between first and second treatment ( $F_{1, 6} = 90.81, p < 0.0001$ ) (Fig. 9f). Post-hoc analysis revealed that percent baseline stimulations were reduced ( $p < 0.0001$ ) in mice treated with 10.0 mg/kg THC, but only when first treated with VEH. RIM (3.0 mg/kg) pre-treatment blocked the reduction in stimulations produced by 10.0 mg/kg THC. These results indicate that CB<sub>1</sub> receptors mediate the rate-decreasing effects of 10.0

mg/kg THC on ICSS. Furthermore, the CB<sub>1</sub> antagonist, RIM (3.0 mg/kg) did not produce any effect on ICSS alone.

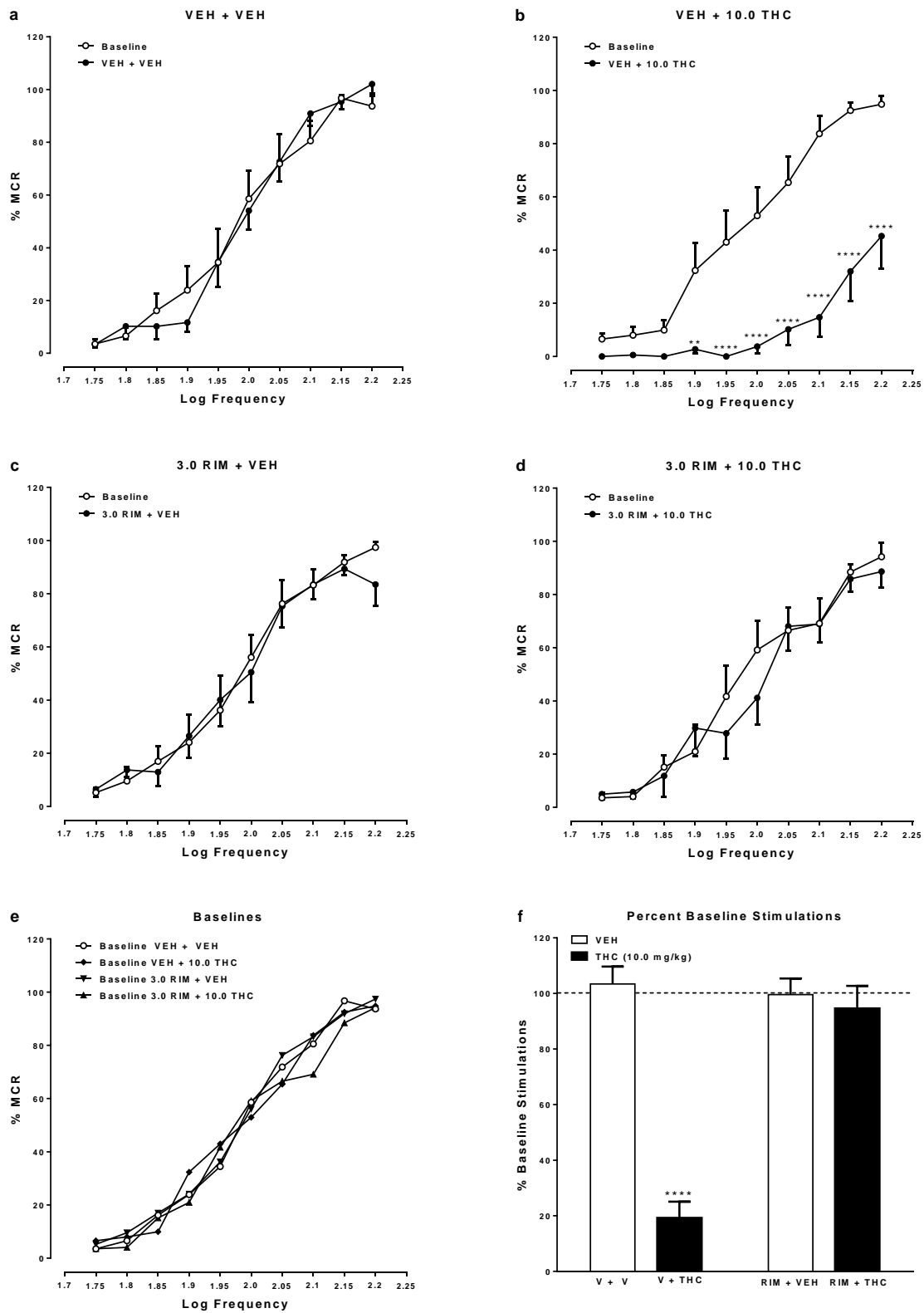


Figure Continues

## Figure Continued

*Figure. 9.* THC CB<sub>1</sub> antagonism assessment. Panel a – d shows rate-frequency data during RIM antagonism tests. Panel e shows all baselines rate-frequency curves during CB<sub>1</sub> testing. Percent maximum control rate is displayed on the Y-axis, log-frequency values are shown on the x-axis. Panel f shows percent baseline stimulations ( $\pm$  SEM) for all treatments. There was a significant interaction with the two-way ANOVA for percent baseline stimulations. Significant interactions were followed up by post-hoc analysis, and differences are denoted by asterisks at values that are significantly different from baseline (a – e), or other treatment conditions (f). \*\*\*\* $p < 0.0001$ , \*\* $p < 0.01$ . Raw overall response rates (stimulations/min; mean  $\pm$  SEM) during baseline (B) and test (T) sessions were as follows: (a) VEH + VEH: B ( $27.67 \pm 3.51$ ) T ( $28.36 \pm 3.48$ ); (b) VEH + 10.0 mg/kg THC: B ( $29.80 \pm 3.86$ ) T ( $6.85 \pm 2.45$ ); (c) 3.0 mg/kg RIM + VEH: B ( $31.01 \pm 4.96$ ) T ( $29.67 \pm 4.01$ ); (d) 3.0 mg/kg RIM + 10.0 mg/kg THC: B ( $28.49 \pm 3.97$ ) T ( $26.48 \pm 3.81$ ).

**THC: Evaluation of CB<sub>2</sub> Receptors.** For this experiment, we evaluated whether the CB<sub>2</sub> antagonist/inverse agonist SR2 would block the reduction of ICSS produced by 10.0 mg/kg THC. Five mice completed testing and the data were analyzed using a within-subjects two-way ANOVA (treatment  $\times$  frequency) for each dose combination. Similar to CB<sub>1</sub> testing, administration of VEH and VEH did not produce a change from baseline ICSS; there was a main effect of frequency ( $F_{9,36} = 60.04$ ,  $p < 0.0001$ ), but no main effect of treatment ( $F_{1,4} = 5.34$ ,  $p > 0.05$ ) and no interaction ( $F_{9,36} = 60.04$ ,  $p > 0.05$ ) (Fig. 10a). Similar to previous tests with 10.0 mg/kg THC, stimulations with VEH and 10.0 mg/kg THC were reduced; there was a main effect of frequency ( $F_{9,36} = 28.93$ ,  $p < 0.0001$ ), treatment ( $F_{1,4} = 240.6$ ,  $p < 0.001$ ) and an interaction ( $F_{9,36} = 4.14$ ,  $p < 0.01$ ) (Fig. 10b). Post-hoc analysis showed reductions at seven frequencies which are denoted on Fig. 10b. SR2 (3.0 mg/kg) given with VEH did not affect baseline ICSS; there was a main effect of frequency ( $F_{9,36} = 28.79$ ,  $p < 0.0001$ ), but no main effect of treatment ( $F_{1,4} = 5.49$ ,  $p > 0.05$ ) and no interaction ( $F_{9,36} = 0.88$ ,  $p > 0.05$ ) (Fig. 10c). SR2 (3.0 mg/kg) was not able to block the reduction in ICSS produced by 10.0 mg/kg THC; there was a main effect of frequency ( $F_{9,36} = 35.66$ ,  $p < 0.0001$ ), treatment ( $F_{1,4} = 39.96$ ,  $p < 0.01$ ) and an interaction ( $F_{9,36} = 6.13$ ,  $p < 0.0001$ ) (Fig. 10d). Post-hoc analysis showed decreases at five

frequencies which are denoted on Fig. 10d. Baseline rate-frequency curves did not differ under any condition tested (Fig. 10e); there was a main effect of frequency ( $F_{9,36} = 36.48, p < 0.0001$ ), but no main effect of baseline day ( $F_{3,12} = 0.91, p > 0.05$ ), and no interaction between baseline day and frequency ( $F_{27,108} = 1.15, p > 0.05$ ).

A two-way ANOVA (3.0 mg/kg SR2 vs. VEH x 10.0 mg/kg THC vs. VEH) was conducted using the percent baseline stimulations data. The reduction of ICSS produced by 10.0 mg/kg THC was not blocked by 3.0 mg/kg SR2 (Fig. 10f). There was no main effect of first treatment (VEH vs. SR2) ( $F_{1,4} = 2.19, p > 0.05$ ), but there was a main effect of second treatment (VEH vs. THC) ( $F_{1,4} = 43.66, p < 0.01$ ), as 10.0 mg/kg THC reduced ICSS. There was no interaction between first treatment and second treatment ( $F_{1,4} = 2.32, p > 0.05$ ). The reduction in stimulations produced by 10.0 mg/kg THC was not blocked by SR2 (3.0 mg/kg), which suggests that the attenuation of ICSS responding is not mediated through CB<sub>2</sub> receptors, which is in agreement with the rate-frequency findings.

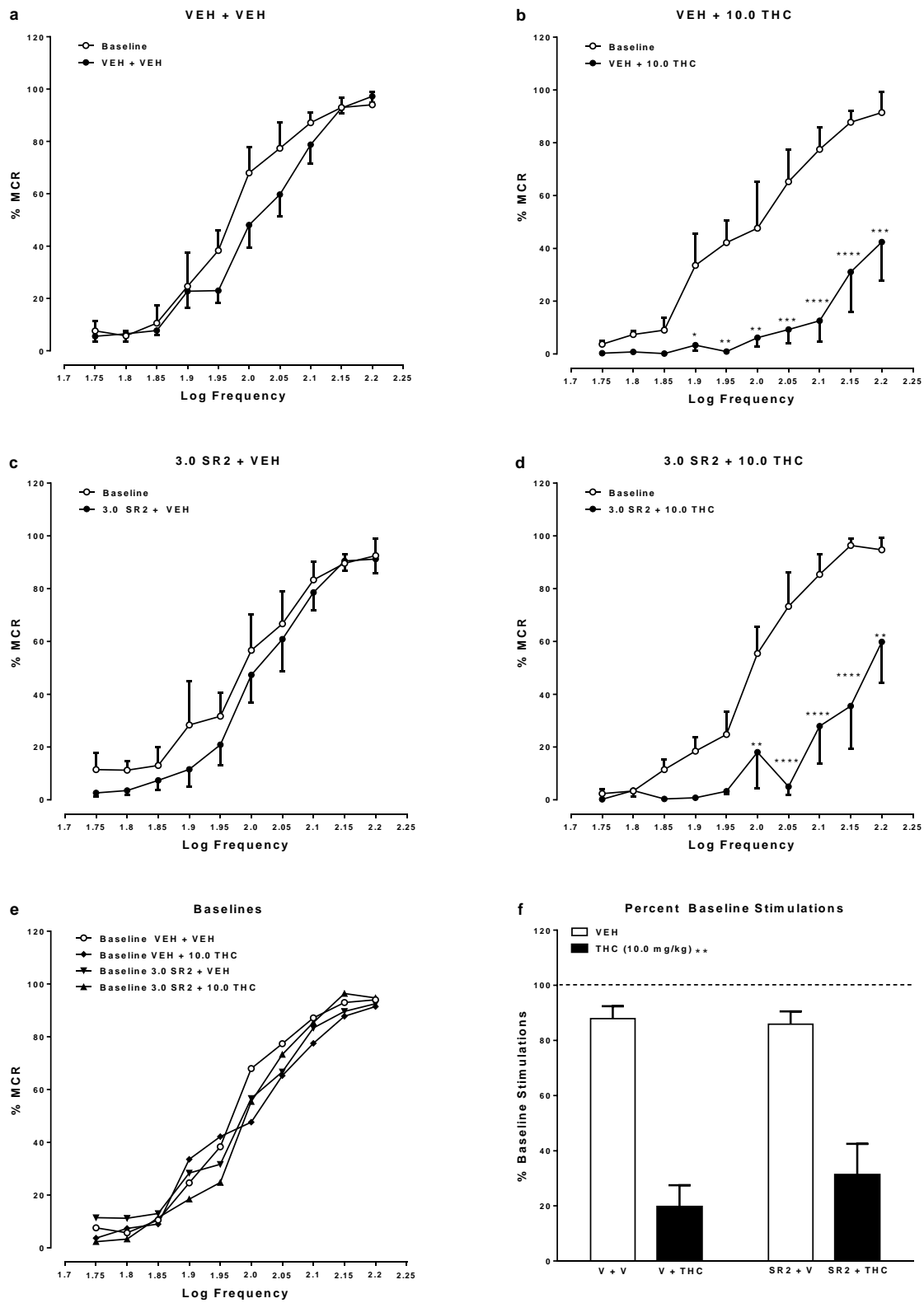


Figure Continues

## Figure Continued

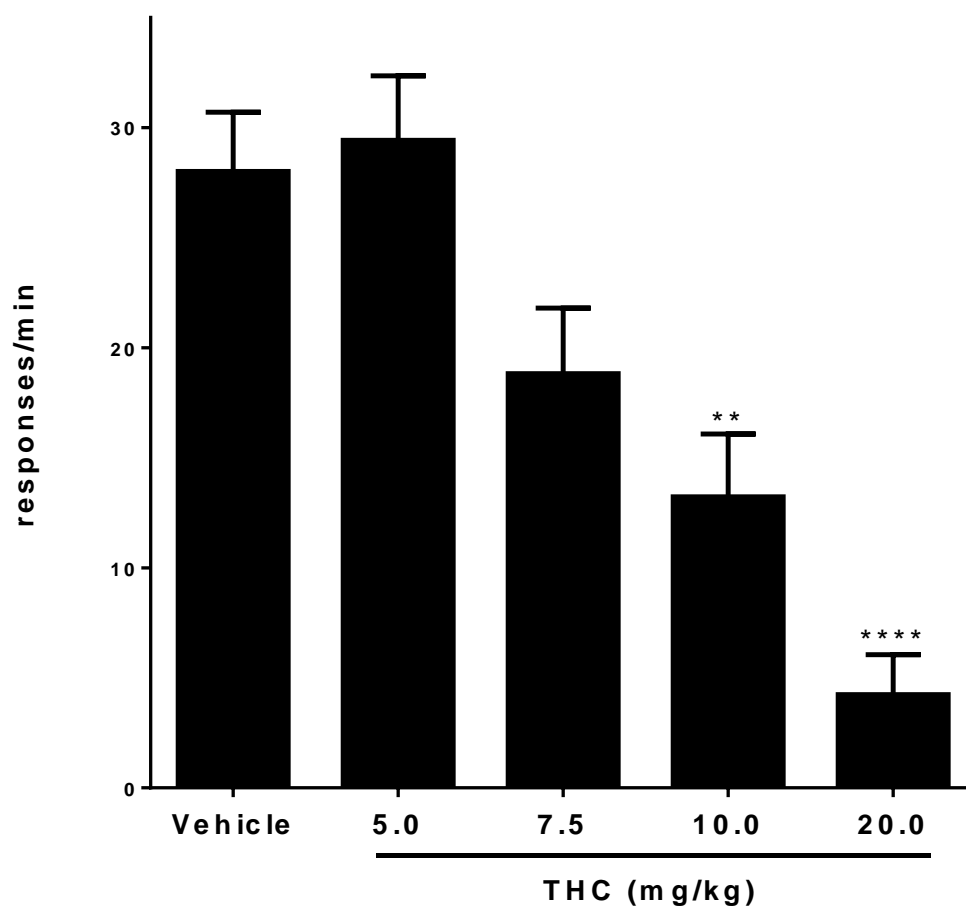
*Figure. 10.* THC CB<sub>2</sub> antagonism assessment. Panels a – d shows rate-frequency data during SR2 antagonism tests. Panel e shows all baselines rate-frequency curves during CB<sub>2</sub> testing. Percent maximum control rate is displayed on the Y-axis, log-frequency values are shown on the x-axis. Panel f shows percent baseline stimulations ( $\pm$  SEM) for all treatments. Significant main effects of treatment are denoted by asterisks by the figure legend. Significant interactions were investigated with post-hoc analysis, and differences from baseline are denoted by asterisks.

\*\*\*\* $p < 0.0001$ , \*\*\* $p < 0.001$ , \*\* $p < 0.01$ , \* $p < 0.05$ . Raw overall response rates (stimulations/min; mean  $\pm$  SEM) during baseline (B) and test (T) sessions were as follows: (a) VEH + VEH: B ( $29.99 \pm 3.54$ ) T ( $26.13 \pm 2.67$ ); (b) VEH + 10.0 mg/kg THC: B ( $28.11 \pm 4.52$ ) T ( $6.72 \pm 3.12$ ); (c) 3.0 mg/kg SR2 + VEH: B ( $28.80 \pm 4.58$ ) T ( $24.11 \pm 3.09$ ); (d) 3.0 mg/kg SR2 + 10.0 mg/kg THC: B ( $26.73 \pm 3.83$ ) T ( $8.91 \pm 3.71$ ).

### THC Operant Responding for Food Assessment

In this experiment the effects of THC (5.0, 7.5, 10.0 and 20.0 mg/kg) on operant responding for food pellets were assessed; thirteen mice were evaluated and response rates were analyzed using a one-way repeated measures ANOVA. THC reduced operant response rates ( $F_{4,59} = 15.46$ ,  $p < 0.0001$ ) (Fig. 11). Post-hoc analysis showed that THC (10.0 and 20.0 mg/kg) produced a reduction ( $p < 0.01$ ;  $p < 0.0001$  respectively) in operant response rates compared to VEH, while 5.0 and 7.5 mg/kg THC group were not affected, though there was a trend toward 7.5 being significantly reduced ( $p = 0.06$ ) (Fig. 11). The reduction of response rates produced by THC was not specific to brain stimulation reinforcement, as it occurred in both ICSS and operant responding for food.





*Figure. 11.* THC operant responding for food. Figure showing response rate ( $\pm$  SEM) on the y-axis, and dose of THC or VEH on the x-axis. A significant one-way ANOVA was investigated with post-hoc analysis, and differences from VEH are denoted by asterisks. \*\*\*\* $p < 0.0001$ , \*\* $p < 0.01$

## JZL184 ICSS Experiments

**JZL184 dose-response assessment.** ICSS was assessed after administration of JZL184 (1.0, 4.0, 16.0 and 40.0 mg/kg) and VEH. Seven mice completed testing, and the rate-frequency stimulation data were analyzed using a two-way, repeated-measures ANOVA (frequency x drug treatment). When analyzing rate-frequency data VEH, 1.0, and 4.0 mg/kg JZL184 did not affect baseline ICSS, while 16.0 and 40.0 mg/kg significantly attenuated stimulations. VEH produced a main effect of frequency ( $F_{9,54} = 39.11, p < 0.0001$ ), but no main effect of treatment ( $F_{1,6} = 0.23, p > 0.05$ ) and no interaction ( $F_{9,54} = 0.92, p > 0.05$ ) (Fig. 12a). JZL184 (1.0 mg/kg) produced a main effect of frequency ( $F_{9,54} = 87.40, p < 0.0001$ ), but no main effect of treatment ( $F_{1,6} = 2.80, p > 0.05$ ) and no interaction ( $F_{9,54} = 0.40, p > 0.05$ ) (Fig. 12b). Similarly, 4.0 mg/kg JZL184 produced a main effect of frequency ( $F_{9,54} = 64.98, p < 0.0001$ ), but no main effect of treatment ( $F_{1,6} = 2.71, p > 0.05$ ) and no interaction ( $F_{9,54} = 0.47, p > 0.05$ ) (Fig. 12c). Higher doses of JZL184 (16.0 and 40.0 mg/kg) attenuated ICSS; after 16.0 mg/kg JZL184 produced a main effect of frequency ( $F_{9,54} = 52.11, p < 0.0001$ ), treatment ( $F_{1,6} = 14.68, p < 0.001$ ) and an interaction ( $F_{9,54} = 2.18, p < 0.05$ ) (Fig. 12d). Post-hoc analysis revealed significant reductions at two frequencies which are denoted on Fig. 12d. JZL184 (40.0 mg/kg) produced a main effect of frequency ( $F_{9,54} = 39.9, p < 0.0001$ ), no main effect of treatment ( $F_{1,6} = 5.78, p = 0.05$ ), but an interaction between treatment and frequency ( $F_{9,54} = 2.43, p < 0.05$ ) (Fig. 12e); post-hoc analysis showed significant reductions at four frequencies which are denoted on Fig. 12e.

All baseline rate-frequency curves were analyzed after all tests were completed to determine if baseline responding differed over time (Fig. 12f). Baseline stimulations slightly differed between test days; there was a main effect of frequency ( $F_{9,54} = 100.90, p < 0.0001$ ) and

baseline day ( $F_{4,24} = 3.19, p < 0.05$ ), but no interaction between treatment and baseline day ( $F_{36,216} = 0.97, p > 0.05$ ). Additionally, a second VEH test was conducted and compared to the initial VEH assessment (data not shown); tests did not differ. There was a main effect of frequency ( $F_{9,54} = 55.33, p < 0.0001$ ), but no main effect of treatment ( $F_{1,6} = 0.36, p > 0.05$ ) and no interaction ( $F_{9,54} = 1.61, p > 0.05$ ). Baselines varied slightly over time, but analysis from daily baseline allows for minimal baseline drift. Data are always assessed in terms of change from that day's baseline to minimize experimental error due to baseline drift.

Percent baseline stimulations were calculated for each test and evaluated using one-way repeated measures ANOVA. VEH was calculated to serve as a comparison to JZL184. JZL184 reduced ICSS percent baseline stimulations ( $F_{4,24} = 4.48, p < 0.01$ ) (Fig. 13); post-hoc analysis showed lower doses of JZL184 (1.0 and 4.0 mg/kg) did not affect percent baseline stimulations, while higher doses of JZL184 (16.0 and 40.0 mg/kg) reduced ( $p < 0.05$ ;  $p < 0.01$ ) percent baseline stimulations (76.28 % and 70.99 %) compared to VEH. These results agreed with the rate-frequency analysis, as the same doses of JZL184 (16.0 and 40.0 mg/kg) produced significant attenuation using both measure of ICSS.

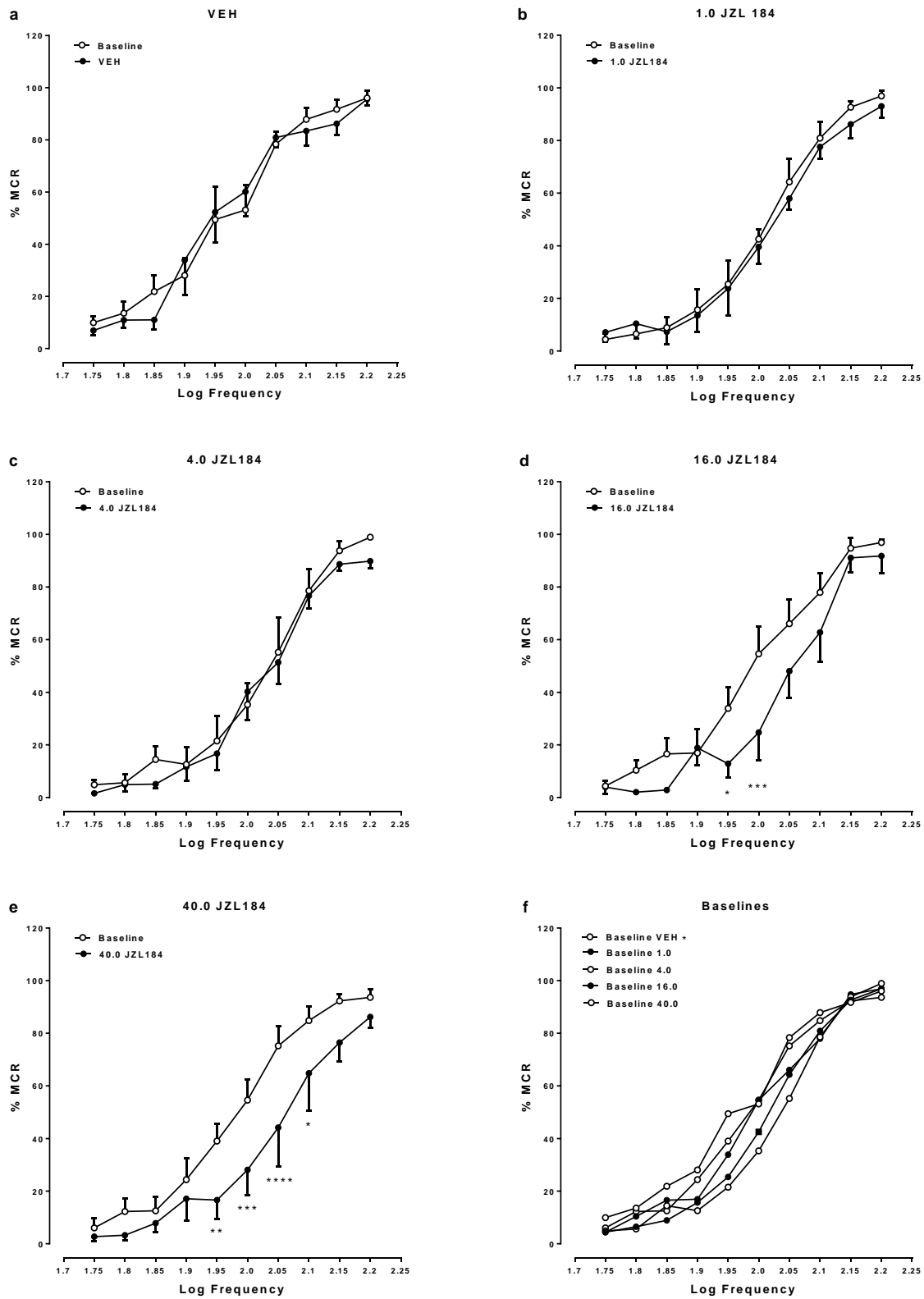
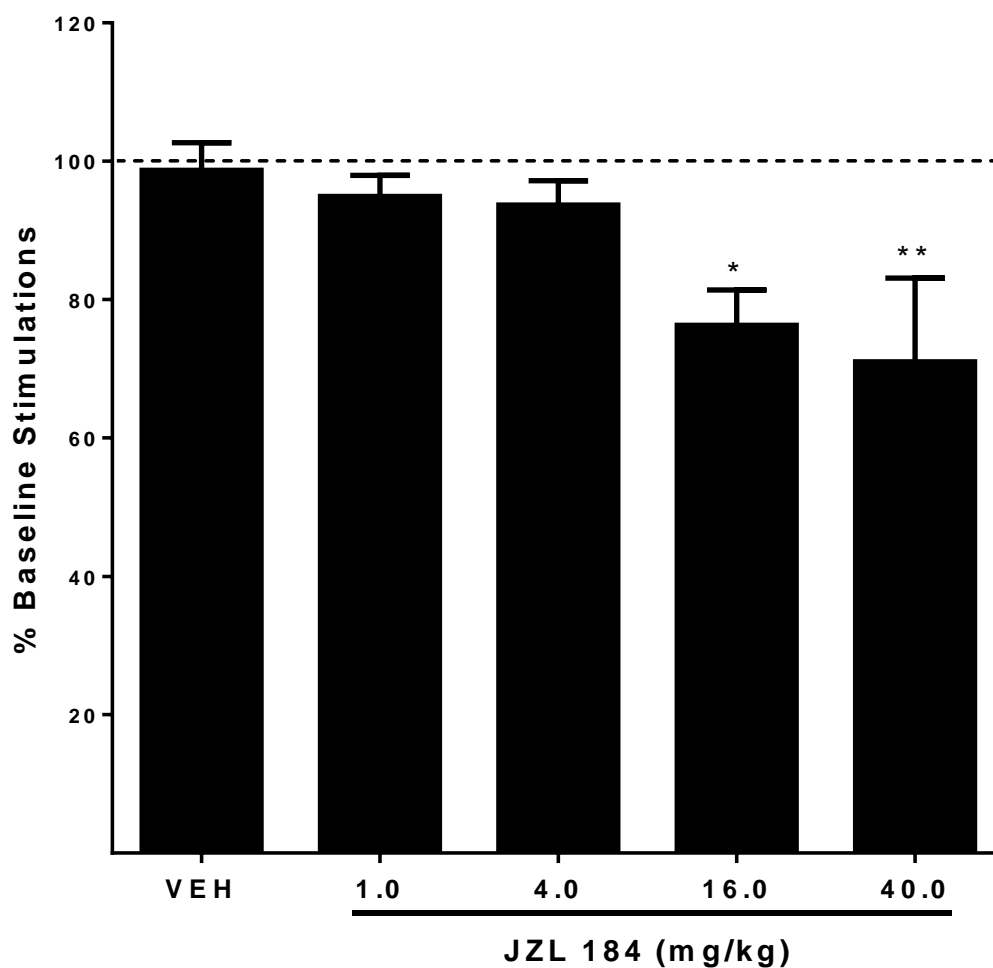


Figure Continues

## Figure Continued

*Figure 12.* JZL184 ICSS dose-response assessment. Panels a – e show rate-frequency stimulation values ( $\pm$  SEM) after VEH or JZL184 (1.0, 4.0, 16.0 and 40.0 mg/kg). Panel f shows all baseline rate-frequency curves during testing. Percent maximum control rate is displayed on the Y-axis, log-frequency values are shown on the x-axis. Significant main effects of treatment are denoted by asterisks by the figure legend. Interactions that achieved significance were investigated with post-hoc analyses, and differences from baseline are denoted by asterisks. \*\*\*\* $p < 0.0001$ , \*\*\* $p < 0.001$ , \*\* $p < 0.01$ , \* $p < 0.05$ . Raw overall response rates (stimulations/min; mean  $\pm$  SEM) during baseline (B) and test (T) sessions were as follows: (a) VEH: B ( $33.99 \pm 2.05$ ) T ( $33.51 \pm 2.31$ ); (b) 1.0 mg/kg JZL184: B ( $28.98 \pm 2.53$ ) T ( $27.55 \pm 2.72$ ); (c) 4.0 mg/kg JZL184: B ( $28.60 \pm 3.90$ ) T ( $26.08 \pm 2.66$ ); (d) 16.0 mg/kg JZL184: B ( $30.76 \pm 3.76$ ) T ( $23.08 \pm 2.62$ ); (e) 40.0 mg/kg JZL184: B ( $34.00 \pm 2.28$ ) T ( $23.91 \pm 3.95$ ).



*Figure. 13.* JZL184 ICSS percent baseline stimulations. Fig. 13 shows percent baseline stimulations ( $\pm$  SEM) from JZL184 dose-response tests. The one-way ANOVA was significant and post-hoc analysis revealed significant differences from VEH, which are denoted with asterisks.  $**p < 0.01$ ,  $*p < 0.05$

**JZL184 Time-course Assessment.** The purpose of this study was to assess the time-course of JZL184-induced suppression of ICSS. Self-stimulations were assessed at 1, 2, 4, 8, 24, and 48 h after administration of VEH or 40.0 mg/kg JZL184. Six mice completed JZL184 time-course testing, and two-way repeated-measures ANOVAs (Treatment x Frequency) were conducted to compare rate-frequency curves between VEH and JZL184 treatment at each time-point. JZL184 (40.0 mg/kg) significantly reduced stimulations compared to VEH at 1, 2, 4, 8 and 24 h after injection. Baselines between the two conditions did not differ from each other; there was a main effect of frequency ( $F_{9,45} = 83.39, p < 0.0001$ ), but no main effect of treatment ( $F_{1,6} = 0.38, p > 0.05$ ) and no interaction ( $F_{9,45} = 0.63, p > 0.05$ ) (Fig. 14a). At the one hour time-point there was a main effect of frequency ( $F_{9,45} = 31.91, p < 0.0001$ ), treatment ( $F_{1,5} = 7.01, p < 0.05$ ) and an interaction ( $F_{9,45} = 2.18, p < 0.05$ ) (Fig. 14b); post-hoc analysis showed reductions at two frequencies which are denoted on Fig. 14b. Similarly, at the two hour time-point there was a main effect of frequency ( $F_{9,45} = 33.43, p < 0.0001$ ), treatment ( $F_{1,5} = 7.80, p < 0.05$ ) and an interaction ( $F_{9,45} = 2.11, p < 0.05$ ) (Fig. 14c). Post-hoc analysis showed reductions at two frequencies which are denoted on Fig. 14c. Four hours after administration, there was a main effect of frequency ( $F_{9,45} = 19.01, p < 0.0001$ ), treatment ( $F_{1,5} = 11.81, p < 0.05$ ), and an interaction ( $F_{9,45} = 2.71, p < 0.05$ ) (Fig. 14d). Post-hoc analysis showed reductions at two frequencies are denoted on Fig. 14d. At the eight hour time-point there was a main effect of frequency ( $F_{9,45} = 28.31, p < 0.0001$ ) and treatment ( $F_{1,5} = 61.41, p < 0.001$ ), but no interaction ( $F_{9,45} = 2.07, p = 0.05$ ) (Fig. 14e). At 24 hours after administrations, JZL184 responding was still attenuated; there was a main effect of frequency ( $F_{9,45} = 63.87, p < 0.0001$ ), treatment ( $F_{1,5} = 14.21, p < 0.001$ ) and an interaction ( $F_{9,45} = 2.82, p < 0.001$ ) (Fig. 14f); post-hoc analysis showed reductions at four frequencies which are denoted on Fig. 14f. 48 hours after

administration, JZL184 stimulation rates returned to normal, as they did not differ from VEH; there was a main effect of frequency ( $F_{9,45} = 48.10, p < 0.0001$ ), but no main effect of treatment ( $F_{1,5} = 0.66, p > 0.05$ ) and no interaction ( $F_{9,45} = 1.41, p > 0.05$ ) (Fig. 14g).

A two-way repeated-measures ANOVA (Time-point x Treatment) was conducted to compare the percent baseline stimulations of VEH and 40.0 mg/kg JZL184 across all time-points (Fig. 14h). JZL184 (40.0 mg/kg) reduced percent baseline stimulations from VEH at several time-points after administration; there was a main effect of time-point ( $F_{5,25} = 12.48, p < 0.0001$ ), treatment ( $F_{1,5} = 11.54, p < 0.05$ ), and an interaction between time-point and treatment ( $F_{5,25} = 2.61, p < 0.05$ ). Post-hoc tests showed that 40.0 mg/kg JZL184 reduced percent baseline stimulations compared to VEH treatment at 2, 4, 8 and 24 hours after treatment as denoted on Fig. 14h. The attenuation of ICSS produced by JZL184 is apparent 2 hours after administration, and returns to baseline within 48 hours after administration according to percent baseline stimulations data. Similarly, the rate-frequency analysis shows reductions at 1 hour after administration, which persist between 24 and 48 h.



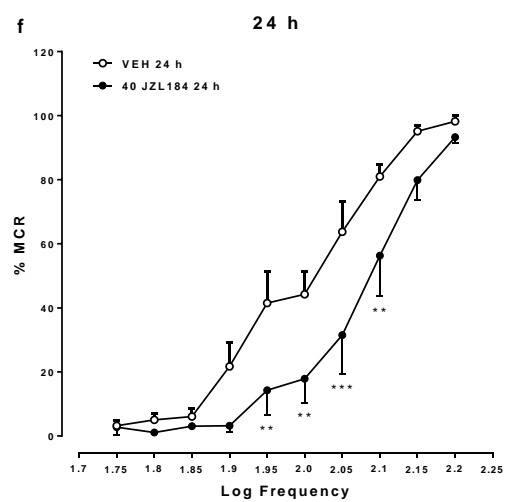
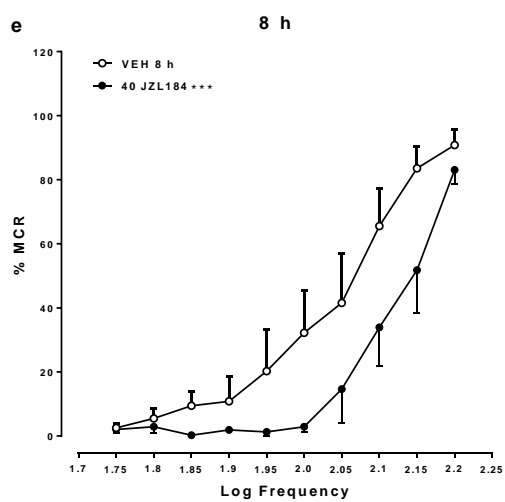
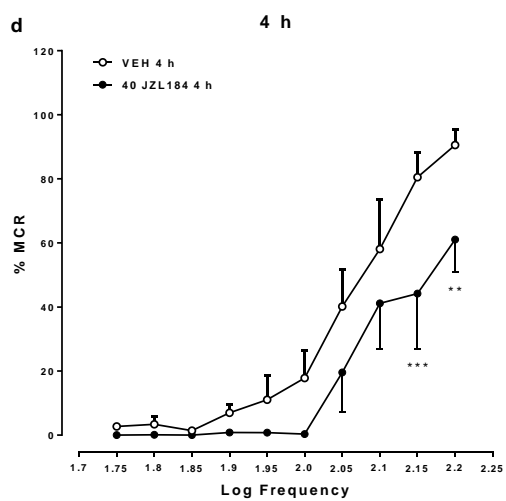
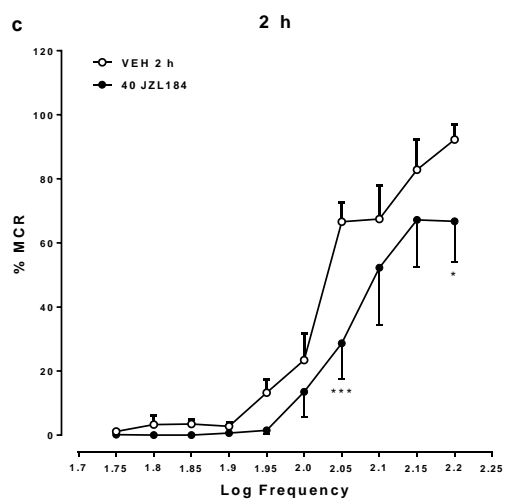
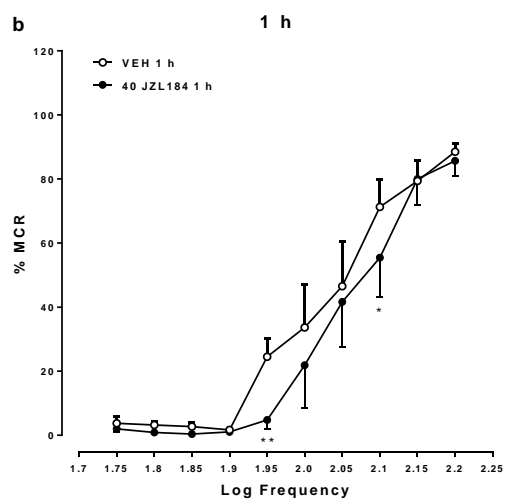
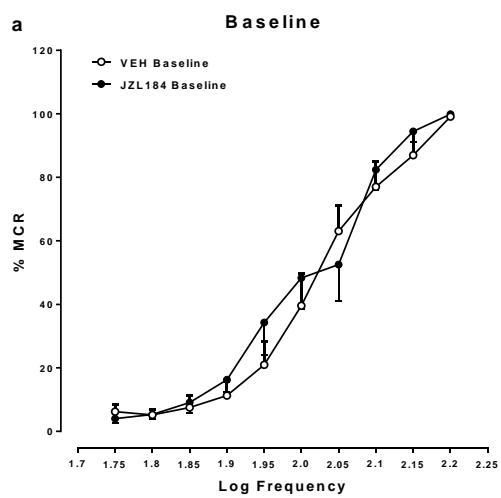
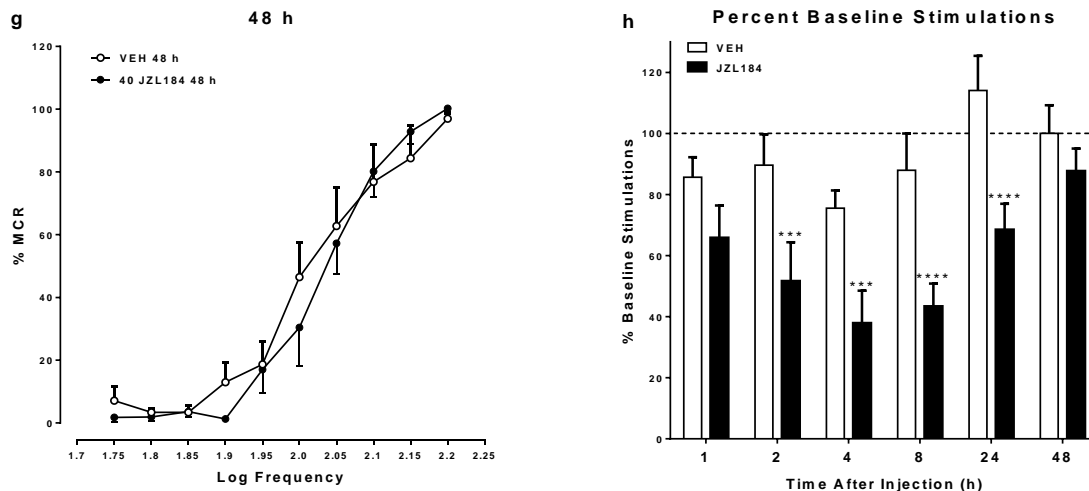


Figure Continues

Figure Continued



**Figure 14.** JZL184 ICSS time-course evaluation. Panels a – g show rate-frequency stimulation values ( $\pm$  SEM) after VEH or JZL184 (40.0 mg/kg) at each time-point (1, 2, 4, 8, 24 and 48 h); percent maximum control rate is displayed on the Y-axis, log-frequency values are shown on the x-axis. Panel h shows percent baseline stimulations data over time. Significant main effects of treatment are denoted by asterisks by the figure legend. Interactions that achieved significance and were accompanied by significant post-hoc analyses are denoted by asterisks at values that are significantly different from baseline VEH. \*\*\*\* $p < 0.0001$ , \*\*\* $p < 0.001$ , \*\* $p < 0.01$ , \* $p < 0.05$ . Raw overall response rates (stimulations/min; mean  $\pm$  SEM) during VEH (V) and 40.0 mg/kg JZL184 (J) conditions were as follows: (a) Baseline: V ( $26.78 \pm 3.41$ ) J ( $28.68 \pm 2.27$ ); (b) 1 h: V ( $23.11 \pm 3.70$ ) J ( $19.48 \pm 4.38$ ); (c) 2 h: V ( $22.73 \pm 2.19$ ) J ( $14.81 \pm 4.01$ ); (d) 4 h: V ( $20.28 \pm 3.27$ ) J ( $11.31 \pm 3.78$ ); (e) 8 h: V ( $23.83 \pm 4.99$ ) J ( $12.93 \pm 3.18$ ); (f) 24 h: V ( $29.33 \pm 3.00$ ) J ( $19.78 \pm 3.56$ ); (g) 48 h: V ( $26.90 \pm 4.55$ ) J ( $25.01 \pm 2.88$ ).

**JZL184: Evaluation of CB<sub>1</sub> receptors.** In these experiments the CB<sub>1</sub> antagonist/inverse agonist RIM (3.0 mg/kg) was administered with JZL184 (40.0 mg/kg) to determine if it would block the JZL184-induced attenuation of ICSS. Eight mice completed testing and the data were analyzed using two-way

(treatment x frequency) repeated-measures ANOVAs. Administration of VEH and VEH did not affect ICSS; there was a main effect of frequency ( $F_{9,63} = 39.01$ ,  $p < 0.0001$ ), but no main effect of treatment ( $F_{1,7} = 2.42$ ,  $p > 0.05$ ) and no interaction ( $F_{9,63} = 0.42$ ,  $p > 0.05$ ) (Fig. 15a).

Administration of VEH and 40.0 mg/kg JZL184 reduced stimulations from baseline; there was a

main effect of frequency ( $F_{9,63} = 34.95, p < 0.0001$ ), treatment ( $F_{1,7} = 29.94, p < 0.001$ ) and an interaction ( $F_{9,63} = 6.97, p < 0.05$ ) (Fig. 15b). Post-hoc analysis revealed reductions at six frequencies which are denoted on Fig. 15b. RIM (3.0 mg/kg) and VEH did not produce a change from baseline responding; there was a main effect of frequency ( $F_{9,63} = 52.30, p < 0.0001$ ), but no main effect of treatment ( $F_{1,7} = 1.71, p > 0.05$ ) and no interaction ( $F_{9,63} = 1.42, p > 0.05$ ) (Fig. 15c). The attenuation of ICSS produced by 40.0 mg/kg JZL184 was blocked by treatment with 3.0 mg/kg RIM, as stimulations did not differ from baseline; there was a main effect of frequency ( $F_{9,63} = 66.55, p < 0.0001$ ), but no main effect of treatment ( $F_{1,7} = 0.13, p > 0.05$ ), and no interaction ( $F_{9,63} = 0.49, p > 0.05$ ) (Fig. 15d). Baseline rate-frequency curves did not differ during any condition tested (Fig. 15e); there was a main effect of frequency ( $F_{9,63} = 66.23, p < 0.0001$ ), but no main effect of baseline day ( $F_{3,21} = 2.78, p > 0.05$ ) and no interaction between baseline day and frequency ( $F_{27,189} = 1.49, p > 0.05$ ).

A two-way, repeated-measures ANOVA (3.0 mg/kg RIM vs. VEH x 40.0 mg/kg JZL184 vs. VEH) was conducted using the percent baseline stimulations data. RIM (3.0 mg/kg) blocked the attenuation of ICSS produced by 40.0 mg/kg JZL184 (Fig. 15f). There was no main effect of first treatment (RIM vs. VEH) ( $F_{1,7} = 3.20, p > 0.05$ ), but there was a main effect of second treatment (JZL184 vs. VEH) ( $F_{1,7} = 6.10, p < 0.05$ ) and an interaction between first and second treatment ( $F_{1,7} = 7.66, p < 0.05$ ). Post-hoc analysis revealed that percent baseline stimulations were reduced ( $p < 0.001$ ) in mice treated with 40.0 mg/kg JZL184, but only when first treated with VEH (Fig. 15f). Administration of 3.0 mg/kg RIM blocked the ICSS reduction produced by 40.0 mg/kg JZL184, demonstrating that the attenuation of stimulations produced by 40.0 mg/kg JZL184 was CB<sub>1</sub> receptor dependent. Rate-frequency findings were consistent with percent

baseline stimulations, as that also showed a blockade of JZL184-induced attenuation of ICSS by RIM. Similar to THC, the attenuation of ICSS was mediated through CB<sub>1</sub> receptors.

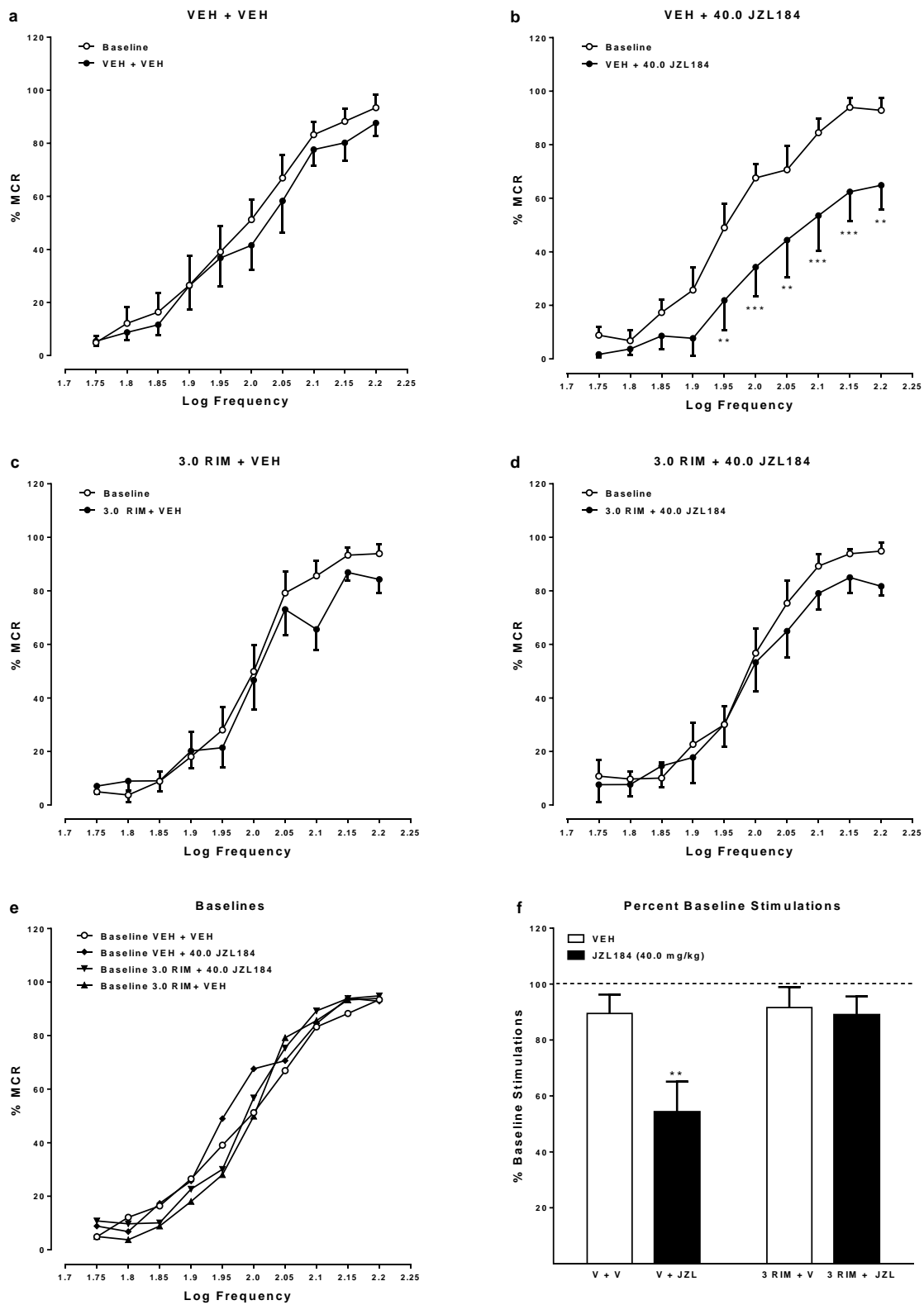


Figure Continues

## Figure Continued

*Figure 15.* JZL184 CB<sub>1</sub> antagonism assessment. Panel a – d shows rate-frequency data during RIM antagonism tests. Panel e shows all baselines rate-frequency curves during CB<sub>1</sub> testing. Percent maximum control rate is displayed on the Y-axis, log-frequency values are shown on the x-axis. Panel f shows percent baseline stimulations ( $\pm$  SEM) for all treatments. Significant interactions were followed up by post-hoc analysis, and differences are denoted by asterisks at values that are significantly different from baseline (a – e), or other treatment conditions (f). \*\*\*\* $p < 0.0001$ , \*\*\* $p < 0.001$ , \*\* $p < 0.01$ . Raw overall response rates (stimulations/min; mean  $\pm$  SEM) during baseline (B) and test (T) sessions were as follows: (a) VEH + VEH: B ( $31.20 \pm 3.46$ ) T ( $29.14 \pm 3.96$ ); (b) VEH + 40.0 mg/kg JZL184: B ( $31.22 \pm 3.18$ ) T ( $19.31 \pm 4.23$ ); (c) 3.0 mg/kg RIM + VEH: B ( $29.18 \pm 2.94$ ) T ( $26.94 \pm 2.88$ ); (d) 3.0 mg/kg RIM + 40.0 mg/kg JZL184: B ( $31.53 \pm 3.22$ ) T ( $28.41 \pm 3.52$ ).

**JZL184: Evaluation of CB<sub>2</sub> receptors.** The purpose of this study was to evaluate whether CB<sub>2</sub> receptor played a necessary role in the suppressive ICSS effects produced by JZL184 (40.0 mg/kg). The CB<sub>2</sub> receptor antagonist/inverse agonist SR2 (10.0 mg/kg) was co-administered to determine if it would block the reduction in stimulation rate produced by JZL184. Eight mice were used for testing and data were analyzed using two-way (treatment x frequency) repeated-measures ANOVAs to compare each drug combination to baseline. Similar to previous tests administration of VEH and VEH did not affect baseline responding; there was a main effect of frequency ( $F_{9,63} = 46.89, p < 0.0001$ ), but no main effect of treatment ( $F_{1,7} = 1.50, p > 0.05$ ), and no interaction ( $F_{9,63} = 1.00, p > 0.05$ ) (Fig. 16a). Administration of VEH and 40.0 mg/kg attenuated ICSS from baseline, similar to results of CB<sub>1</sub> tests; there was a main effect of frequency ( $F_{9,63} = 43.63, p < 0.0001$ ), treatment ( $F_{1,7} = 92.02, p < 0.0001$ ) and an interaction ( $F_{9,63} = 2.06, p < 0.05$ ) (Fig. 16b). Post-hoc analysis showed reductions at six frequencies which are denoted on Fig. 16b. SR2 (10.0 mg/kg) co-administered with VEH did not affect baseline ICSS; there was a main effect of frequency ( $F_{9,63} = 35.02, p < 0.0001$ ), but no main effect of drug treatment ( $F_{1,7} = 1.78, p > 0.05$ ) and no interaction ( $F_{9,63} = 0.79, p > 0.05$ ) (Fig. 16c). SR2 (10.0 mg/kg) was not able to block the effects of JZL184, as it still produced a

main effect of frequency ( $F_{9,63} = 33.23, p < 0.0001$ ), treatment ( $F_{1,7} = 5.87, p < 0.05$ ) and an interaction ( $F_{9,63} = 2.053, p < 0.05$ ) (Fig. 16d). Post-hoc analysis revealed reductions at 2 frequencies which are denoted on Fig. 16d. Baseline rate-frequency curves did not differ during any condition tested (Fig. 16e); there was a main effect of frequency ( $F_{9,63} = 62.05, p < 0.0001$ ), but no main effect of baseline day ( $F_{3,21} = 3.00, p > 0.05$ ) and no interaction between baseline day and frequency ( $F_{27,189} = 1.43, p > 0.05$ ).

Percent baseline stimulations data were analyzed using a two-way, repeated-measures ANOVA (10.0 mg/kg SR2 vs. VEH x 40.0 mg/kg JZL184 vs. VEH). The attenuation of ICSS produced by 40.0 mg/kg JZL184 was not blocked by co-administration with SR2 (10.0 mg/kg) (Fig. 16f). There was no main effect of first treatment (SR2 vs. VEH) ( $F_{1,7} = 0.56, p > 0.05$ ), but there was a main effect of second treatment (JZL184 vs. VEH) ( $F_{1,7} = 34.59, p < 0.001$ ) and an interaction between first treatment and second treatment ( $F_{1,7} = 7.17, p < 0.05$ ). Post-hoc analysis revealed that JZL184 significantly reduced percent baseline stimulations when co-administered with SR2 or VEH, indicating that JZL184 induced attenuation of ICSS was CB<sub>2</sub> receptor independent. These results completely agreed with rate-frequency analysis. These results were also similar to findings with THC which produced attenuation mediated through CB<sub>1</sub> receptors, and not affected by CB<sub>2</sub> receptor antagonism.

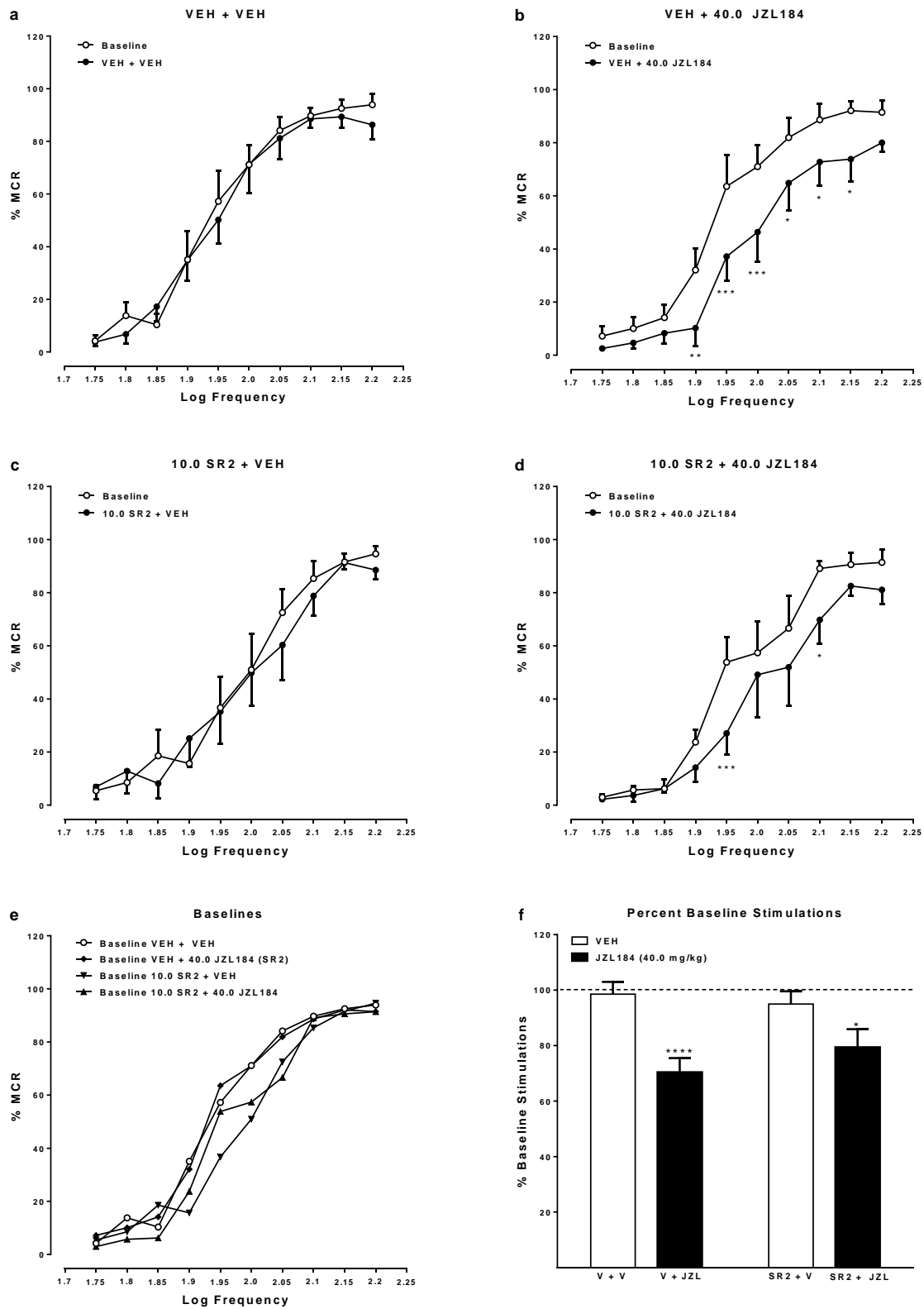


Figure Continues

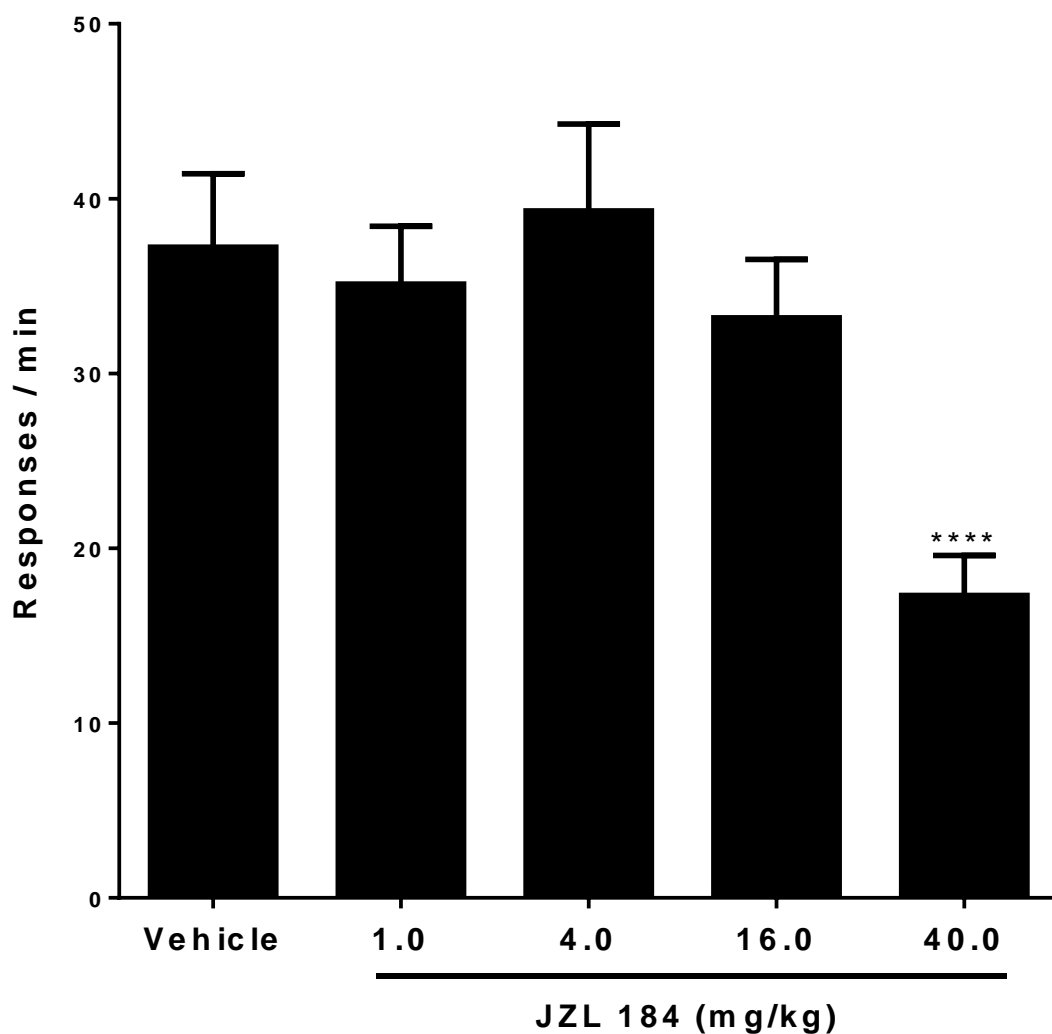


## Figure Continued

*Figure 16.* JZL184 CB<sub>2</sub> antagonism assessment. Panels a – d shows rate-frequency data during SR2 antagonism tests. Panel e shows all baselines rate-frequency curves during CB<sub>2</sub> testing. Percent maximum control rate is displayed on the Y-axis, log-frequency values are shown on the x-axis. Panel f shows percent baseline stimulations ( $\pm$  SEM) for all treatments. Significant interactions with were investigated with post-hoc analysis, and differences are denoted by asterisks at values that are significantly different from baseline (a – e), or other treatment conditions (f). \*\*\*\* $p < 0.0001$ , \*\*\* $p < 0.001$ , \*\* $p < 0.01$ , \* $p < 0.05$ . Raw overall response rates (stimulations/min; mean  $\pm$  SEM) during baseline (B) and test (T) sessions were as follows: (a) VEH + VEH: B ( $34.04 \pm 3.06$ ) T ( $33.88 \pm 3.54$ ); (b) VEH + 40.0 mg/kg JZL184: B ( $36.02 \pm 3.38$ ) T ( $26.98 \pm 3.40$ ); (c) 10.0 mg/kg SR2 + VEH: B ( $30.98 \pm 4.31$ ) T ( $29.47 \pm 4.31$ ); (d) 10.0 mg/kg SR2 + 40.0 mg/kg JZL184: B ( $30.81 \pm 3.44$ ) T ( $25.09 \pm 3.47$ ).

### JZL184 Operant Responding for Food Assessment

In this study, operant responding for food pellets was assessed after VEH and JZL184 (1.0, 4.0, 16.0 and 40.0 mg/kg). A one-way repeated-measures ANOVA was used to analyze response rates. JZL184 reduced operant response rates ( $F_{4,32} = 9.89$ ,  $p < 0.0001$ ) (Fig. 17). Post-hoc analysis showed that 40.0 mg/kg JZL184 significantly attenuated ( $p < 0.0001$ ) response rates, while 1.0, 4.0, and 16.0 mg/kg JZL184 did not differ from VEH as denoted on Fig. 17. These findings with high dose JZL184 (40.0 mg/kg) support ICSS findings, in which both 16.0 and 40.0 mg/kg JZL184 attenuated responding. This was also similar to THC, as THC (10.0 mg/kg) reduced operant response rates for both food and brain stimulation.



*Figure 17.* JZL184 effects on operant responding for food. Mean response rates ( $\pm$  SEM) are displayed on the y-axis, and dose of JZL184 or VEH is on the x-axis. A significant one-way ANOVA was investigated with post-hoc analysis, and significant differences from VEH are denoted by asterisks. \*\*\*\* $p < 0.0001$

### **JZL184 Spontaneous Locomotor Activity Assessment**

Spontaneous locomotor activity was assessed after administration of JZL184 (4.0, 16.0 and 40.0 mg/kg) or VEH. Distance travelled (m), mean speed (m/s) and time immobile (s) were recorded and are displayed on Fig. 18. A significant main effect of JZL184 treatment on distance travelled was observed ( $F_{3,31} = 8.91, p < 0.001$ ) (Fig. 18a). Post-hoc analysis showed that 40.0 mg/kg JZL184 reduced ( $p < 0.001$ ) distance travelled, while 4.0 and 16.0 mg/kg did not differ

from VEH. JZL184 also increased time-immobile ( $F_{3,31} = 30.60, p < 0.0001$ ). Post-hoc analysis showed that 40.0 and 16.0 mg/kg JZL184 increased ( $p < 0.0001$ ;  $p < 0.01$ ) time-immobile compared to VEH, while 4.0 mg/kg did not differ from VEH (Fig. 18b). JZL184 also significantly reduced speed of locomotion ( $F_{3,31} = 3.07, p < 0.05$ ) (Fig. 18c). Post-hoc analysis showed that 40.0 mg/kg JZL184 decreased ( $p < 0.05$ ) mean adjusted speed from VEH, while 4.0 and 16.0 mg/kg did not differ from VEH. These results support ICSS and operant responding data, as distance travelled, operant responding, and ICSS were all reduced with high dose JZL184 (40.0 mg/kg), while time-immobile and ICSS were also affected by 16.0 mg/kg JZL184.

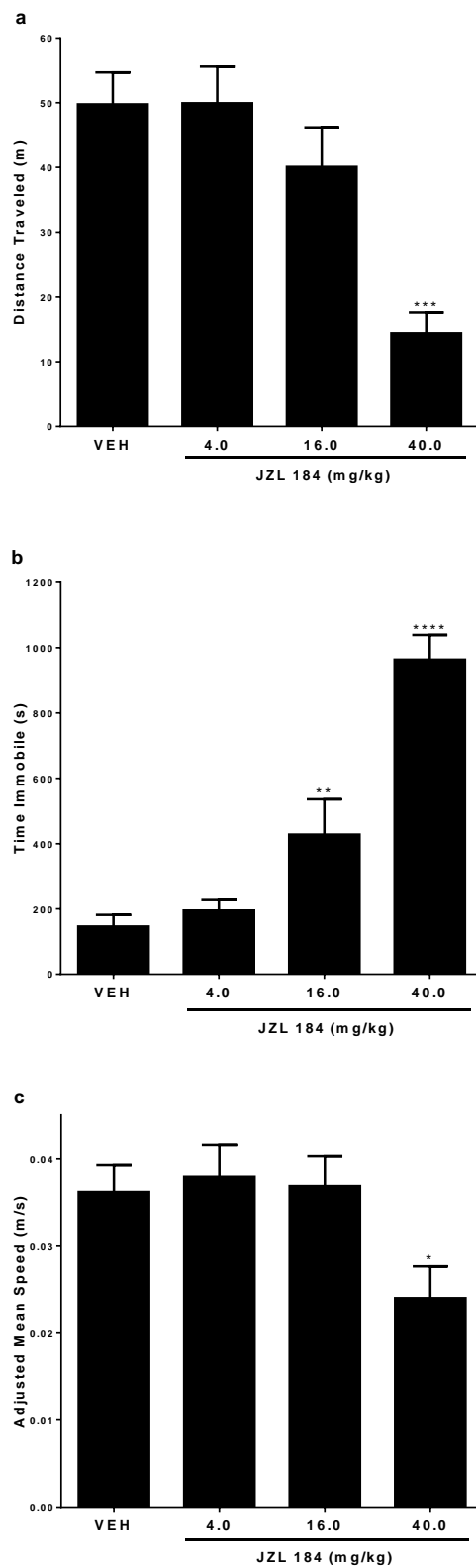


Figure Continues

Figure Continued

*Figure 18.* JZL184 effects on spontaneous locomotor activity. All panels show dose of JZL184 and VEH on the x-axis. Panel a shows distance travelled on the y-axis, panel b shows time-immobile, and panel c shows adjusted speed. Significant one-way ANOVAs were investigated with post-hoc analysis, and significant differences from VEH are denoted by asterisks. \*\*\*\* $p < 0.0001$ , \*\*\* $p < 0.001$ , \*\* $p < 0.01$ , \* $p < 0.05$

### **JZL184 Endocannabinoid Quantification**

Concentrations of 2-AG, AEA, and AA were determined from tissue samples from PFC, NAc, VMB, amygdala, and cerebellum after treatment with VEH, or JZL184 (4.0 or 40.0 mg/kg). Separate one-way ANOVAs were conducted within each brain region to analyze concentration changes from VEH treatment. JZL184 dose-dependently increased 2-AG concentrations in the PFC ( $F_{2,12} = 124.2$ ,  $p < 0.0001$ ); post-hoc analysis showed that 40.0 mg/kg JZL184 produced a 7-fold increase ( $p < 0.0001$ ) of 2-AG from VEH, while treatment 4.0 mg/kg JZL184 did not differ from VEH (Fig. 19a). JZL184 decreased AEA in the PFC by JZL184 ( $F_{2,12} = 4.45$ ,  $p < 0.05$ ); post-hoc analysis showed that 40.0 mg/kg JZL184 reduced ( $p < 0.05$ ) AEA concentrations to 49 % of VEH, while 4.0 mg/kg JZL184 AEA concentrations did not differ from VEH (Fig. 19b). JZL184 decreased concentrations of AA in the PFC ( $F_{2,12} = 12.32$ ,  $p < 0.01$ ); post-hoc analysis showed that 40.0 mg/kg JZL184 reduced AA concentrations to 78 % of VEH, while 4.0 mg/kg JZL184 did not affect AA (Fig. 19c).

JZL184 increased 2-AG concentrations in the NAc ( $F_{2,11} = 130.9$ ,  $p < 0.0001$ ); post-hoc analysis showed that 4.0 and 40.0 mg/kg JZL184 increased ( $p < 0.001$ ;  $p < 0.0001$ ) 2-AG levels over 4-fold and 10-fold respectively from VEH (Fig. 19a). JZL184 decreased AEA in the NAc ( $F_{2,11} = 4.92$ ,  $p < 0.05$ ); post-hoc analysis showed that 40.0 mg/kg JZL184 reduced ( $p < 0.05$ ) AEA concentrations to 46 % of VEH levels, while 4.0 mg/kg JZL184 did not affect AEA levels

(Fig. 19b). JZL184 did not affect AA concentrations in the NAc ( $F_{2,11} = 3.88, p = 0.05$ ); however, there was a trend toward reduction of AA (Fig. 19c).

Administration of JZL184 increased 2-AG concentrations in the VMB ( $F_{2,12} = 33.41, p < 0.0001$ ); post-hoc analysis showed that 4.0 and 40.0 mg/kg JZL184 increased ( $p < 0.01$ ;  $p < 0.0001$ ) 2-AG over 7-fold and 13-fold VEH levels (Fig. 19a). JZL184 did not affect AEA in the VMB ( $F_{2,12} = 0.42, p > 0.05$ ), as concentrations did not differ from VEH (Fig. 19b). JZL184 reduced AA concentrations in the VMB ( $F_{2,12} = 4.35, p < 0.05$ ); post-hoc analysis showed that 40.0 mg/kg JZL184 decreased ( $p < 0.05$ ) AA concentrations ( $p < 0.05$ ) to 61 % of VEH AA, while 4.0 mg/kg JZL184 AA levels did not differ from VEH (Fig. 19c).

JZL184 increased 2-AG levels in the amygdala ( $F_{2,12} = 87.70, p < 0.0001$ ); post-hoc analysis showed that both 4.0 and 40.0 mg/kg JZL184 increased ( $p < 0.05$ ;  $p < 0.0001$ ) 2-AG over 2-fold and 8-fold of VEH levels respectively (Fig. 19a). JZL184 did not affect AEA levels in the amygdala ( $F_{2,12} = 3.69, p > 0.05$ ) (Fig. 19b). JZL184 reduced AA concentrations in the amygdala ( $F_{2,12} = 10.12, p < 0.01$ ). Post-hoc analysis showed that 40.0 mg/kg JZL184 reduced ( $p < 0.01$ ) AA levels to 65 % of VEH concentrations, while 4.0 mg/kg JZL184 AA levels did not differ from VEH (Fig. 19c).

Administration of JZL184 increased 2-AG in the cerebellum after ( $F_{2,12} = 75.91, p < 0.0001$ ); post-hoc analysis showed that 4.0 and 40.0 mg/kg JZL184 increased ( $p < 0.001$ ;  $p < 0.0001$ ) 2-AG concentrations by over 2-fold and nearly 4-fold of VEH levels respectively (Fig. 19a). JZL184 did not affect AEA concentrations in the cerebellum ( $F_{2,12} = 1.09, p > 0.05$ ); (Fig. 19b). JZL184 lowered AA concentrations in the cerebellum ( $F_{2,12} = 30.192, p < 0.0001$ ). Post-hoc analysis showed that 40.0 mg/kg JZL184 decreased AA to 69 % of VEH levels, while 4.0 mg/kg JZL184 AA concentrations did not differ from VEH (Fig. 19c).

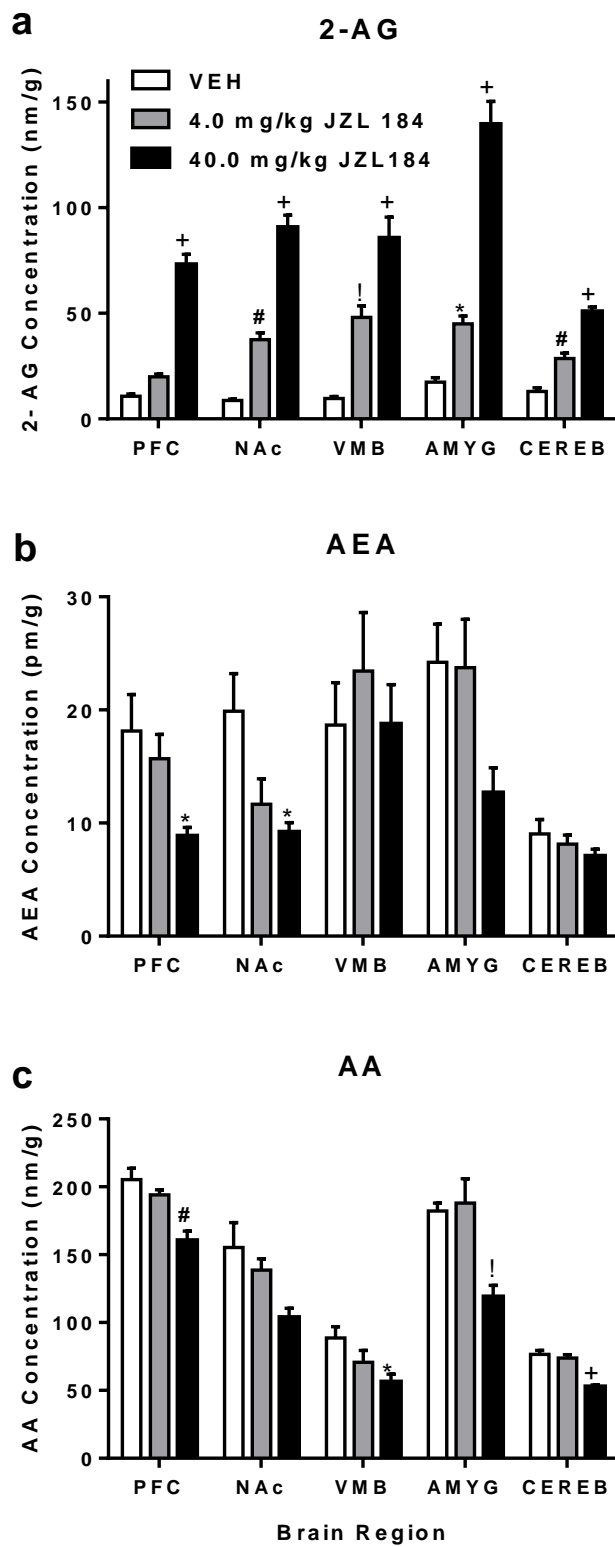


Figure Continues

## Figure Continued

*Figure 19.* JZL184 effects on endocannabinoid and AA concentrations. All panels show brain region on the x-axis (AMYG = amygdala, CEREB = cerebellum). Panel a shows 2-AG levels on the y-axis, panel b shows AEA, and panel c displays AA. One-way ANOVAs were calculated within each brain region and substrate measured. Significant one-way ANOVAs were investigated with post-hoc analyses, and significant differences from corresponding VEH treatment are denoted by symbols. <sup>+</sup>  $p < 0.0001$ , <sup>#</sup>  $p < 0.001$ , <sup>!</sup>  $p < 0.01$ , \* $p < 0.05$

## PF-3845 ICSS Experiments

**PF-3845 dose-response assessment.** This study examined the effects of FAAH inhibition on ICSS. ICSS was assessed after administration of VEH and PF-3845 (1.0, 3.0, 10.0, and 30.0 mg/kg). ICSS was not affected by VEH, 1.0, 3.0, or 10.0 mg/kg PF-3845, but it was significantly attenuated by 30.0 mg/kg PF-3845. It is important to note that no other behavioral studies have assessed doses over 10.0 mg/kg, which we will discuss further in the discussion. VEH produced a main effect of frequency ( $F_{9,72} = 103.2, p < 0.0001$ ), but no main effect of treatment ( $F_{1,8} = 0.09, p > 0.05$ ) and no interaction ( $F_{9,72} = 0.30, p > 0.05$ ) (Fig. 20a). As mentioned above, PF-3845 (1.0, 3.0 and 10.0 mg/kg) did not affect ICSS; with 1.0 mg/kg there was a main effect of frequency ( $F_{9,72} = 63.36, p < 0.0001$ ), but there was no main effect of treatment ( $F_{1,8} = 5.17, p = 0.05$ ) and no interaction ( $F_{9,72} = 0.72, p > 0.05$ ) (Fig. 20b). PF-3845 (3.0 mg/kg) produced a main effect of frequency ( $F_{9,72} = 64.84, p < 0.0001$ ), but no main effect of treatment ( $F_{1,8} = 2.60, p > 0.05$ ) and no interaction ( $F_{9,72} = 0.82, p > 0.05$ ) (Fig. 20c). PF-3845 (10.0 mg/kg) produced a main effect of frequency ( $F_{9,72} = 63.50, p < 0.0001$ ), but no main effect of treatment ( $F_{1,8} = 2.07, p > 0.05$ ) and no interaction ( $F_{9,72} = 0.61, p > 0.05$ ) (Fig. 20d). The highest dose of PF-3845 (30.0 mg/kg) reduced ICSS rate; there was a main effect of frequency ( $F_{9,72} = 61.19, p < 0.0001$ ), treatment ( $F_{1,8} = 8.32, p < 0.05$ ) and an interaction ( $F_{9,72} = 3.72, p < 0.001$ ) (Fig. 20e). Post-hoc analysis showed reductions at four frequencies which are denoted on Fig. 20e. All baseline rate-frequency stimulation rates were analyzed after testing to



determine if there were any differences (Fig. 20f). There was a main effect of frequency ( $F_{9,72} = 110.10, p < 0.0001$ ), but no main effect of baseline day ( $F_{4,32} = 0.24, p > 0.05$ ) and no interaction between treatment and baseline day ( $F_{36,288} = 0.64, p > 0.05$ ), indicating that baselines did not differ over time. Additionally, a second VEH test was conducted and compared to the initial VEH assessment; VEH tests did not differ from each other (data not shown), as there was a main effect of frequency ( $F_{9,72} = 79.31, p < 0.0001$ ), but there was no main effect of treatment ( $F_{1,8} = 2.39, p > 0.05$ ) and no interaction ( $F_{9,72} = 0.99, p > 0.05$ ). Similar to JZL184 treatment, the high dose of PF-3845 produced a significant reduction of ICSS.

Percent baseline stimulations were calculated for each condition and evaluated using one-way repeated measures ANOVA. PF-3845 reduced percent baseline stimulations ( $F_{4,24} = 4.48, p < 0.01$ ) (Fig. 21). Post-hoc analysis showed that 30.0 mg/kg (79.10 %) produced significant attenuation ( $p < 0.05$ ) of percent baseline stimulations from VEH. While other doses of PF-3845 (1.0, 3.0 and 10.0 mg/kg) did not affect percent baseline stimulations. These results are in agreement with the rate-frequency data analysis, as only the high dose of PF-3845 (30.0 mg/kg) produced a reduction of ICSS.

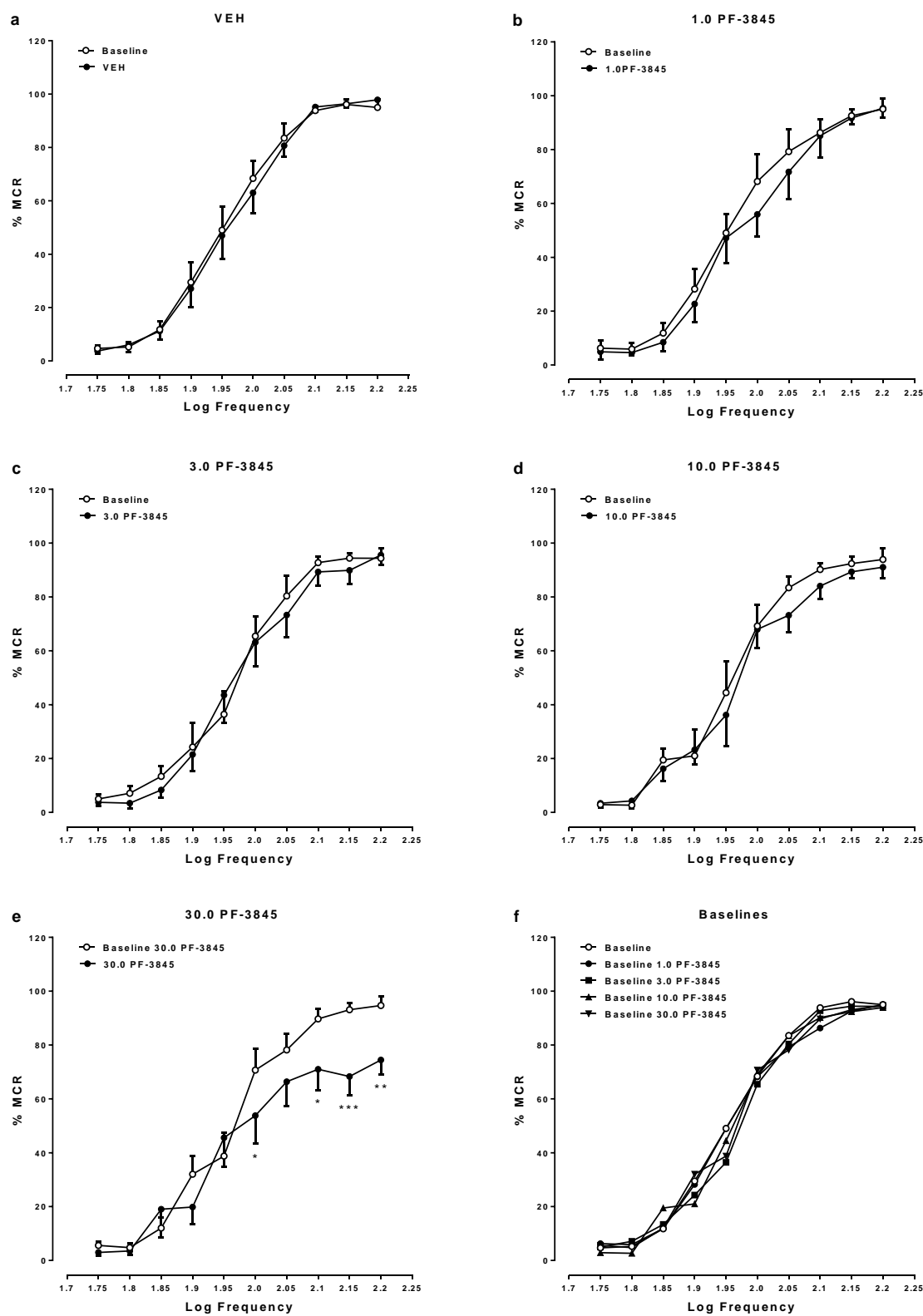
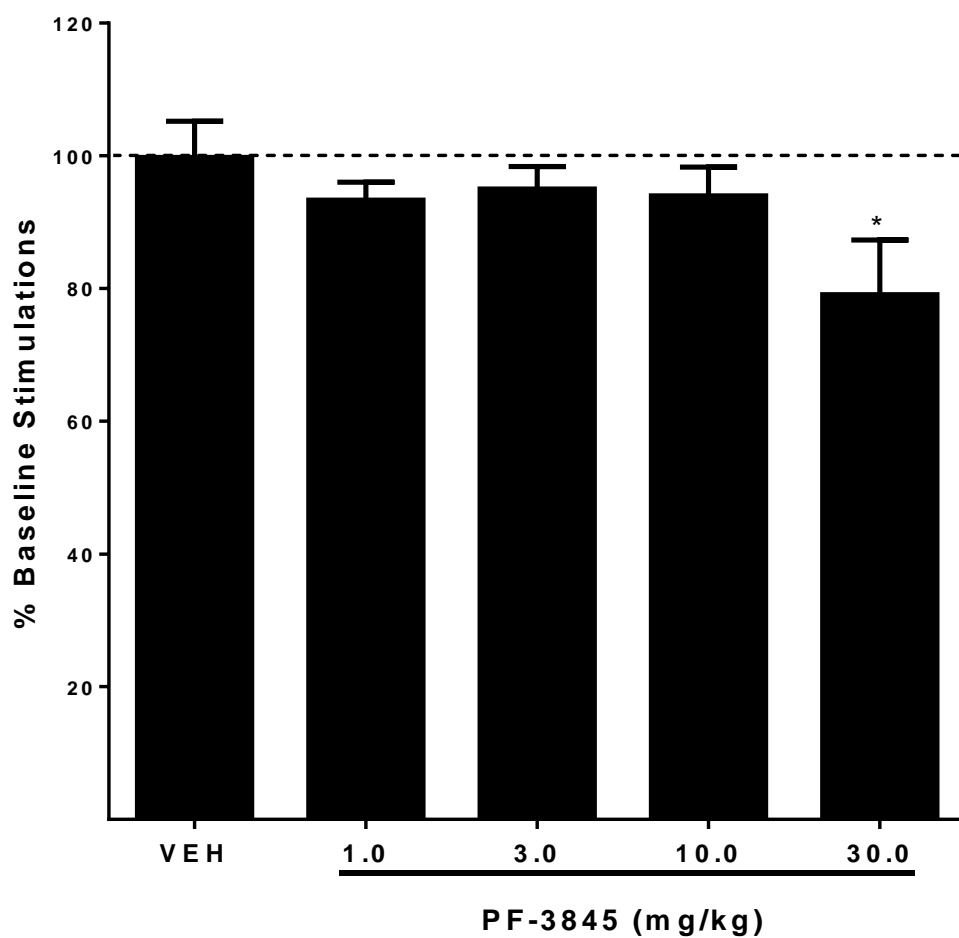


Figure Continues

## Figure Continued

*Figure 20.* PF-3845 ICSS dose-response assessment. Panels a – e show rate-frequency stimulation values ( $\pm$  SEM) after VEH or PF-3845 (1.0, 3.0, 10.0 and 30.0 mg/kg). Panel f shows all baseline rate-frequency curves during testing. Percent maximum control rate is displayed on the Y-axis, log-frequency values are shown on the x-axis. Interactions that achieved significance were investigated with post-hoc analyses, and significant differences from baseline are denoted by asterisks. \*\*\* $p < 0.001$ , \*\* $p < 0.01$ , \* $p < 0.05$ . Raw overall response rates (stimulations/min; mean  $\pm$  SEM) during baseline (B) and test (T) sessions were as follows: (a) VEH: B ( $32.81 \pm 2.19$ ) T ( $32.13 \pm 1.94$ ); (b) 1.0 mg/kg PF-3845: B ( $32.82 \pm 2.90$ ) T ( $30.73 \pm 2.83$ ); (c) 3.0 mg/kg PF-3845: B ( $32.59 \pm 2.58$ ) T ( $31.28 \pm 2.91$ ); (d) 10.0 mg/kg PF-3845: B ( $34.08 \pm 2.40$ ) T ( $32.10 \pm 2.72$ ); (e) 30.0 mg/kg PF-3845: B ( $32.96 \pm 2.55$ ) T ( $27.08 \pm 3.68$ ).



*Figure 21.* PF-3845 ICSS percent baseline stimulations. Fig. 21 shows percent baseline stimulations ( $\pm$  SEM) after PF-3845 or VEH. The one-way ANOVA was significant and post-hoc analysis revealed a significant difference from VEH, which is denoted with an asterisk.  $*p < 0.05$

**PF-3845 time-course assessment.** The purpose of this experiment was to assess the time-course of PF-3845 induced attenuation of ICSS. Self-stimulations were assessed at 1, 2, 4, 8, 24, and 48 h after administration of 30.0 mg/kg PF-3845 or VEH. Eight mice completed testing, and two-way repeated-measures ANOVAs (Treatment x Frequency) were conducted to compare rate-frequency curves between VEH and PF-3845 treatment at each time-point. Rate-frequency data shows that PF-3845 (30.0 mg/kg) attenuated stimulations compared to VEH at 1, 2, 4, 8 and 24 h after injection, but did not differ from each other 48 h after administration. Baseline rate-frequency curves between the two conditions did not differ from each other, as there was a main effect of frequency ( $F_{9, 63} = 71.80, p < 0.0001$ ), but no main effect of treatment ( $F_{1, 7} = 1.1, p > 0.05$ ) and no interaction ( $F_{9, 63} = 0.90, p > 0.05$ ) (Fig. 22a). One hour after administration there was a main effect of frequency ( $F_{9, 63} = 46.84, p < 0.0001$ ), treatment ( $F_{1, 7} = 7.99, p < 0.05$ ) and an interaction ( $F_{9, 63} = 2.12, p > 0.05$ ), indicating a significant reduction of ICSS (Fig. 22b). Post-hoc analysis showed a difference at one frequency which is denoted on Fig. 22b. Two hours after injection, there was a main effect of frequency ( $F_{9, 63} = 55.03, p < 0.0001$ ) and a main effect of treatment ( $F_{1, 7} = 3.66, p < 0.05$ ), but no interaction ( $F_{9, 63} = 1.36, p > 0.05$ ) (Fig. 22c). At the four hour time-point there was a main effect of frequency ( $F_{9, 63} = 64.03, p < 0.0001$ ) and treatment ( $F_{1, 7} = 6.80, p < 0.05$ ), but no interaction ( $F_{9, 63} = 0.82, p > 0.05$ ) (Fig. 22d). Eight hours after injection, there was a main effect of frequency ( $F_{9, 63} = 51.67, p < 0.0001$ ) and treatment ( $F_{1, 7} = 6.56, p < 0.05$ ), but there was no interaction ( $F_{9, 63} = 1.09, p > 0.05$ ) (Fig. 22e). At the 24 h time-point, there was a main effect of frequency ( $F_{9, 63} = 62.67, p < 0.0001$ ), treatment ( $F_{1, 7} = 6.47, p < 0.05$ ) and an interaction ( $F_{9, 63} = 3.33, p < 0.01$ ) (Fig. 22f). Post-hoc analysis showed differences at two frequencies which are denoted on Fig. 22f. After 48 hours, stimulations with VEH and 30.0 mg/kg PF-3845 did not differ; there was a main effect of

frequency ( $F_{9,63} = 86.94, p < 0.0001$ ), but no main effect of treatment ( $F_{1,7} = 3.13, p > 0.05$ ) and no interaction ( $F_{9,63} = 0.86, p > 0.05$ ) (Fig. 22g). The reduction of ICSS produced by PF-3845 (30.0 mg/kg) persisted between 24 and 48 hours after treatment, similar to the reduction produced by the MAGL inhibitor JZL184.

Percent baseline stimulations data were analyzed using a two-way repeated-measures ANOVA (Time-point x Treatment) to assess changes in ICSS across all time-points (Fig. 22h). PF-3845 (30.0 mg/kg) reduced percent baseline stimulations compared to VEH, as there was a main effect of time-point ( $F_{5,35} = 4.66, p < 0.01$ ), treatment ( $F_{1,7} = 14.32, p < 0.01$ ) and an interaction between time-point and treatment ( $F_{5,35} = 3.75, p < 0.01$ ). Post-hoc analysis shows that 30.0 mg/kg PF-3845 attenuated percent baseline stimulations compared to VEH treatment at 1, 2, 4, 8 and 24 hours after treatment as denoted on Fig. 22h. These results are in agreement with the rate-frequency analysis, as nearly all time-points were shown to be significantly reduced with both measures. Percent baseline stimulations analysis showed that PF-3845 (30.0 mg/kg) also reduced responding 2 h after administration, in agreement with our initial findings.

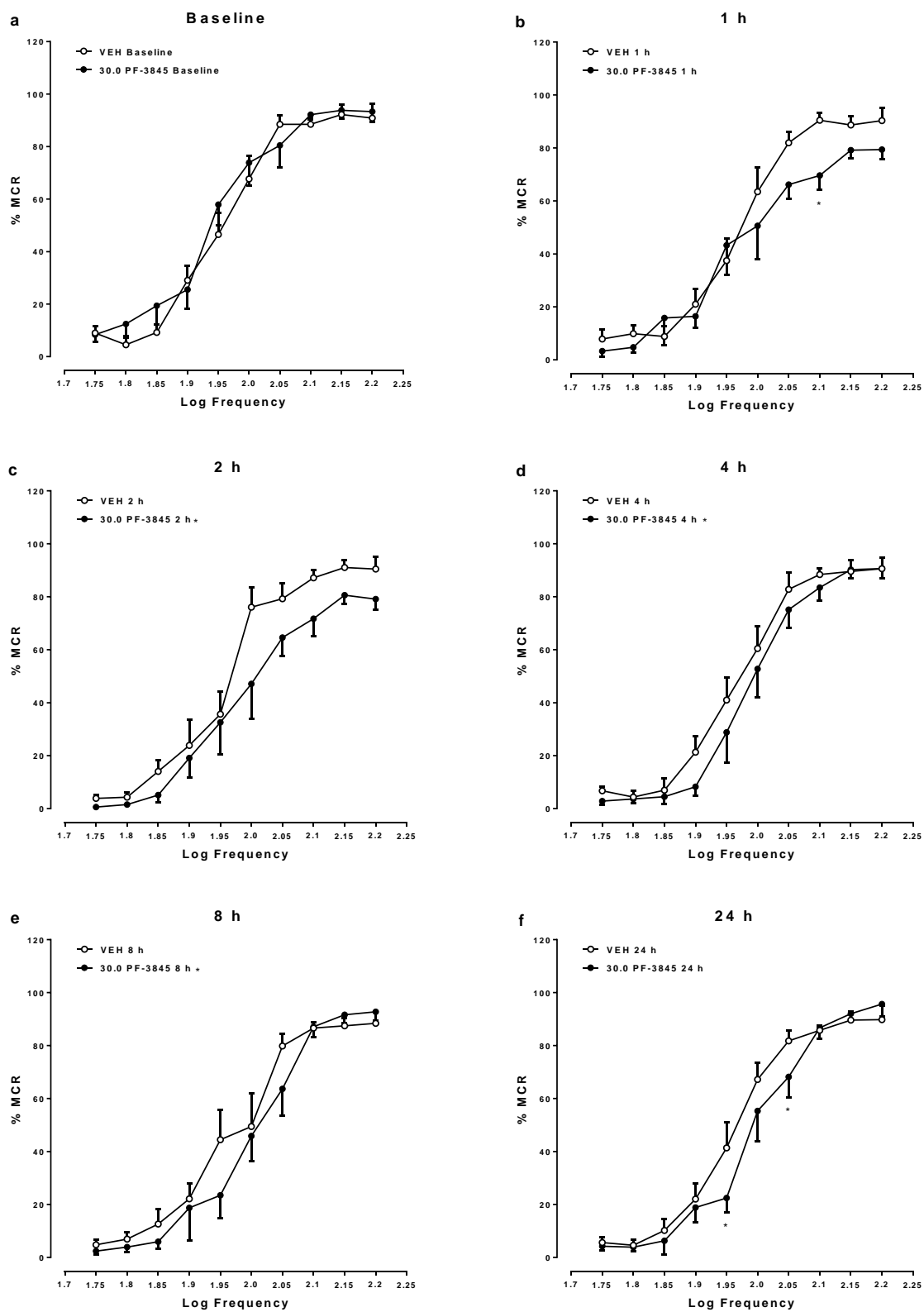
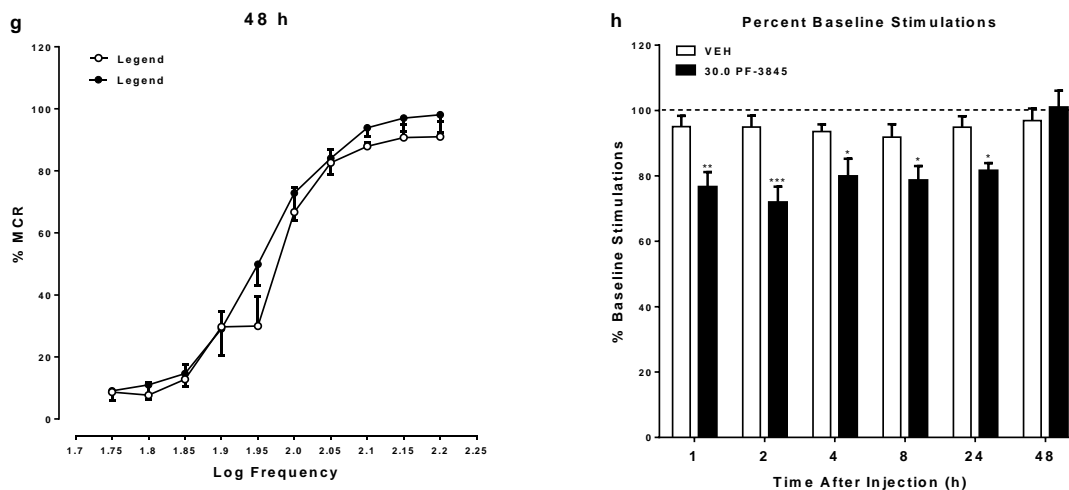


Figure Continues

Figure Continued



**Figure 22.** PF-3845 ICSS time-course evaluation. Panels a – g show rate-frequency stimulation values ( $\pm$  SEM) after VEH or PF-3845 (30.0 mg/kg) at each time-point (1, 2, 4, 8, 24 and 48 h); percent maximum control rate is displayed on the y-axis, log-frequency values are shown on the x-axis. Panel h shows percent baseline stimulations data over time. Significant main effects of treatment are denoted by asterisks on the figure legend. Interactions that achieved significance and were accompanied by significant post-hoc analyses are denoted by asterisks at values that are significantly different from VEH. \*\*\* $p < 0.001$ , \*\* $p < 0.01$ , \* $p < 0.05$ . Raw overall response rates (stimulations/min; mean  $\pm$  SEM) during VEH (V) and 30.0 mg/kg PF-3845 (P) conditions were as follows: (a) Baseline: V ( $35.24 \pm 2.05$ ) P ( $34.84 \pm 2.48$ ); (b) 1 h: V ( $33.54 \pm 2.26$ ) P ( $26.86 \pm 2.87$ ); (c) 2 h: V ( $33.69 \pm 2.75$ ) P ( $25.18 \pm 2.67$ ); (d) 4 h: V ( $33.01 \pm 2.19$ ) P ( $27.53 \pm 2.20$ ); (e) 8 h: V ( $32.37 \pm 2.27$ ) P ( $27.26 \pm 2.32$ ); (f) 24 h: V ( $33.29 \pm 1.96$ ) P ( $28.29 \pm 1.97$ ); (g) 48 h: V ( $33.94 \pm 1.90$ ) P ( $34.88 \pm 2.69$ ).

**PF-3845: Evaluation of CB<sub>1</sub> receptors.** The purpose of this study was to examine whether the reduction of ICSS produced by PF-3845 was CB<sub>1</sub> dependent. Similar to JZL184 antagonist experiments, we tested whether rimonabant was able to block PF-3845 induced attenuation of ICSS. Six mice completed testing and data were analyzed using two-way repeated-measures ANOVAs (treatment x frequency) for each dose combination tested. Administration of VEH and VEH did not produce a change from baseline; there was a main effect of frequency ( $F_{9,45} = 56.60$ ,  $p < 0.0001$ ), but no main effect of treatment ( $F_{1,5} = 2.36$ ,  $p > 0.05$ ) and no



interaction ( $F_{9,45} = 0.94, p > 0.05$ ) (Fig. 23a). PF-3845 (30.0 mg/kg) given in combination with VEH attenuated stimulations from baseline as expected; there was a main effect of frequency ( $F_{9,45} = 51.72, p < 0.0001$ ), treatment ( $F_{1,5} = 43.55, p < 0.01$ ) and an interaction ( $F_{9,45} = 2.72, p < 0.05$ ) (Fig. 23b). Post-hoc analysis showed reductions at four frequencies which are denoted on Fig. 23b. RIM (3.0 mg/kg) and VEH did not affect baseline ICSS; there was a main effect of frequency ( $F_{9,45} = 94.34, p < 0.0001$ ), but no main effect of treatment ( $F_{1,5} = 3.73, p > 0.05$ ) and no interaction ( $F_{9,45} = 1.04, p > 0.05$ ) (Fig. 23c). RIM (3.0 mg/kg) did not block the attenuation of ICSS produced by 30.0 mg/kg PF-3845, as the combination reduced responding compared to baseline ICSS; there was a main effect of frequency ( $F_{9,45} = 47.41, p < 0.0001$ ), treatment ( $F_{1,5} = 20.23, p < 0.01$ ), and an interaction ( $F_{9,45} = 3.51, p < 0.01$ ) (Fig. 23d). Post-hoc analysis showed reductions at six frequencies which are denoted on Fig. 23d. Next, we assessed a higher dose (10.0 mg/kg) of RIM with VEH and PF-3845. ICSS after administration of 10.0 mg/kg RIM and VEH did not differ from baseline; there was a main effect of frequency ( $F_{9,45} = 31.09, p < 0.0001$ ), but no main effect of treatment ( $F_{1,5} = 202, p > 0.05$ ) and no interaction ( $F_{9,45} = 0.36, p > 0.05$ ) (Fig. 23e). However, RIM (10.0 mg/kg) was also not able to block the PF-3845-induced attenuation of ICSS, as there was a main effect of frequency ( $F_{9,45} = 22.09, p < 0.0001$ ), treatment ( $F_{1,5} = 41.64, p < 0.01$ ), and an interaction ( $F_{9,45} = 3.48, p < 0.01$ ) (Fig. 23f). Post-hoc analysis revealed reductions at five frequencies which are denoted on Fig. 23f. Baseline rate-frequency responses slightly differed during the course of these experiments (Fig. 23g); there was a main effect of frequency ( $F_{9,45} = 62.28, p < 0.0001$ ) and baseline day ( $F_{5,25} = 3.23, p > 0.05$ ), but there was no interaction between baseline day and frequency ( $F_{45,225} = 1.26, p > 0.05$ ). Baseline rate-frequency curves during 10.0 mg/kg RIM antagonism tests did not differ; there was a main effect of frequency ( $F_{9,45} = 48.20, p < 0.0001$ ), but no main effect of

baseline day ( $F_{3, 15} = 0.63, p > 0.05$ ) and no interaction between baseline day and frequency ( $F_{27, 135} = 0.94, p > 0.05$ ).

A two-way ANOVA (RIM vs. VEH x 30.0 mg/kg PF-3845 vs. VEH) was conducted with the percent baseline stimulations data. RIM (3.0 and 10.0 mg/kg) was not able to block the reduction in ICSS produced by 30.0 mg/kg PF-3845 (Fig. 23h); there was no main effect of first treatment (VEH, 3.0 vs. 10.0 mg/kg RIM) ( $F_{2, 10} = 0.34, p > 0.05$ ), but there was a main effect of second treatment (VEH vs. PF-3845) ( $F_{2, 10} = 6.10, p < 0.0001$ ); however there was no interaction between first treatment and second treatment ( $F_{2, 10} = 0.46, p > 0.05$ ) (Fig. 23h). PF-3845 (30.0 mg/kg) reduced ICSS regardless of pre-treatment. The percent baseline stimulations assessment confirmed the rate-frequency analysis. In contrast to the effects observed with THC and JZL184, the attenuation of stimulations after administration of 30.0 mg/kg PF-3845 was not mediated through CB<sub>1</sub> receptors.

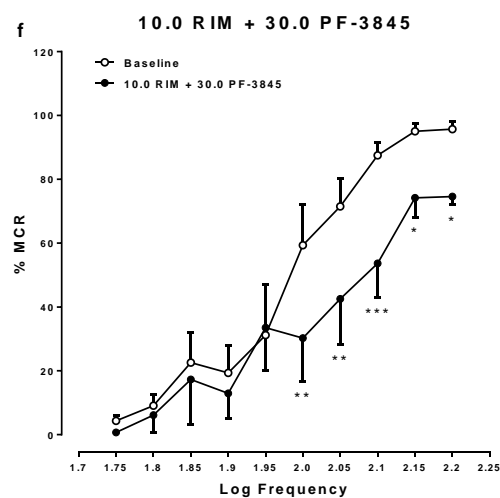
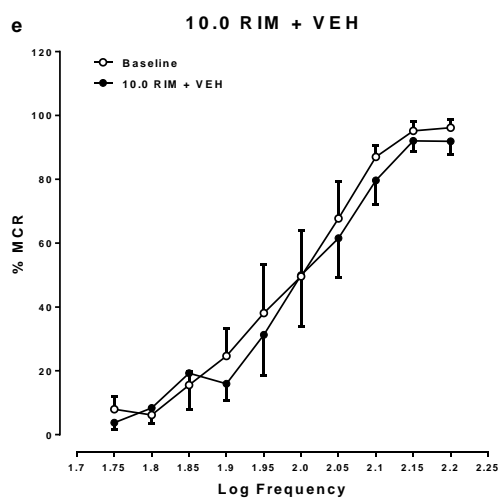
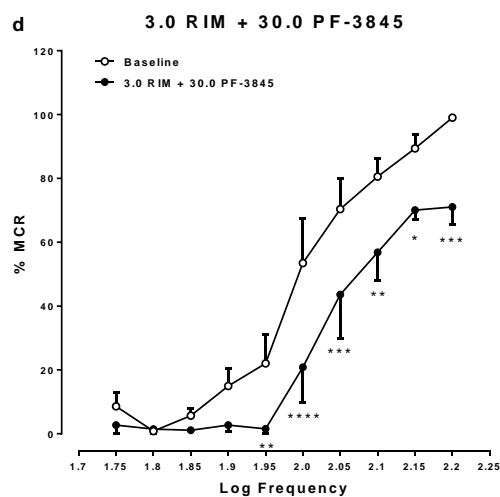
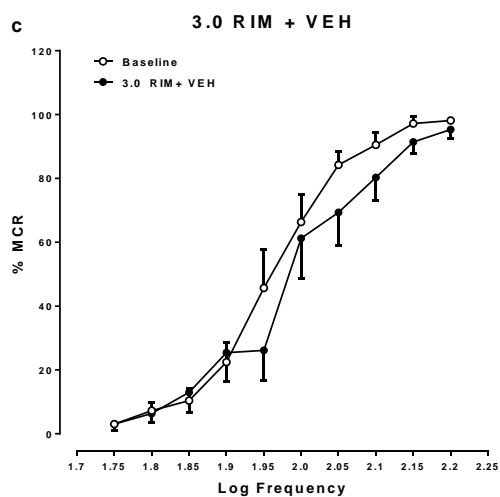
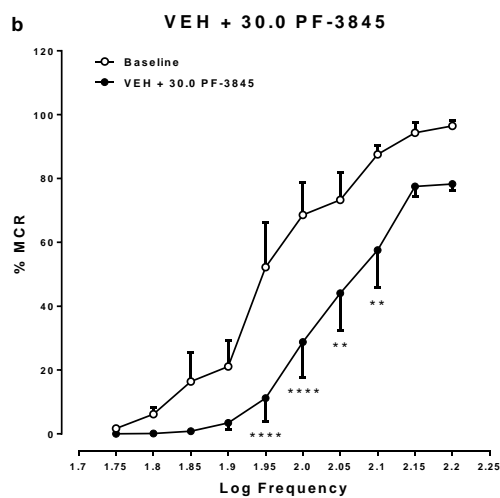
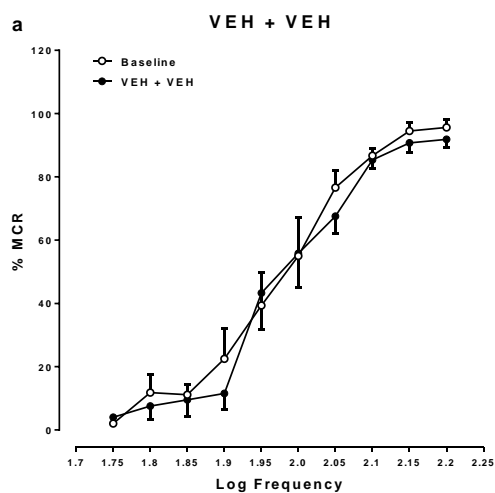
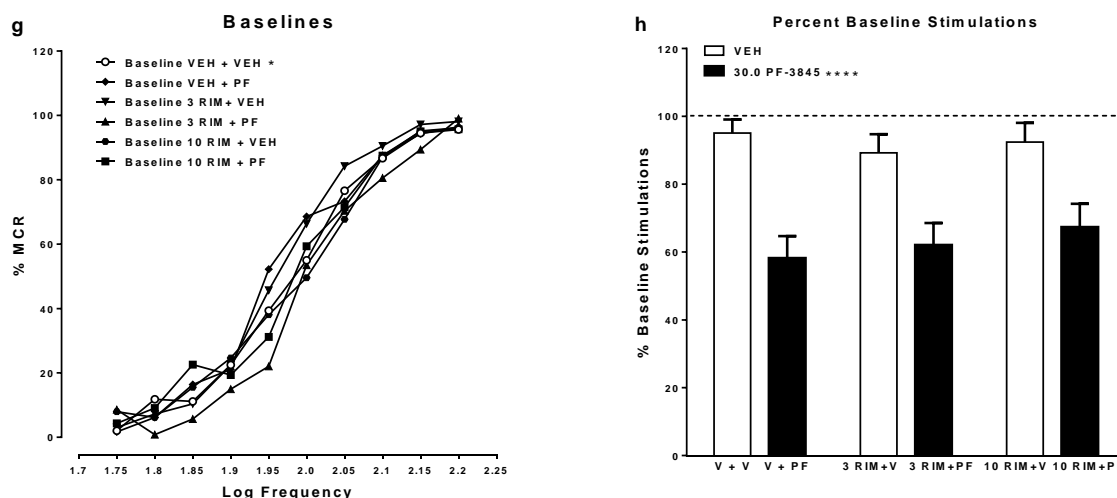


Figure Continues

Figure Continued



**Figure 23.** PF-3845 CB<sub>1</sub> antagonism assessment. Panels a – f shows rate-frequency data during RIM antagonism tests with 30.0 mg/kg PF-3845. Panel g shows all baselines rate-frequency curves during CB<sub>1</sub> testing. Percent maximum control rate is displayed on the Y-axis, log-frequency values are shown on the x-axis. Panel h shows percent baseline stimulations ( $\pm$  SEM) for all treatments. Significant main effects are denoted by asterisks by the figure legend. Significant interactions were followed up by post-hoc analysis, and differences are denoted by asterisks at values that are significantly different from baseline (a – e). \*\*\*\* $p < 0.0001$ , \*\*\* $p < 0.001$ , \*\* $p < 0.01$ . Raw overall response rates (stimulations/min; mean  $\pm$  SEM) during baseline (B) and test (T) sessions were as follows: (a) VEH + VEH: B ( $31.53 \pm 3.11$ ) T ( $29.56 \pm 2.23$ ); (b) VEH + 30.0 mg/kg PF-3845: B ( $33.49 \pm 2.96$ ) T ( $19.70 \pm 2.99$ ); (c) 3.0 mg/kg RIM + VEH: B ( $32.86 \pm 2.87$ ) T ( $29.37 \pm 3.27$ ); (d) 3.0 mg/kg RIM + 30.0 mg/kg PF-3845: B ( $26.87 \pm 3.61$ ) T ( $16.47 \pm 2.58$ ); (e) 10.0 mg/kg RIM + VEH: B ( $30.97 \pm 4.24$ ) T ( $28.84 \pm 4.51$ ); (f) 10.0 mg/kg RIM + 30.0 mg/kg PF-3845: B ( $31.32 \pm 4.50$ ) T ( $22.03 \pm 4.52$ ).

**PF-3845: Evaluation of CB<sub>2</sub> receptors.** These experiments were conducted to determine if the PF-3845 induced attenuation of ICSS was mediated through CB<sub>2</sub> receptors. ICSS was assessed after SR2 (10.0 mg/kg) co-administered with PF-3845 (30.0 mg/kg) to determine if the CB<sub>2</sub> antagonist/inverse agonist SR2 could block the reduction produced by 30.0 mg/kg PF-3845. Six mice completed testing, and data were analyzed using two-way repeated measure ANOVAs (treatment x frequency) for each dose-combination tested, similar to JZL184 antagonist testing. Administration of VEH and VEH did not affect ICSS rates (Fig. 24a); there was a main effect of

frequency ( $F_{9,45} = 43.20, p < 0.0001$ ), but no main effect of treatment ( $F_{1,5} = 2.13, p > 0.05$ ) and no interaction ( $F_{9,45} = 1.003, p > 0.05$ ). VEH co-administered with 30.0 mg/kg PF-3845 attenuated ICSS from baseline (Fig. 24b), as there was a main effect of frequency ( $F_{9,45} = 27.15, p < 0.0001$ ), treatment ( $F_{1,5} = 15.10, p < 0.05$ ) and an interaction ( $F_{9,45} = 2.41, p < 0.05$ ). Post-hoc analysis revealed reductions at two frequencies which are denoted on Fig. 24b. Similar to VEH, SR2 (10.0 mg/kg) and VEH treatment did not affect self-stimulation rate (Fig. 24c); there was a main effect of frequency ( $F_{9,45} = 30.18, p < 0.0001$ ), but no main effect of treatment ( $F_{1,5} = 1.52, p > 0.05$ ) and no interaction ( $F_{9,45} = 0.48, p > 0.05$ ). Administration of SR2 (10.0 mg/kg) did not block the attenuation produced by 30.0 mg/kg PF-3845, as there was a main effect of frequency ( $F_{9,45} = 22.91, p < 0.0001$ ), no main effect of treatment ( $F_{1,5} = 6.05, p = 0.05$ ), but a significant interaction between treatment and frequency ( $F_{9,45} = 2.11, p < 0.05$ ) (Fig. 24d); post-hoc analysis showed reductions at three frequencies which are denoted on Fig. 24d. Baseline rate-frequency response rates did not differ across conditions tested (Fig. 24e); there was a main effect of frequency ( $F_{9,45} = 33.86, p < 0.0001$ ), but no main effect of baseline day ( $F_{3,15} = 1.14, p > 0.05$ ), and no interaction between baseline day and frequency ( $F_{27,135} = 1.51, p > 0.05$ ).

Percent baseline stimulations data were analyzed with a repeated-measures two-way ANOVA (SR2 vs. VEH x PF-3845 vs. VEH). SR2 (10.0 mg/kg) did not block the attenuation of stimulations produced by 30.0 mg/kg PF-3845 (Fig. 24f). There was no main effect of first treatment (VEH vs. SR2) ( $F_{1,5} = 4.87, p > 0.05$ ), but there was a main effect of second treatment (VEH vs. PF-3845) ( $F_{1,5} = 7.96, p < 0.05$ ) and an interaction between first treatment and second treatment ( $F_{1,5} = 8.46, p < 0.05$ ). Post-hoc analysis revealed that PF-3845-induced attenuation of ICSS was not blocked by the CB<sub>2</sub> antagonist SR2, as ICSS was reduced ( $p < 0.01; p < 0.001$ ) regardless of co-administration with either VEH or SR2 (Fig. 24f). These results confirm the

findings from rate-frequency analysis, that the attenuation of ICSS is not mediated through CB<sub>2</sub> receptors. Interestingly, the attenuation of ICSS produced by PF-3845 (30.0 mg/kg) was not mediated through CB<sub>1</sub> or CB<sub>2</sub> receptors.

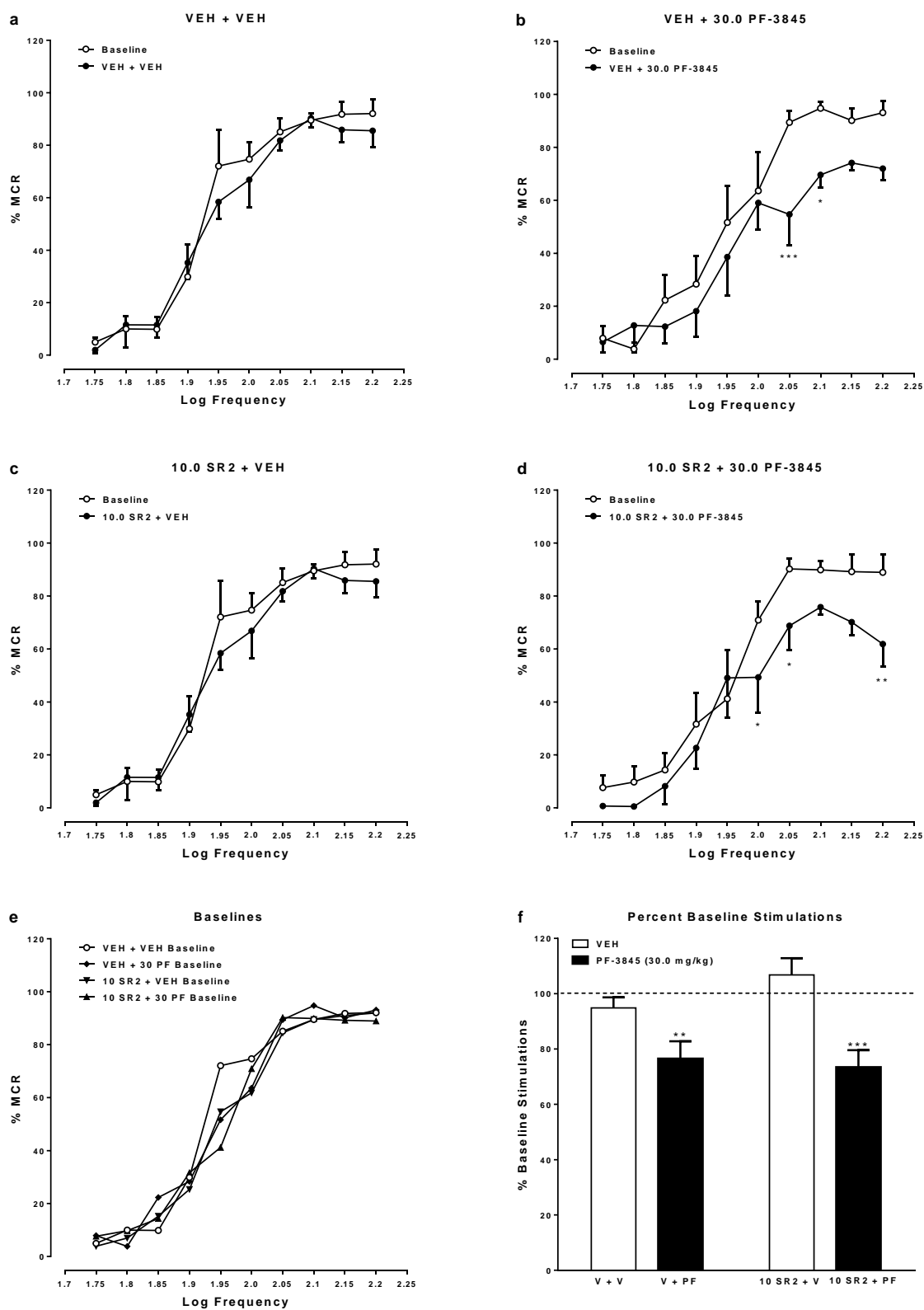


Figure Continues

## Figure Continued

**Figure 24.** PF-3845 CB<sub>2</sub> antagonism assessment. Panels a – d shows rate-frequency data during SR2 antagonism tests with 30.0 mg/kg PF-3845. Panel e shows all baselines rate-frequency curves during CB<sub>2</sub> testing. Percent maximum control rate is displayed on the Y-axis, log-frequency values are shown on the x-axis. Panel f shows percent baseline stimulations ( $\pm$  SEM) for all treatments. Significant interactions were investigated with post-hoc analyses, and differences from baseline (a – e) or other conditions (f) are denoted by asterisks. \*\*\* $p < 0.001$ , \*\* $p < 0.01$ , \* $p < 0.05$ . Raw overall response rates (stimulations/min; mean  $\pm$  SEM) during baseline (B) and test (T) sessions were as follows: (a) VEH + VEH: B ( $37.10 \pm 2.66$ ) T ( $35.20 \pm 2.92$ ); (b) VEH + 30.0 mg/kg PF-3845: B ( $35.12 \pm 3.24$ ) T ( $27.06 \pm 3.57$ ); (c) 10.0 mg/kg SR2 + VEH: B ( $34.10 \pm 2.39$ ) T ( $36.31 \pm 2.92$ ); (d) 10.0 mg/kg SR2 + 30.0 mg/kg PF-3845: B ( $37.02 \pm 3.05$ ) T ( $27.71 \pm 4.33$ ).

## SA-57 ICSS Experiments

**SA-57 dose-response assessment.** This study evaluated the effect of combined inhibition of FAAH and MAGL on ICSS. ICSS was examined after administration of the dual FAAH/MAGL inhibitor SA-57 (1.0, 3.0, 10.0, and 17.8 mg/kg) or VEH. Seven mice completed the study; the rate-frequency response data was analyzed using two-way repeated measures ANOVAs (treatment  $\times$  frequency) for each dose. Low dose SA-57 (1.0 mg/kg) did not affect self-stimulation rate, while 3.0, 10.0, and 17.8 mg/kg SA-57 significantly attenuated ICSS responding. VEH produced a main effect of frequency ( $F_{9,54} = 4.9$ ,  $p < 0.0001$ ), but no main effect of treatment ( $F_{1,6} = 0.05$ ,  $p > 0.05$ ) and no interaction ( $F_{9,54} = 1.12$ ,  $p > 0.05$ ) (Fig. 25a). Similarly, SA-57 (1.0 mg/kg) produced a main effect of frequency ( $F_{9,54} = 48.64$ ,  $p < 0.0001$ ), but no main effect of treatment ( $F_{1,6} = 0.81$ ,  $p > 0.05$ ) and no interaction ( $F_{9,54} = 0.39$ ,  $p > 0.05$ ) (Fig. 25b). As mentioned above, higher doses of SA-57 (3.0, 10.0, and 17.8 mg/kg) reduced ICSS; 3.0 mg/kg produced a main effect of frequency ( $F_{9,54} = 29.95$ ,  $p < 0.0001$ ), treatment ( $F_{1,6} = 40.88$ ,  $p < 0.001$ ) and an interaction ( $F_{9,54} = 2.62$ ,  $p < 0.05$ ) (Fig. 25c). Post-hoc analysis showed reduced stimulations at five frequencies which are denoted on Fig. 25c. SA-57 (10.0 mg/kg) produced a main effect of frequency ( $F_{9,54} = 34.51$ ,  $p < 0.0001$ ), treatment ( $F_{1,6} = 55.49$ ,  $p < 0.001$ ) and an interaction ( $F_{9,54} = 8.17$ ,  $p < 0.0001$ ) (Fig. 25d); post-hoc analysis



showed significant attenuation of ICSS at five frequencies which are denoted on Fig. 25d. SA-57 (17.8 mg/kg) also produced a main effect of frequency ( $F_{9,54} = 40.05, p < 0.0001$ ), treatment ( $F_{1,6} = 170.8, p < 0.0001$ ) and an interaction ( $F_{9,54} = 6.06, p < 0.0001$ ) (Fig. 25e). Post-hoc analysis revealed significant reductions at seven frequencies which are denoted on Fig. 25e.

Baseline rate-frequency curves were analyzed after tests were completed to determine if there were any differences in basal responding; they did not differ as there was a main effect of frequency ( $F_{9,54} = 61.21, p < 0.0001$ ), but no main effect of baseline day ( $F_{4,24} = 0.47, p > 0.05$ ), and no interaction between treatment and baseline day ( $F_{36,216} = 0.81, p > 0.05$ ) (Fig. 25f). The effect of VEH on ICSS was re-assessed after testing and compared to the initial VEH assessment; they did not differ as there was a main effect of frequency ( $F_{9,54} = 56.79, p < 0.0001$ ), but no main effect of treatment ( $F_{1,6} = 0.56, p > 0.05$ ) and no interaction ( $F_{9,54} = 0.84, p > 0.05$ ).

A one-way repeated measures ANOVA was used to analyze percent baseline stimulations data that were calculated for each condition. SA-57 attenuated percent baseline stimulations ( $F_{4,24} = 49.25, p < 0.0001$ ) (Fig. 26); post-hoc analysis revealed that SA-57 (3.0, 10.0, and 17.8 mg/kg) (67.80 %, 35.06 % and 27.61 %) significantly reduced ( $p < 0.001$ ;  $p < 0.0001$ ;  $p < 0.0001$ ) percent baseline stimulations compared to VEH, while low dose SA-57 (1.0 mg/kg) did not affect percent baseline stimulations. The results of the percent baseline stimulations analysis agrees with rate-frequency data, as the same doses of SA-57 (3.0, 10.0, and 17.8 mg/kg) produced attenuation with both measures.

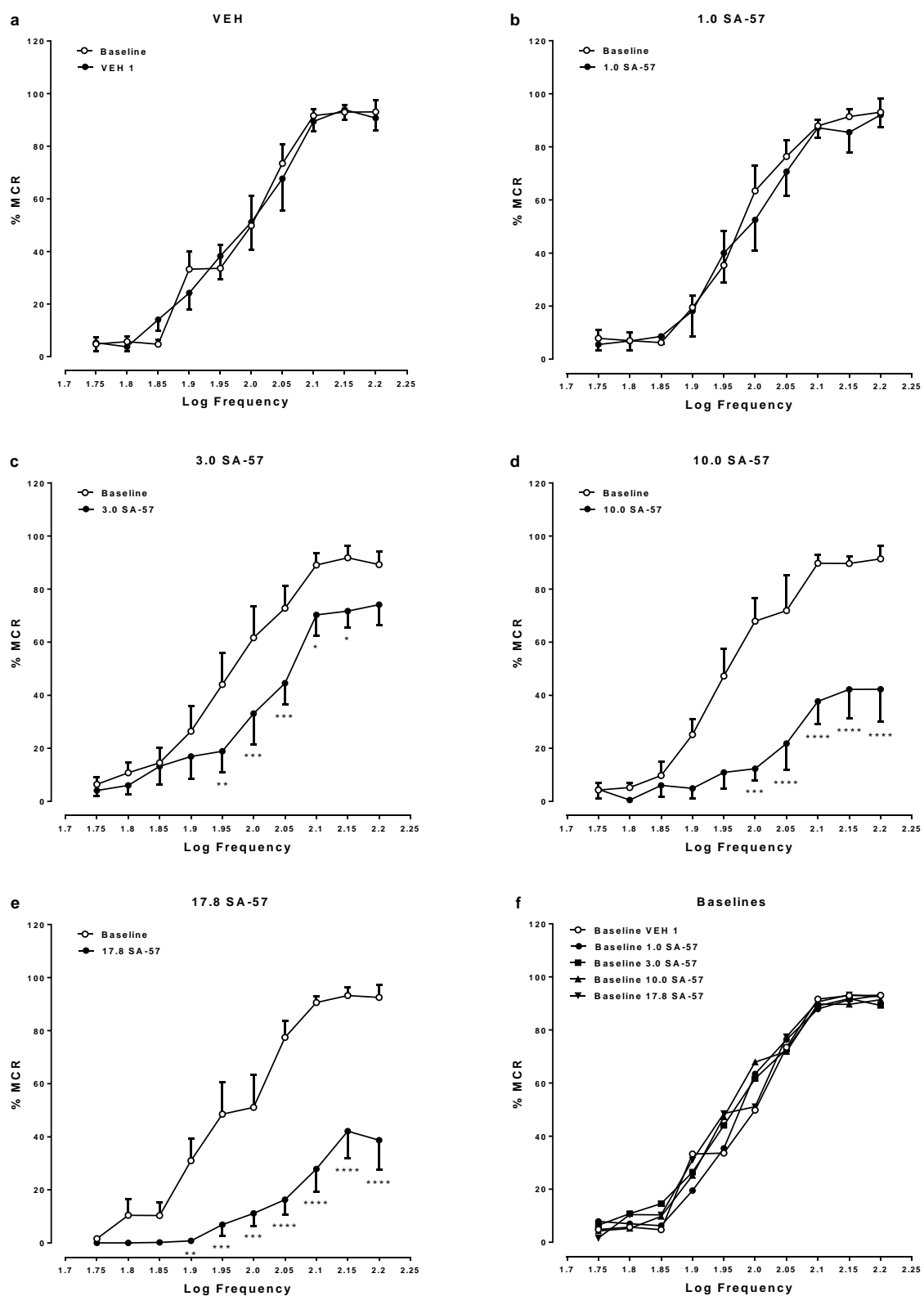
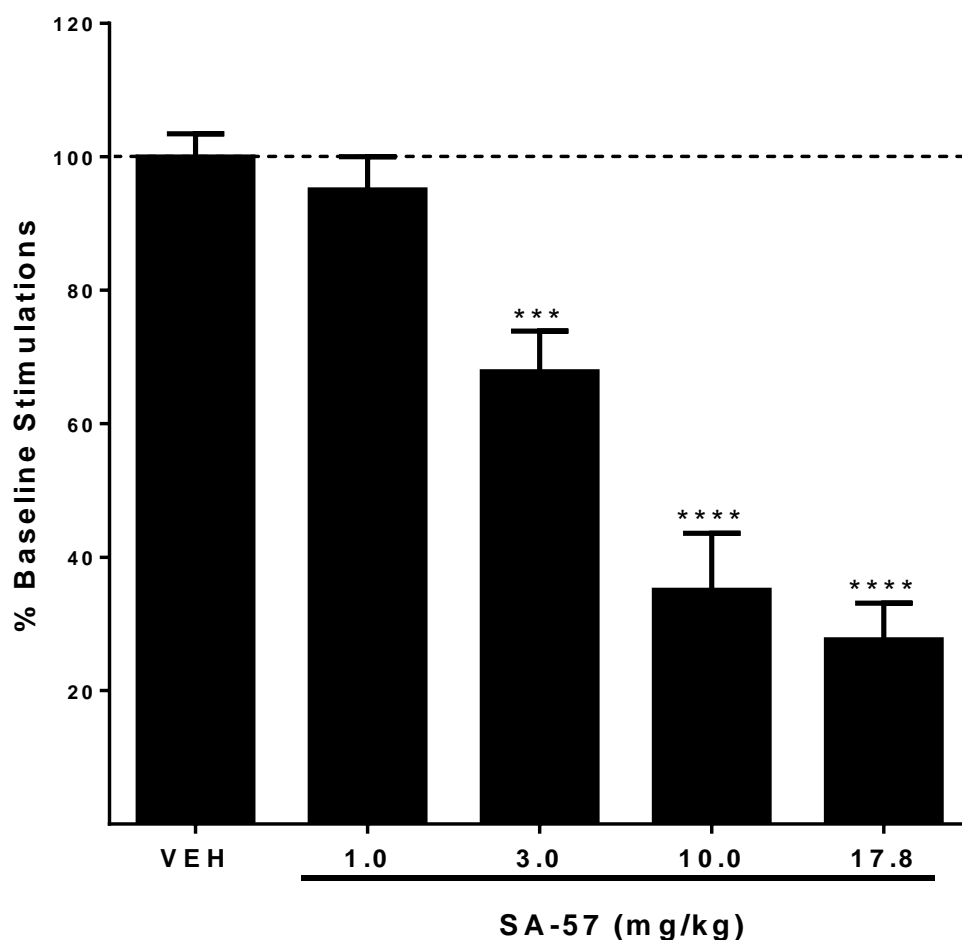


Figure Continues

## Figure Continued

*Figure 25.* SA-57 ICSS dose-response assessment. Panels a – e show rate-frequency stimulation values ( $\pm$  SEM) after VEH or JZL184 (1.0, 4.0, 16.0 and 40.0 mg/kg). Panel f shows all baseline rate-frequency curves during testing. Percent maximum control rate is displayed on the Y-axis, log-frequency values are shown on the x-axis. Interactions that achieved significance were investigated with post-hoc analyses, and differences from baseline are denoted by asterisks.

\*\*\*\* $p < 0.0001$ , \*\*\* $p < 0.001$ , \*\* $p < 0.01$ . Raw overall response rates (stimulations/min; mean  $\pm$  SEM) during baseline (B) and test (T) sessions were as follows: (a) VEH: B ( $32.21 \pm 3.47$ ) T ( $32.44 \pm 3.89$ ); (b) 1.0 mg/kg SA-57: B ( $33.21 \pm 2.85$ ) T ( $31.98 \pm 3.80$ ); (c) 3.0 mg/kg SA-57: B ( $33.45 \pm 3.41$ ) T ( $23.56 \pm 3.81$ ); (d) 10.0 mg/kg SA-57: B ( $32.83 \pm 4.02$ ) T ( $12.19 \pm 3.80$ ); (e) 17.8 mg/kg SA-57: B ( $33.98 \pm 2.98$ ) T ( $9.89 \pm 2.20$ ).



*Figure 26.* SA-57 ICSS percent baseline stimulations. Values represent mean percent baseline stimulations ( $\pm$  SEM) after VEH or SA-57. The one-way ANOVA was significant and post-hoc analysis revealed significant differences from VEH, which are denoted with asterisks. \*\*\*\* $p < 0.0001$ , \*\*\* $p < 0.001$

**SA-57 time-course assessment.** This study assessed the attenuation of ICSS produced by 10.0 mg/kg SA-57 over time. ICSS was assessed after VEH and 10.0 mg/kg SA-57 at 1, 2, 4, 8, 24 and 48 h after injection. Similar to PF-3845 time-course tests, data were analyzed using two-way repeated measures ANOVAs (Treatment x Frequency) to compare rate-frequency responding between VEH and SA-57 at each time-point. SA-57 (10.0 mg/kg) attenuated ICSS at 1, 2, 4 and 8 h after injection and returned to baseline 24 hours after injection. Baseline rate-frequency responding for VEH and SA-57 tests did not differ from each other; there was a main

effect of frequency ( $F_{9,45} = 28.10, p < 0.0001$ ), but no main effect of treatment ( $F_{1,6} = 0.12, p > 0.05$ ) and no interaction ( $F_{9,45} = 0.28, p > 0.05$ ) (Fig. 27a). At the one hour time-point there was a main effect of frequency ( $F_{9,45} = 22.06, p < 0.0001$ ), treatment ( $F_{1,5} = 56.31, p < 0.001$ ) and an interaction ( $F_{9,45} = 4.73, p < 0.001$ ) (Fig. 27b); post-hoc analysis revealed reductions at six frequencies which are denoted on Fig. 27b. Two hours after administration there was a main effect of frequency ( $F_{9,45} = 22.63, p < 0.0001$ ), treatment ( $F_{1,5} = 64.32, p < 0.001$ ) and an interaction ( $F_{9,45} = 7.21, p < 0.0001$ ) (Fig. 27c); post-hoc analysis showed reductions at six frequencies which are denoted on Fig. 27c. Four hours after injection there was a main effect of frequency ( $F_{9,45} = 47.54, p < 0.0001$ ), treatment ( $F_{1,5} = 109.9, p < 0.001$ ) and an interaction ( $F_{9,45} = 3.09, p < 0.01$ ) (Fig. 27d); post-hoc analysis revealed reductions at five frequencies which are denoted on Fig. 27d. Eight hours after administration, there was a main effect of frequency ( $F_{9,45} = 38.47, p < 0.0001$ ) and treatment ( $F_{1,5} = 17.35, p < 0.01$ ), but no interaction ( $F_{9,45} = 1.50, p > 0.05$ ) (Fig. 27e). At the 24 hour time-point 10.0 mg/kg SA-57 stimulations did not differ from VEH; there was a main effect of frequency ( $F_{9,45} = 34.50, p < 0.0001$ ), but no main effect of treatment ( $F_{1,5} = 0.72, p > 0.05$ ) and no interaction ( $F_{9,45} = 0.34, p > 0.05$ ) (Fig. 27f). Similarly, at the 48 hour time-point, there was a main effect of frequency ( $F_{9,45} = 34.69, p < 0.0001$ ), but no main effect of treatment ( $F_{1,5} = 0.003, p > 0.05$ ) and no interaction ( $F_{9,45} = 0.80, p > 0.05$ ) (Fig. 27g).

A repeated-measures two-way ANOVA (Time-point x Treatment) was used to analyze percent baseline stimulations of VEH and 10.0 mg/kg SA-57 across all time-points (Fig. 27h). SA-57 decreased ICSS at several time-points, as there was a main effect of time-point ( $F_{5,25} = 34.73, p < 0.0001$ ), treatment ( $F_{1,5} = 22.06, p < 0.01$ ), and an interaction between time-point and treatment ( $F_{5,25} = 33.30, p < 0.0001$ ). Post-hoc analysis revealed significant attenuation of ICSS

1, 2, 4 and 8 hours after treatment. These data are concordant with results of rate-frequency analysis; the attenuation of ICSS produced by SA-57 (10.0 mg/kg) was apparent 1 h after administration and returned to normal responding within 24 h.

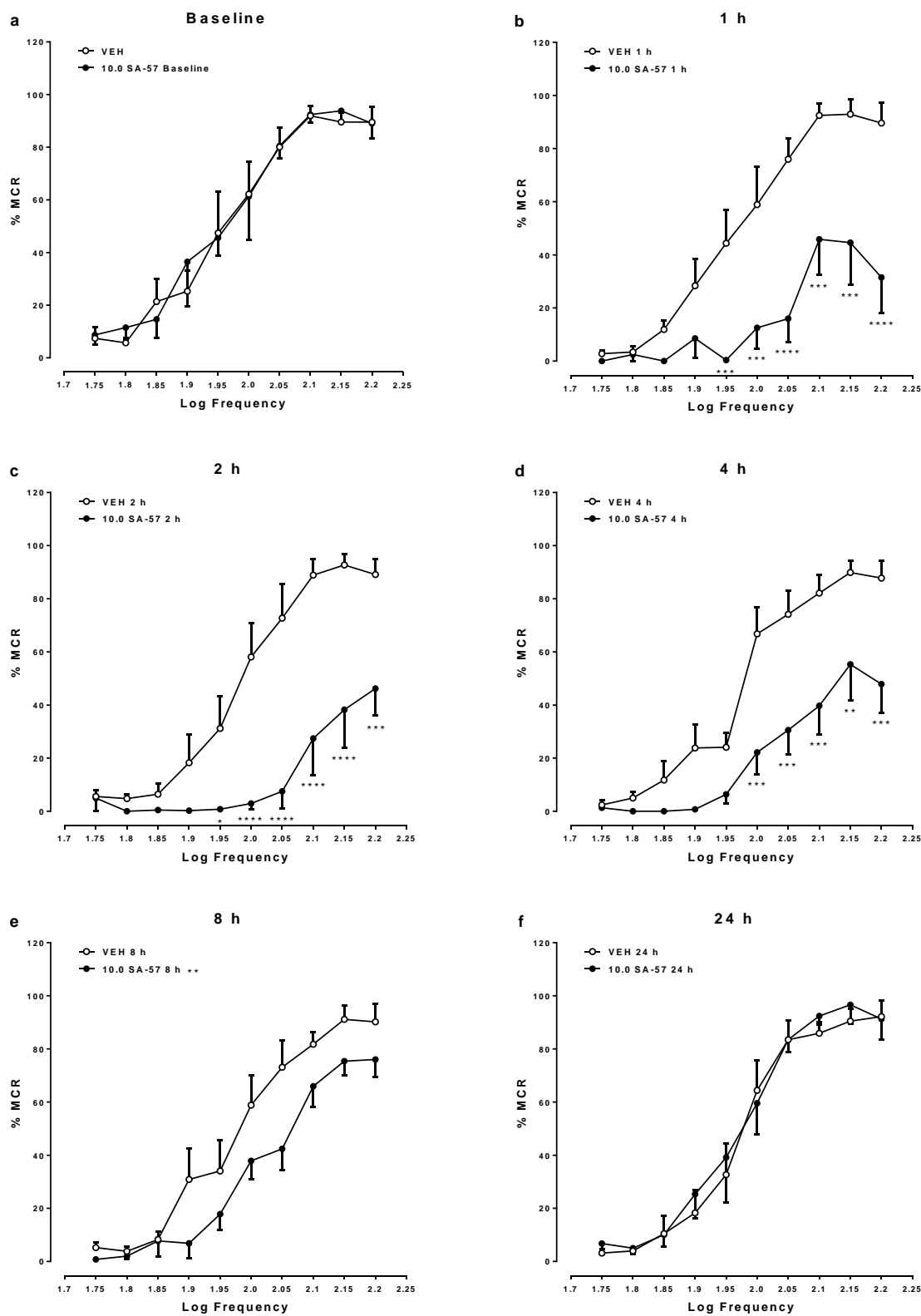
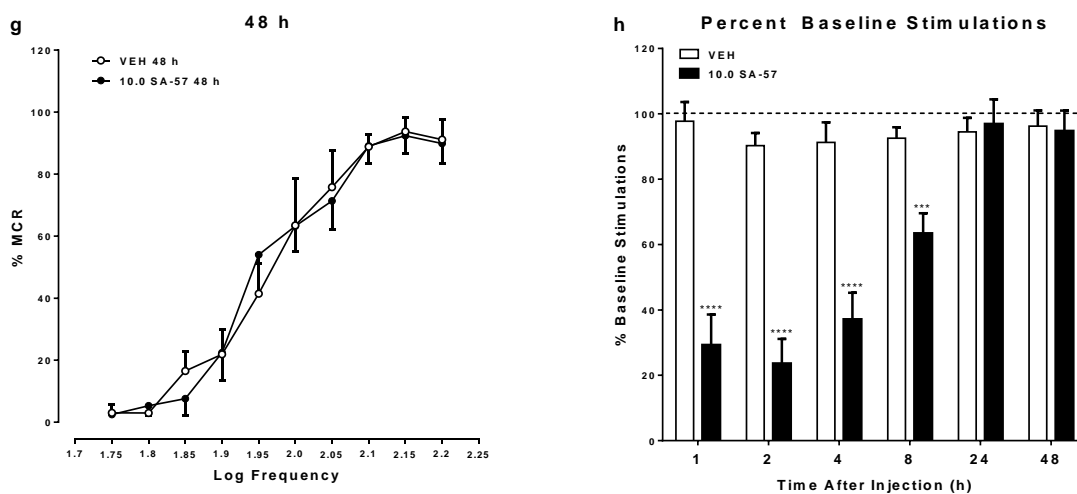


Figure Continues

Figure Continued



**Figure 27.** SA-57 ICSS time-course evaluation. Panels a – g show rate-frequency stimulation values ( $\pm$  SEM) after VEH and SA-57 (10.0 mg/kg) at each time-point (1, 2, 4, 8, 24 and 48 h); percent maximum control rate is displayed on the y-axis, log-frequency values are shown on the x-axis. Panel h shows percent baseline stimulations data over time. Significant main effects of treatment are denoted by asterisks next on the figure legend. Significant interactions were investigated with post-hoc analyses, and differences from VEH are denoted by asterisks. \*\*\*\* $p < 0.0001$ , \*\*\* $p < 0.001$ , \*\* $p < 0.01$ , \* $p < 0.05$ . Raw overall response rates (stimulations/min; mean  $\pm$  SEM) during VEH (V) and 10.0 mg/kg SA-57 (S) conditions were as follows: (a) Baseline: V ( $34.53 \pm 4.98$ ) S ( $35.09 \pm 3.93$ ); (b) 1 h: V ( $32.78 \pm 3.76$ ) S ( $11.13 \pm 4.10$ ); (c) 2 h: V ( $30.93 \pm 4.16$ ) S ( $8.58 \pm 3.11$ ); (d) 4 h: V ( $30.68 \pm 3.70$ ) S ( $13.85 \pm 3.51$ ); (e) 8 h: V ( $31.50 \pm 4.20$ ) S ( $21.78 \pm 2.77$ ); (f) 24 h: V ( $31.76 \pm 3.73$ ) S ( $33.77 \pm 4.65$ ); (g) 48 h: V ( $33.14 \pm 4.96$ ) S ( $32.72 \pm 3.52$ ).

**SA-57: Evaluation of CB<sub>1</sub> receptors.** This experiment was conducted to determine if SA-57 induced attenuation of ICSS can be blocked by the CB<sub>1</sub> antagonist/inverse agonist RIM. Six mice completed the experiments, and data were analyzed using a two-way repeated measures ANOVA (treatment x frequency) for each dose combination tested. Administration of VEH and VEH did not differ from baseline, as there was a main effect of frequency ( $F_{9,63} = 35.71$ ,  $p < 0.0001$ ), but no main effect of treatment ( $F_{1,5} = 0.01$ ,  $p > 0.05$ ), and no interaction ( $F_{9,45} = 0.19$ ,  $p > 0.05$ ) (Fig. 28a). VEH and 10.0 mg/kg SA-57 administration reduced ICSS rates; there was a



main effect of frequency ( $F_{9,45} = 30.82, p < 0.0001$ ), treatment ( $F_{1,5} = 116.60, p < 0.001$ ) and an interaction ( $F_{9,45} = 5.55, p < 0.0001$ ) (Fig. 28b). Post-hoc analysis revealed reductions at six frequencies which are denoted on Fig. 28b. Treatment with 3.0 mg/kg RIM and VEH did not affect baseline ICSS; there was a main effect of frequency ( $F_{9,45} = 28.08, p < 0.0001$ ), but no main effect of treatment ( $F_{1,5} = 0.38, p > 0.05$ ). There was an interaction ( $F_{9,45} = 2.36, p < 0.05$ ), but post-hoc analysis revealed that there were no differences at any frequency (Fig. 28c). RIM (3.0 mg/kg) was not able to block SA-57 induced attenuation of ICSS, as co-administration with 10.0 mg/kg SA-57 reduced ICSS from baseline; there was a main effect of frequency ( $F_{9,45} = 27.37, p < 0.0001$ ), but no main effect of treatment ( $F_{1,5} = 5.01, p > 0.05$ ). However, there was an interaction ( $F_{9,45} = 2.41, p < 0.05$ ) (Fig. 28d); post-hoc analysis showed a reduction at one frequency which is denoted on Fig. 28d. RIM (10.0 mg/kg) co-administered with VEH did not affect baseline ICSS, as there was a main effect of frequency ( $F_{9,45} = 28.04, p < 0.0001$ ), but no main effect of treatment ( $F_{1,5} = 0.37, p > 0.05$ ) and no interaction ( $F_{9,45} = 1.92, p > 0.05$ ) (Fig. 28e). Administration of RIM (10.0 mg/kg) blocked the rate-reducing effects produced by SA-57, as stimulations after co-administration with 10.0 mg/kg SA-57 did not differ from baseline. There was a main effect of frequency ( $F_{9,45} = 21.27, p < 0.0001$ ), but no main effect of treatment ( $F_{1,5} = 0.11, p > 0.05$ ), and no interaction ( $F_{9,45} = 1.76, p > 0.05$ ) (Fig. 28f). Baselines did not differ during any condition tested (Fig. 28g), as there was a main effect of frequency ( $F_{9,45} = 39.17, p < 0.0001$ ), but no main effect of baseline day ( $F_{5,25} = 2.01, p > 0.05$ ) and no interaction between baseline day and frequency ( $F_{45,225} = 1.07, p > 0.05$ ).

A repeated-measures two-way ANOVA (RIM vs. VEH x 10.0 mg/kg SA-57 vs. VEH) was conducted to analyze the percent baseline stimulations data. RIM blocked the attenuation of percent baseline stimulations produced by 10.0 mg/kg SA-57 (Fig. 28h). There was a main effect

of first treatment (VEH vs. RIM) ( $F_{2,10} = 11.40, p < 0.01$ ), second treatment (VEH vs. SA-57) ( $F_{1,5} = 43.68, p < 0.01$ ) and an interaction between first treatment and second treatment ( $F_{2,10} = 25.90, p < 0.05$ ). Post-hoc analysis revealed reductions in percent baseline stimulations after 10.0 mg/kg SA-57, but only when first treated with VEH or 3.0 mg/kg RIM. The reduction in stimulations produced by 10.0 mg/kg SA-57 was blocked by pre-treatment with 10.0 mg/kg RIM, and therefore was mediated through CB<sub>1</sub> receptors. These data confirm the results of rate-frequency analysis, and also coincide with results of JZL184 and THC studies, which were also found to produce effects mediated through CB<sub>1</sub> receptors. Though, they were blocked with 3.0 mg/kg RIM, while the antagonism of SA-57s attenuation required 10.0 mg/kg RIM. It is also noteworthy that high dose RIM (10.0 mg/kg) did not affect basal ICSS.

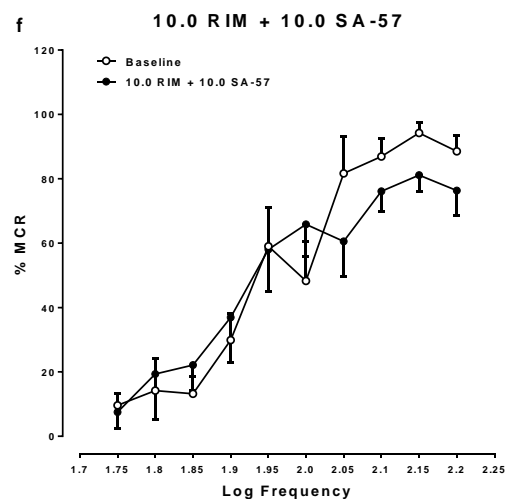
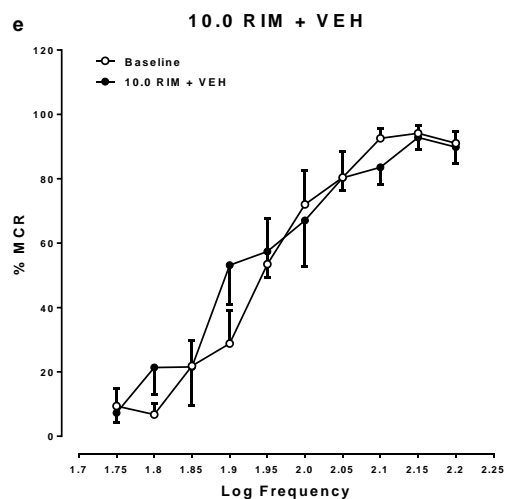
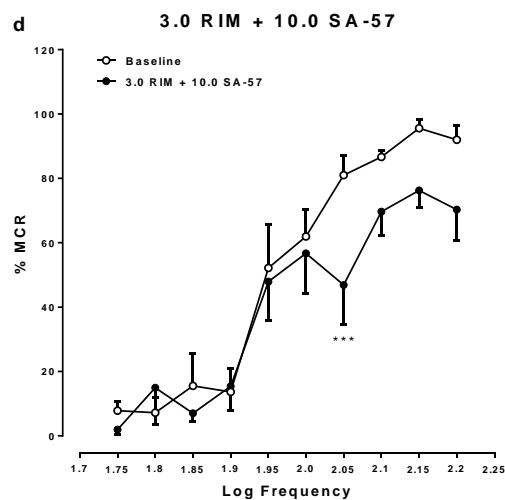
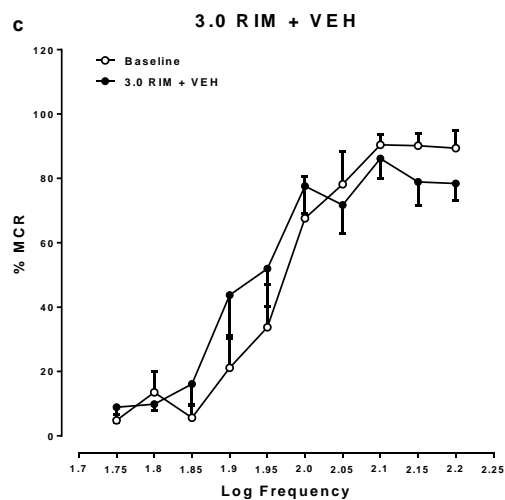
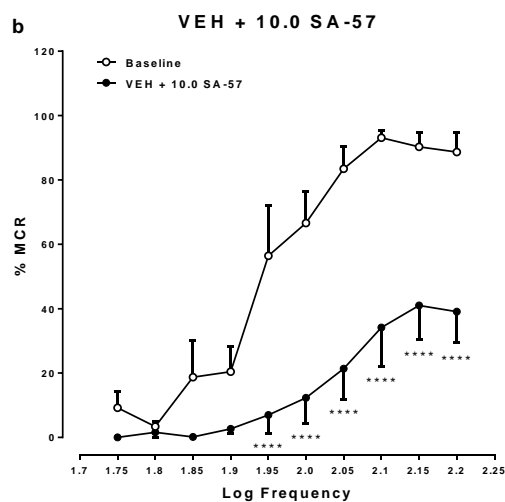
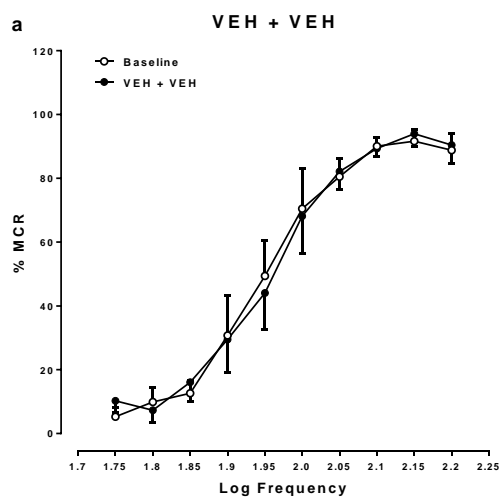
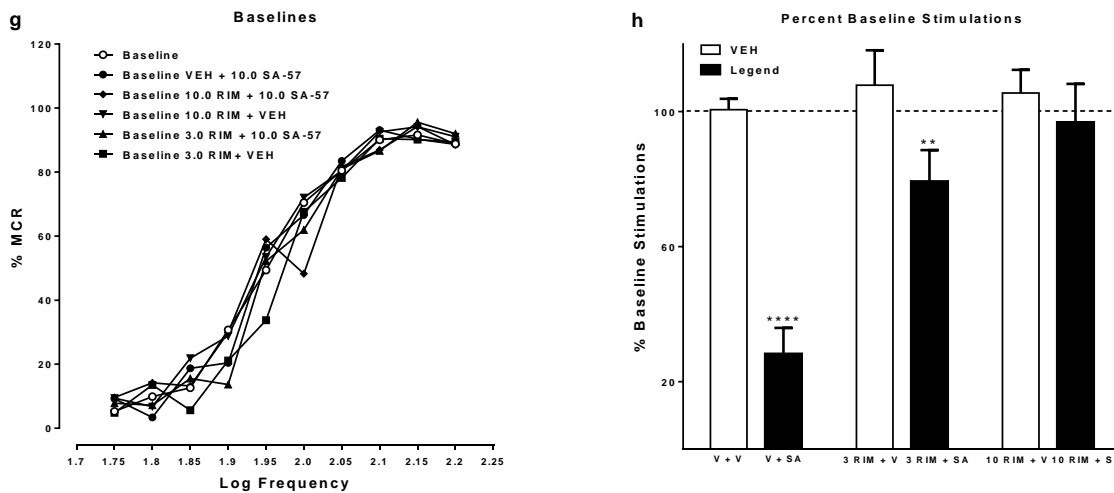


Figure Continues

## Figure Continued



**Figure 28.** SA-57 CB<sub>1</sub> antagonism assessment. Panels a – f shows rate-frequency data during RIM antagonism tests with 10.0 mg/kg SA-57. Panel g shows all baselines rate-frequency curves during CB<sub>1</sub> testing. Percent maximum control rate is displayed on the y-axis, log-frequency values are shown on the x-axis. Panel h shows percent baseline stimulations ( $\pm$  SEM) for all treatments. Significant interactions were followed up by post-hoc analyses, and differences from baseline (a – g) or other conditions (h) are denoted by asterisks. \*\*\*\* $p < 0.0001$ , \*\*\* $p < 0.001$ , \*\* $p < 0.01$ . Raw overall response rates (stimulations/min; mean  $\pm$  SEM) during baseline (B) and test (T) sessions were as follows: (a) VEH + VEH: B ( $34.83 \pm 3.65$ ) T ( $34.88 \pm 3.64$ ); (b) VEH + 10.0 mg/kg SA-57: B ( $36.48 \pm 3.76$ ) T ( $10.97 \pm 3.30$ ); (c) 3.0 mg/kg RIM + VEH: B ( $33.64 \pm 4.43$ ) T ( $35.88 \pm 5.23$ ); (d) 3.0 mg/kg RIM + 10.0 mg/kg SA-57: B ( $34.32 \pm 3.50$ ) T ( $28.11 \pm 5.18$ ); (e) 10.0 mg/kg RIM + VEH: B ( $36.69 \pm 5.45$ ) T ( $38.65 \pm 6.61$ ); (f) 10.0 mg/kg RIM + 10.0 mg/kg SA-57: B ( $35.07 \pm 4.71$ ) T ( $34.02 \pm 6.63$ ).

**SA-57: Evaluation of CB<sub>2</sub> receptors.** This study assessed the involvement of CB<sub>2</sub> receptors in the reduction of ICSS produced by SA-57 using the CB<sub>2</sub> antagonist/inverse agonist SR2 (10.0 mg/kg). Six subjects completed tests, and data were analyzed using two-way repeated measures ANOVAs (treatment x frequency). VEH and VEH administration did not affect ICSS behavior; there was a main effect of frequency ( $F_{9,45} = 32.61$ ,  $p < 0.0001$ ), but no main effect of treatment ( $F_{1,5} = 0.004$ ,  $p > 0.05$ ) and no interaction ( $F_{9,45} = 1.06$ ,  $p > 0.05$ ) (Fig. 29a). Administration of VEH and 10.0 mg/kg SA-57 reduced self-stimulation rate; there was a main effect of frequency ( $F_{9,45} = 46.55$ ,  $p < 0.0001$ ), treatment ( $F_{1,5} = 73.80$ ,  $p < 0.001$ ) and an

interaction ( $F_{9,45} = 5.24, p < 0.0001$ ) (Fig. 29b). Post-hoc analysis showed significant decreases at seven frequencies which are denoted on Fig. 29b. SR2 (10.0 mg/kg) co-administered with VEH did not affect ICSS; there was a main effect of frequency ( $F_{9,45} = 29.76, p < 0.0001$ ), but no main effect of treatment ( $F_{1,5} = 0.05, p > 0.05$ ) and no interaction ( $F_{9,45} = 0.85, p > 0.05$ ) (Fig. 29c). SR2 (10.0 mg/kg) did not block the reduction of ICSS produced by 10.0 mg/kg SA-57, as there was a main effect of frequency ( $F_{9,45} = 30.81, p < 0.0001$ ), treatment ( $F_{1,5} = 84.46, p < 0.001$ ) and an interaction ( $F_{9,45} = 4.84, p < 0.0001$ ) (Fig. 29d). Post-hoc analysis revealed reductions at seven frequencies which are denoted on Fig. 29d. Baseline rate-frequency stimulations did not differ during any condition tested (Fig. 29e); there was a main effect of frequency ( $F_{9,45} = 41.88, p < 0.0001$ ), but no main effect of baseline day ( $F_{3,15} = 1.14, p > 0.05$ ), and no interaction between baseline day and frequency ( $F_{27,135} = 0.98, p > 0.05$ ).

Percent baseline stimulations data were analyzed using a two-way, repeated-measures ANOVA (RIM vs. VEH x 10.0 mg/kg SA-57 vs. VEH). SR2 (10.0 mg/kg) did not block the attenuation of stimulations produced by 10.0 mg/kg SA-57 (Fig. 29f); there was no main effect of first treatment (VEH vs. SR2) ( $F_{1,5} = 1.07, p > 0.05$ ), but there was a main effect of second treatment (VEH vs. SA-57) ( $F_{1,5} = 153.6, p < 0.0001$ ), as SA-57 produced a reduction of ICSS. There was no interaction between first treatment and second treatment ( $F_{1,5} = 1.14, p > 0.05$ ). SA-57 (10.0 mg/kg) reduced ICSS when co-administered with either 10.0 mg/kg SR2 or VEH, indicating that the reduction in stimulations is not mediated through CB<sub>2</sub> receptors. Results are confirmed by rate-frequency analysis, where SR2 (10.0 mg/kg) was unable to block the reduction attenuation produced by SA-57. These findings are similar to those obtained with THC, PF-3845, and JZL184 as none of these drugs produce ICSS modulating effects that are CB<sub>2</sub> receptor dependent.

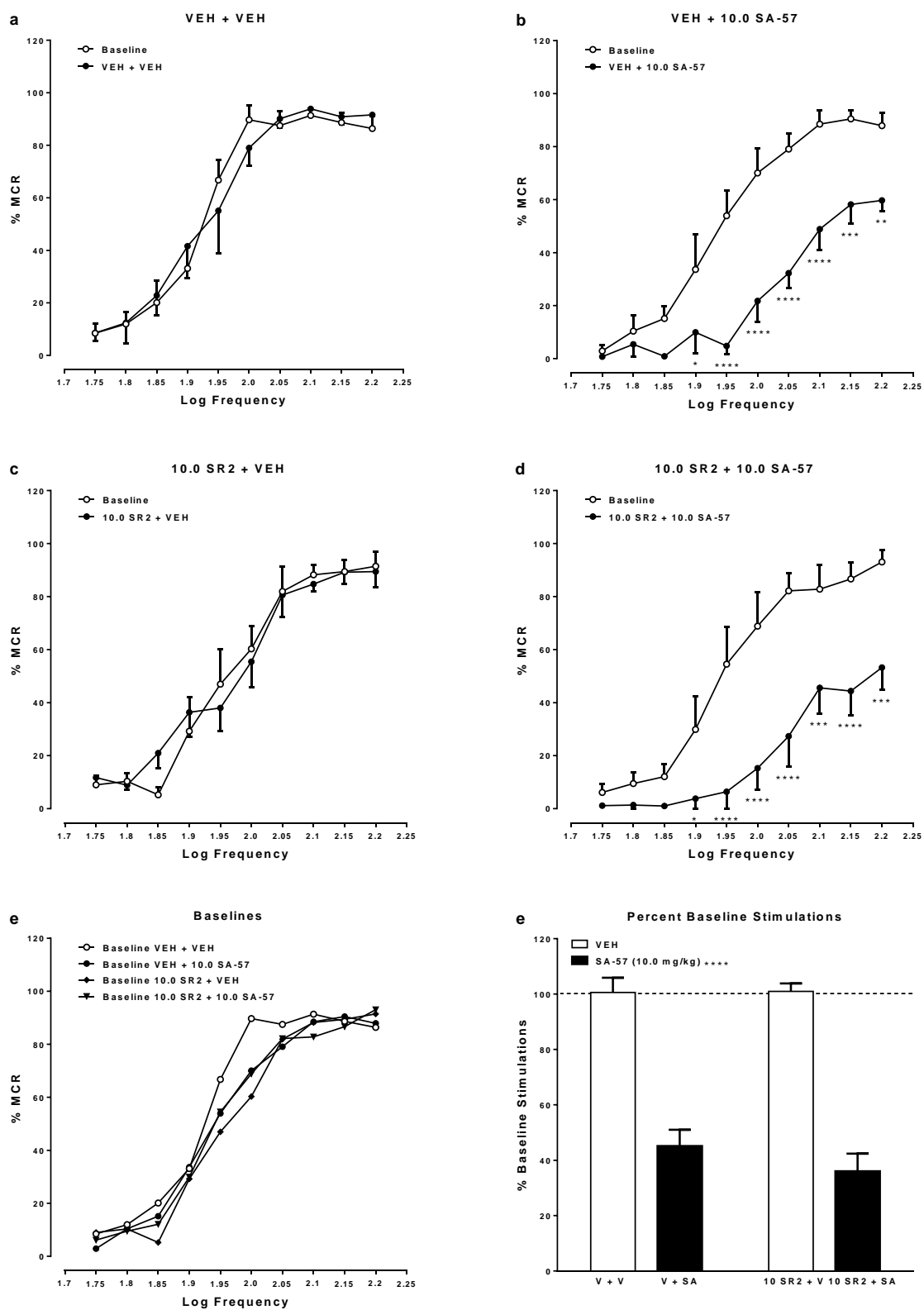


Figure Continues

## Figure Continued

*Figure 29.* SA-57 CB<sub>2</sub> antagonism assessment. Panels a – d shows rate-frequency data during SR2 antagonism tests with 10.0 mg/kg SA-57. Panel e shows all baselines rate-frequency curves during CB<sub>2</sub> testing. Percent maximum control rate is displayed on the y-axis, log-frequency values are shown on the x-axis. Panel f shows percent baseline stimulations ( $\pm$  SEM) for all treatments. Significant main effects were denoted on figure legends. Significant interactions with were investigated with post-hoc analyses, and differences from baseline (a – e) are denoted by asterisks. \*\*\* $p < 0.001$ , \*\* $p < 0.01$ , \* $p < 0.05$ . Raw overall response rates (stimulations/min; mean  $\pm$  SEM) during baseline (B) and test (T) sessions were as follows: (a) VEH + VEH: B (38.23  $\pm$  3.87) T (38.37  $\pm$  3.91); (b) VEH + 10.0 mg/kg SA-57: B (35.89  $\pm$  3.45) T (16.99  $\pm$  3.57); (c) 10.0 mg/kg SR2 + VEH: B (34.09  $\pm$  2.78) T (34.20  $\pm$  2.46); (d) 10.0 mg/kg SR2 + 10.0 mg/kg SA-57: B (34.45  $\pm$  4.37) T (13.63  $\pm$  4.09).

### SA-57 Spontaneous Locomotor Activity Assessment

Spontaneous locomotor activity was assessed after VEH or SA-57 treatment (1.0, 3.0 and 10.0 mg/kg). Distance travelled (m), mean speed (m/s) and time immobile (s) were analyzed using one-way ANOVAs (Fig. 30). SA-57 reduced distance travelled ( $F_{3,32} = 14.78$ ,  $p < 0.0001$ ). Post-hoc analysis revealed that 10.0 mg/kg SA-57 significantly reduced ( $p < 0.0001$ ) distance travelled, while 1.0 and 3.0 mg/kg SA-57 did not differ from VEH (Fig. 30a). Time-immobile increased with SA-57 ( $F_{3,32} = 85.14$ ,  $p < 0.0001$ ). Post-hoc analysis showed that 10.0 mg/kg SA-57 increased ( $p < 0.0001$ ) time-immobile, while 1.0 and 3.0 mg/kg did not produce an effect (Fig. 30b). SA-57 did not affect adjusted speed of locomotion ( $F_{3,32} = 0.33$ ,  $p > 0.05$ ) (Fig. 30c). Similar to ICSS results, 10.0 mg/kg SA-57 reduced locomotor behavior, but in contrast, 3.0 mg/kg SA-57 did not disrupt spontaneous locomotor activity, but reduced ICSS.

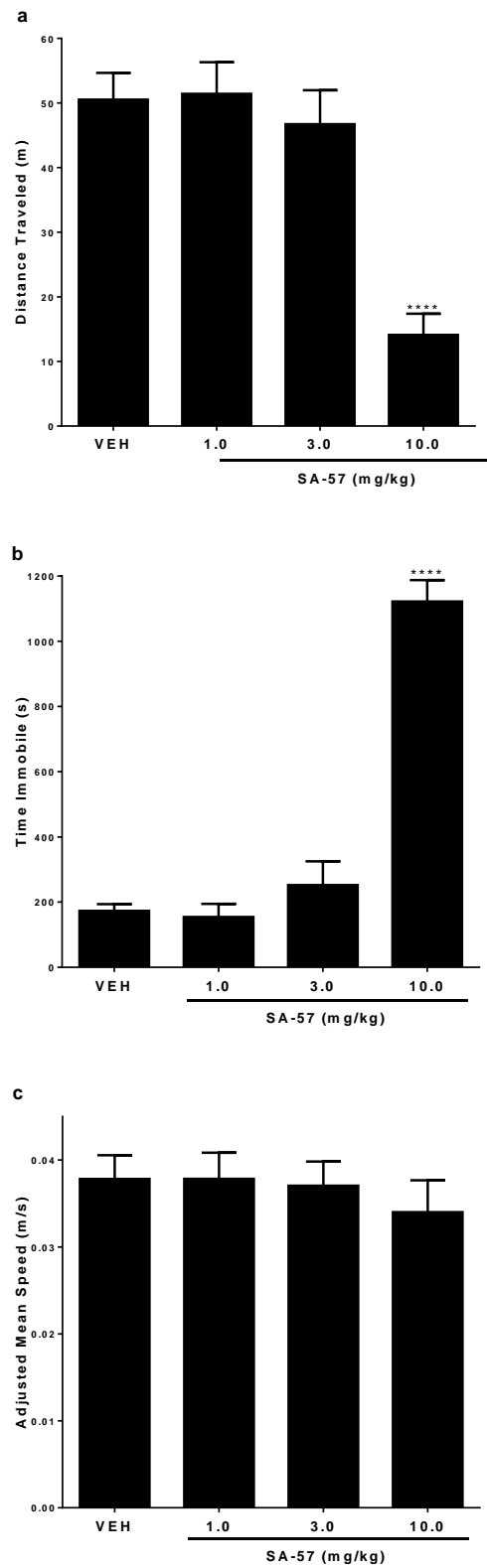


Figure Continues



Figure Continued

*Figure 30.* SA-57 spontaneous locomotor activity. All panels show dose of SA-57 and VEH on the x-axis. Panel a shows distance travelled on the y-axis, panel b shows time-immobile, and panel c shows adjusted speed. Significant one-way ANOVAs were investigated with post-hoc analysis, and significant differences from VEH are denoted by asterisks. \*\*\*\* $p < 0.0001$

### **SA-57 Endocannabinoid Quantification Experiment**

This experiment assessed the ability of SA-57 to alter endocannabinoid levels in specific brain regions. Concentrations of 2-AG, AEA, and AA were determined from samples of PFC, NAc, VMB, amygdala, and cerebellum after treatment with VEH, or SA-57 (1.0 or 10.0 mg/kg). Separate one-way ANOVAs were conducted within each brain region to analyze concentration changes from VEH treatment.

SA-57 increased 2-AG in the PFC ( $F_{2, 12} = 161.4, p < 0.0001$ ); post-hoc analysis showed that treatment with 10.0 mg/kg SA-57 increased ( $p < 0.0001$ ) concentrations of 2-AG to over 16-fold of VEH concentrations, while treatment 1.0 mg/kg did not differ from VEH (Fig. 31a). Administration of SA-57 increased AEA in the PFC ( $F_{2, 12} = 7.07, p < 0.01$ ); post-hoc analysis showed that both 1.0 and 10.0 mg/kg SA-57 increased ( $p < 0.01$ ) AEA to almost 3-fold of VEH levels (Fig. 31b). SA-57 decreased AA concentrations in the PFC ( $F_{2, 12} = 9.36, p < 0.01$ ); post-hoc analysis showed that 10.0 mg/kg SA-57 reduced AA concentrations to 66 % of VEH levels, while 1.0 mg/kg SA-57 did not affect AA levels (Fig. 31c).

Treatment with SA-57 increased 2-AG in the NAc ( $F_{2, 12} = 708.0, p < 0.0001$ ); post-hoc analysis showed that 1.0 mg/kg SA-57 did not differ from VEH, but 10.0 mg/kg SA-57 increased ( $p < 0.0001$ ) 2-AG to over 12-fold VEH concentrations (Fig. 31a). SA-57 increased AEA in the NAc by ( $F_{2, 12} = 7.55, p < 0.01$ ); post-hoc analysis showed that 1.0 and 10.0 mg/kg SA-57 increased ( $p < 0.01; p < 0.05$ ) AEA to over 2-fold VEH concentrations (Fig. 31b).

Administration of SA-57 reduced AA tissue concentration in the NAc; there was a main effect of

SA-57 treatment ( $F_{2,12} = 4.05, p < 0.05$ ), but post-hoc analysis showed that 1.0 and 10.0 mg/kg SA-57 AA levels did not differ from VEH (Fig. 31c).

SA-57 treatment increased 2-AG in the VMB ( $F_{2,12} = 33.41, p < 0.0001$ ); post-hoc analysis showed that 10.0 mg/kg SA-57 increased 2-AG to over 6-fold of VEH concentrations, while 1.0 mg/kg SA-57 did not differ from VEH levels (Fig. 31a). SA-57 increased AEA in the VMB ( $F_{2,12} = 9.49, p < 0.01$ ); both 1.0 and 10.0 mg/kg SA-57 increased ( $p < 0.05$ ;  $p < 0.01$ ) AEA concentrations to 5-fold and 8-fold VEH levels respectively (Fig. 31b). SA-57 did not modulate AA concentrations in the VMB ( $F_{2,12} = 0.01, p > 0.05$ ) (Fig. 31c).

SA-57 increased 2-AG in the amygdala ( $F_{2,12} = 112.00, p < 0.0001$ ); post-hoc analysis showed that 10.0 mg/kg SA-57 increased ( $p < 0.0001$ ) 2-AG to over 11-fold VEH concentrations, while 1.0 mg/kg SA-57 did not affect 2-AG levels (Fig. 31a). Treatment with SA-57 increased AEA in the amygdala ( $F_{2,12} = 3.69, p < 0.01$ ); post-hoc analysis showed that low dose SA-57 (1.0 mg/kg) increased ( $p < 0.01$ ) AEA to over 3-fold VEH levels, while the high dose SA-57 (10.0 mg/kg) did not affect AEA concentrations (Fig. 31b). SA-57 reduced AA tissue concentrations in the amygdala ( $F_{2,12} = 32.71, p < 0.0001$ ); post-hoc analysis showed that low dose SA-57 (1.0 mg/kg) did not affect AA, while high dose SA-57 (10.0 mg/kg) reduced ( $p < 0.0001$ ) AA concentrations to 46 % of VEH levels (Fig. 31c).

Administration of SA-57 increased 2-AG in the cerebellum after treatment with SA-57 ( $F_{2,12} = 165.4, p < 0.0001$ ); post-hoc analysis showed that high dose SA-57 (10.0 mg/kg) increased 2-AG increased ( $p < 0.0001$ ) to over 4-fold VEH concentrations. In contrast, low dose SA-57 (1.0 mg/kg) 2-AG levels did not differ from VEH (Fig. 31a). SA-57 produced an increase of AEA in the cerebellum ( $F_{2,12} = 27.30, p < 0.0001$ ); post-hoc analysis showed that both 1.0 and 10.0 mg/kg SA-57 increased ( $p < 0.001$ ;  $p < 0.0001$ ) AEA concentrations to over 5-fold and 6-

fold VEH levels respectively (Fig. 31b). SA-57 treatment decreased AA in the cerebellum ( $F_{2,12} = 32.15, p < 0.0001$ ); post-hoc analysis showed that high dose SA-57 (10.0 mg/kg) significantly reduced ( $p < 0.0001$ ) AA to 57 % of VEH levels, while low dose SA-57 (1.0 mg/kg) did not affect AA concentrations (Fig. 31c).

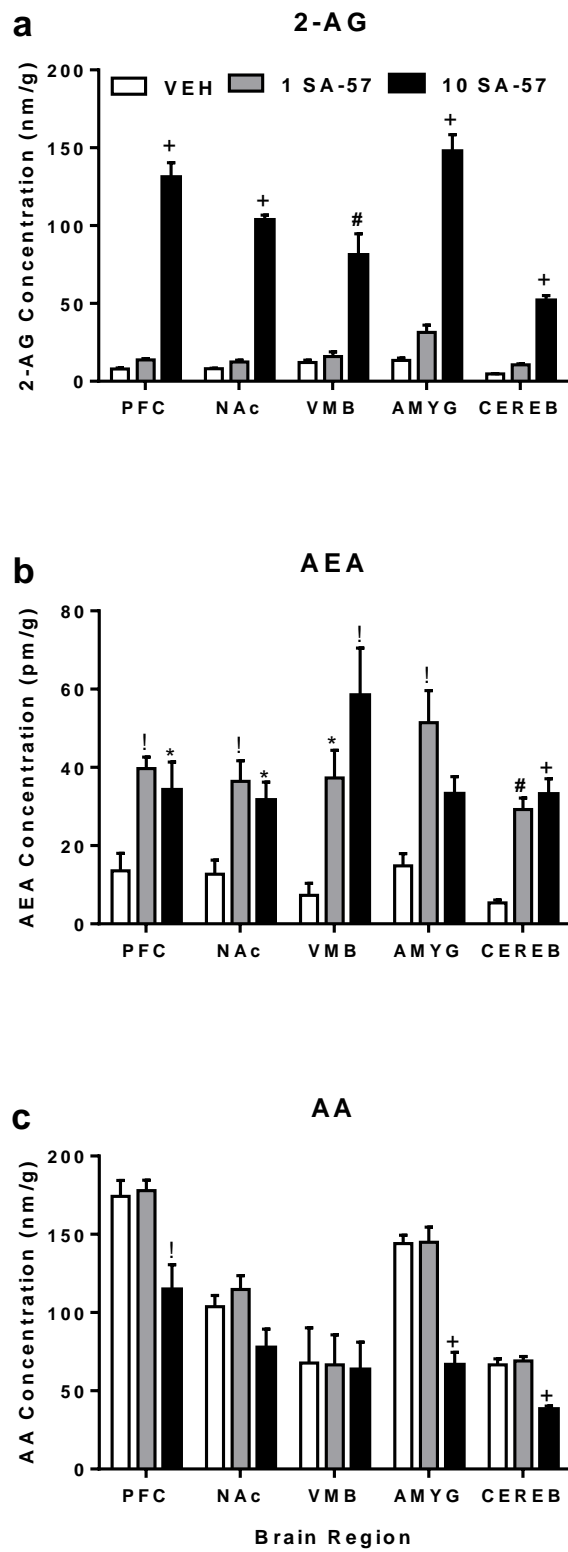


Figure Continues

## Figure Continued

*Figure 31.* SA-57 endocannabinoid and AA concentrations. All panels show brain region on the x-axis (AMYG = amygdala, CEREB = cerebellum). Panel a shows 2-AG levels on the y-axis, panel b shows AEA, and panel c displays AA. One-way ANOVAs were calculated within each brain region and substrate measured. Significant one-way ANOVAs were investigated with post-hoc analyses, and significant differences from corresponding VEH treatment are denoted by symbols. <sup>+</sup>  $p < 0.0001$ , <sup>#</sup>  $p < 0.001$ , <sup>!</sup>  $p < 0.01$ , \* $p < 0.05$

## Discussion

These studies were conducted to evaluate the effects of the exogenous agonist THC, and catabolic enzyme inhibitors of the endocannabinoids AEA and 2-AG on ICSS and other behaviors. One of the goals of this study was to determine the impact of THC on operant responding for brain stimulation reward. In general, pharmacological treatments that reduce the amount of electrical stimulation needed to maintain reinforcement are interpreted as reducing brain reward thresholds, or potentiating reinforcement produced by brain stimulation (R. A. Wise, 2005). Conversely, treatments that reduce ICSS, or increase the amount of stimulation needed to maintain reinforcement are interpreted as increasing reward thresholds or producing anhedonia, or general motor impairment (Cryan, et al., 2003; Markou & Koob, 1991). The goal of these studies is to test whether exogenous and endogenous cannabinoids alter reward motivated behaviors mediated through the mesolimbic system. In these studies, we utilized JZL184 to inhibit MAGL, PF-3845 to inhibit FAAH, and SA-57 the dual FAAH/MAGL inhibitor in order to discern the effects of 2-AG, AEA, and the combination, respectively. To our knowledge, none of these drugs have been tested with ICSS in mice, and PF-3845, JZL184, and SA-57 have not been tested in any species.

## **Cocaine**

Cocaine (5.0 and 10.0 mg/kg) produced a dose-related increase of ICSS, or leftward shift in the frequency response curve, indicating decreased reward thresholds, or increased brain-reward function. These studies were conducted because cocaine reliably increases mouse ICSS behavior, indicative of enhanced brain-reward function. Thus, cocaine was employed as a positive control for this study (Fish, et al., 2012; Robinson, et al., 2012). These data show that psychomotor stimulants potentiate ICSS in the C57BL/6 mouse, as cocaine produced a robust facilitation of ICSS.

Table. 2

*Summary of THC and Enzyme Inhibitor Results*

Results	ICSS	% Baseline		ICSS	Receptor Mediation		Operant (food)	Spontaneous
Drug		R-F	Stimulations	Time-course	CB <sub>1</sub>	CB <sub>2</sub>	Responding	locomotor Activity
<b>THC (s.c.)</b>		↓	↓	↓	<b>YES</b>	<b>NO</b>	↓	N.T.
<b>Doses (mg/kg):</b>	(3, 5.6 & 10)		(5.6 & 10)	(10)	(10)	(10)	(10 & 20)	
<b>Antagonist Doses:</b>				<b>R-F = 5 min – 8 h</b> <b>% baseline = .5 – 8 h</b>	(3.0 RIM)	(3.0 SR2)		
<b>JZL184 (i.p.)</b>		↓	↓	↓	<b>YES</b>	<b>NO</b>	↓	↓
<b>Doses (mg/kg):</b>	(16 & 40)		(16 & 40)	(40)	(40)	(40)	(40)	(40)
<b>Antagonist Doses:</b>				<b>R-F = 1 – 24 h</b> <b>% baseline = 1 – 24 h</b>	(3.0 RIM)	(10.0 SR2)		(16: immobility only)
<b>PF-3845 (i.p.)</b>		↓	↓	↓	<b>NO</b>	<b>NO</b>	N.T.	N.T.
<b>Doses (mg/kg):</b>	(30)		(30)	(30)	(30)	(30)		
<b>Antagonist Doses:</b>				<b>R-F = 1 – 24 h</b> <b>% baseline = 2 – 24 h</b>	(10.0 RIM)	(10.0 SR2)		
<b>SA-57 (i.p.)</b>		↓	↓	↓	<b>YES</b>	<b>NO</b>	N.T.	↓
<b>Doses (mg/kg):</b>	(3, 10 & 17.8)		(3, 10 & 17.8)	(10)	(10)	(10)		(10)
<b>Antagonist Doses:</b>				<b>R-F = 1 – 8 h</b> <b>% baseline = 1 – 8 h</b>	(10.0 RIM)	(10.0 SR2)		
<b>JZL184</b>	<b>2-AG</b>	<b>AEA</b>	<b>AA</b>	<b>SA-57</b>	<b>2-AG</b>	<b>AEA</b>	<b>AA</b>	
<b>Brain Region: PFC:</b>	↑(40)	↓(40)	↓(40)	<b>PFC:</b>	↑(10)	↑(1&10)	↓(10)	
<b>NAc:</b>	↑(4&40)	↓(40)	—	<b>NAc:</b>	↑(10)	↑(1&10)	—	
<b>VMB:</b>	↑(4&40)	—	↓(40)	<b>VMB:</b>	↑(10)	↑(1&10)	—	
<b>Amygdala:</b>	↑(4&40)	—	↓(40)	<b>Amygdala:</b>	↑(10)	↑(1)	↓(10)	
<b>Cerebellum:</b>	↑(4&40)	—	↓(40)	<b>Cerebellum:</b>	↑(10)	↑(1&10)	↓(10)	

## **CB<sub>1</sub> and CB<sub>2</sub> Antagonists**

One intriguing finding of this study was that the CB<sub>1</sub> antagonist, RIM (3.0 and 10.0 mg/kg), did not produce a disruption of ICSS in C57BL/6 mice as shown on Table 2. This may be a species-specific effect, as all other studies that have reported disruption of ICSS with CB<sub>1</sub> antagonists assessed rats. Some rat studies have reported decreases in baseline ICSS after moderate doses of RIM including 1.0 and 3.0 mg/kg (De Vry, et al., 2004; Deroche-Gamonet, et al., 2001; Trujillo-Pisanty, et al., 2011) while others don't report disruption of ICSS until higher doses of 10.0 mg/kg RIM (Xi, et al., 2008) or higher (Arnold, et al., 2001). Specifically, Arnold and colleagues observed that doses up to 10.0 mg/kg RIM did not disrupt ICSS in Lewis rats (Arnold, et al., 2001), so our findings in mice are not unprecedented. It is well-accepted that CB<sub>1</sub> antagonists disrupt ICSS behavior, and it has been cited as indirect evidence that cannabinoids can enhance dopamine levels in the mesolimbic system (Tanda & Goldberg, 2003). The inconsistency of RIM effects in this assay raises questions about how endocannabinoids function in the mesolimbic system. The present data indicate that C57BL/6 mice may not be sensitive to ICSS-facilitating effects of cannabinoids, since they were not affected by high doses of RIM. Interestingly though, the only other study that has shown no effect on ICSS with a dose of 10.0 mg/kg RIM was conducted in Lewis rats, which is a strain that has produced facilitation of ICSS with THC (Arnold, et al., 2001; Gardner, et al., 1988; Lepore, et al., 1996). The lack of rate-decreasing effects with CB<sub>1</sub> antagonism does not necessarily mean that agonists would be precluded from producing the opposite effect. Thus, C57BL/6 mice may still show facilitating effects of ICSS with cannabinoid agonists. Similar to the CB<sub>1</sub> antagonist RIM, the CB<sub>2</sub> antagonist SR2 (3.0 and 10.0 mg/kg) did not produce an effect on ICSS alone. We did not expect SR2 to reduce ICSS when administered alone, but given recent reports about the role of CB<sub>2</sub> in



drug reward, it was interesting to confirm that it had no effect (Ignatowska-Jankowska, Muldoon, et al., 2013; Xi, et al., 2011). This is a novel finding, as CB<sub>2</sub> antagonists have not been assessed in ICSS in any species to our knowledge.

## **THC**

As summarized on Table 2, THC produced a dose-related attenuation of ICSS behavior or a rightward shift in the frequency-response curve. Two potential interpretations of this finding are that THC reduced brain reward functioning, or simply reduced behavior through a non-mesolimbic mediated, or non-specific motor effect. Although we hypothesized that THC would facilitate ICSS in the mouse, there is much evidence to the contrary reported in the rat (see table 1). In fact, the majority of studies that reported low doses of THC facilitating ICSS used a specific strain of rat (Lewis), so it is likely a strain-specific effect (Gardner, et al., 1988; Lepore, et al., 1996). Similar to many studies discussed in the introduction, low doses of THC (0.3 and 1.0 mg/kg) did not affect ICSS. The most recent study with THC and ICSS showed that only low-dose THC (0.1 mg/kg) produced facilitating effects, while 1.0 mg/kg THC reduced responding in Sprague-Dawley rats (Katsidoni, et al., 2013). We did not assess any doses lower than 0.3 mg/kg THC, as there was no effect with 1.0 or 0.3 mg/kg THC. Lower doses may have potentially facilitated ICSS in the C57BL/6J mouse, but we assessed a 10-fold range of ineffective doses, and there was no trend toward increasing ICSS with the lowest dose of THC (0.3 mg/kg) ( $p = 0.08$ ). If anything there was a trend toward decreasing responding as shown on Fig. 4d. Additionally, time-course evaluations with low doses of THC may have unmasked facilitating effects. In the present study, we elected to focus on the ICSS decreasing effects of THC, as they were the prominent effects observed during initial dose-response tests. The

attenuation of ICSS produced by 10.0 mg/kg returned to baseline between 8 and 24 hours after administration.

It is important to note that the 3 studies that show ICSS is facilitated by THC analyzed changes in “threshold,” which typically uses linear regression to determine calculate the frequency which maintains a given level of responding (e.g. 0%, 50%, etc.). Threshold analysis is a common way to analyze ICSS data; the methods used to determine thresholds and changes in thresholds however, vary greatly between laboratories. ICSS rate-frequency response curves are typically curvilinear or sigmoidal shaped. Thus, by using linear regression to determine lines, some of the data is usually left out of analysis to create a straight slope. In addition to removing data from analysis, some investigators also “interpolate” data points, or add data when data doesn’t follow a linear trend. These practices make can make the data analysis process more subjective than traditional statistical measures such as ANOVA. Also, because of the wide range of response patterns shown between subjects and tests, exhaustive lists of “rules” for inclusion and exclusion of data must be created by experimenter. These rules are almost never reported in the methods section of papers, making it very difficult to infer how the experimental values were actually calculated. Because of all of these potential issues with analyzing ICSS thresholds, we chose to analyze data using standard repeated-measures, two-way (frequency x treatment) ANOVAs. By doing so, we were able to analyze all data without altering or removing any information, thereby limiting subjectivity. We also calculated percent baseline stimulations, which similar to threshold determinations is a summation measure, but more objective.

Although there is supporting biochemical evidence that exogenous cannabinoids increase both tonic and phasic dopamine in the nucleus accumbens, it is important to note that many of those reports used synthetic agonists such as WIN 55,212-2 (Cheer, et al., 2004; Cheer, Wassum,

et al., 2007), although some showed a similar effect with THC (J. Chen, et al., 1990; J. P. Chen, et al., 1991). It is important to note also that nearly all of those studies examined cannabinoid agonists that were administered i.v., as route of administration can have a considerable influence on drug effects. The i.v. route of administration may provide the most relevant data to human cannabinoid use, as humans typically inhale marijuana. Both inhalation and i.v. administration allow drugs reach the brain much more quickly, and with less early metabolism than other routes, which has been associated with higher abuse and dependence liability. It remains to be determined whether i.v. administration of THC could potentially produce a facilitation of ICSS in C57BL/6 mice. Additionally, although THC and other cannabinoid agonists produce increases in dopamine levels in the nucleus accumbens, the magnitude of the increase is not nearly as large as that produced by cocaine, or other psychomotor stimulants. THC can produce increases in extracellular dopamine in the nucleus accumbens up to about 150 % of baseline dopamine (Tanda, et al., 1997). In contrast, cocaine has been shown to increase dopamine levels to over 400% of basal levels (Panos & Baker, 2010). Furthermore, stimuli other than drugs of abuse can also produce increases in dopamine in the nucleus accumbens. For example, studies have shown that an aversive tail-pinch can produce increases in dopamine measured by *in vivo* voltammetry (D'Angio, Serrano, Rivy, & Scatton, 1987). This suggests that dopamine release in the nucleus accumbens may be related to salient stimuli in general, rather than being specific to drugs of abuse. This is important to take into consideration when evaluating data on cannabinoid agonist-induced increases in extracellular dopamine.

In the present study, THC-induced reduction of ICSS was found to be mediated through CB<sub>1</sub> receptors, as it was blocked with RIM (3.0 mg/kg) pre-treatment. This effect has been established in rat studies using ICSS, as rightward shifts of rate-frequency response curves

produced by THC were blocked by pre-treatment with RIM (Kwilasz & Negus, 2012; Vlachou, et al., 2007). Even though we found that CB<sub>1</sub> antagonism blocked THC-induced reduction of ICSS, we wanted to rule out the involvement of CB<sub>2</sub> receptors. As expected, CB<sub>2</sub> blockade did not prevent the attenuation of ICSS produced by THC. Although CB<sub>2</sub> receptors have recently been implicated in cocaine reinforcement and nicotine reward (Ignatowska-Jankowska, Muldoon, et al., 2013; Xi, et al., 2011), they do not appear to be involved in the reduction of self-stimulations produced by THC.

The rate-reducing effects produced by THC were not specific to ICSS alone; we found that high dose THC (10.0 mg/kg) also reduced operant responding for food pellets, while 5.0 mg/kg did not affect response rates. These results may seem incongruent with studies in the literature, as cannabinoid agonists have been shown to increase food intake (Wiley et al., 2005). But it is important to note that food intake is a different measure than performing an operant response for food pellets. The latter requires a trained behavior that has a large motor component, while the former requires a less complex, more naturalistic feeding behavior. The reduction of nose-poking to receive food-pellets may represent more of an overall motor effect than a feeding-specific effect. Similar to these effects seen on ICSS and food-maintained operant responding, THC also reduced spontaneous locomotor activity. A recent study in rats reported that 1.0 mg/kg THC reduced locomotor activity, while a lower dose (0.1 mg/kg) increased locomotor activity (Katsidoni, et al., 2013). Many other studies in mice have reported decreases in locomotor activity with 3.0 mg/kg or higher doses of THC (Kinsey, O'Neal, et al., 2011; L. E. Long et al., 2010; Varvel et al., 2006; Wiley et al., 2013). This decrease in overall activity produced by cannabinoids makes interpretation of the ICSS effects presented here difficult.

## **Cannabinoid Catabolic Enzyme Inhibitors**

**JZL184.** The MAGL inhibitor JZL184 (16.0 and 40.0 mg/kg) produced a decrease in ICSS responding, or a rightward shift of the rate-frequency curve, which might be interpreted as a decrease in brain-reward function in the mesolimbic system. These results are similar to what we and others have reported with THC and other cannabinoid agonists, but with a lesser magnitude of reduction. This finding is also concurrent with the one study in the ICSS literature that has assessed a 2-AG modulating drug. Vlachou and colleagues have shown the purported AEA transport inhibitor AM-404 reduces ICSS responding in rats through a CB<sub>1</sub> dependent mechanism (Vlachou, et al., 2008). The relevance of this finding to the present study is related to findings by Wiskerke and colleagues, who have reported that AM-404 (1.0 mg/kg) causes increases of extracellular 2-AG in the nucleus accumbens shell of rats, while not affecting AEA levels (Wiskerke, et al., 2012). AM-404 may be attenuating ICSS via increased 2-AG in brain-reward relevant regions (e.g. NAc) which converges with our JZL184 data in ICSS. The MAGL inhibitor JZL184 also affects ICSS through a similar mechanism. Similar to our data with THC, the attenuation of ICSS produced by JZL184 was a CB<sub>1</sub> dependent effect, and it was not affected by CB<sub>2</sub> antagonism (Vlachou, et al., 2006; Vlachou, et al., 2008). The time-course of JZL184-induced suppression of ICSS behavior lasted up to 48 h after treatment, which was longer than THC-induced attenuation of ICSS, though the reduction of responding was not as drastic. This finding is consistent with the fact that JZL184 is an irreversible MAGL inhibitor that can produce increases in brain 2-AG that last up to 24 h (J. Z. Long, Li, et al., 2009).

In addition to attenuating ICSS, JZL184 (40.0 mg/kg) reduced operant responding for food and decreased spontaneous locomotor activity. As mentioned above, the reduction in nose-poke behavior is probably not reflective of a decrease in motivation for feeding, but more of a

general motor effect. Studies that have shown increases in food-intake with cannabinoids have not assessed feeding via an operant response (Wiley, et al., 2005). The reduction in operant responding for food by JZL184 (40.0 mg/kg) was of a similar magnitude ( $\approx 50\%$ ) to that produced by THC (10.0 mg/kg).

**PF-3845.** The FAAH inhibitor PF-3845 reduced ICSS responding, but only at a very high dose (30.0 mg/kg). It is important to note that no other studies in the literature have assessed a dose of 30.0 mg/kg PF-3845. The results were followed up with a time-course study, where we found it produced a moderate attenuation of ICSS that persisted until between 24 and 48 h after treatment. The effect was recapitulated during antagonism tests, where we found that PF-3845-induced reduction of ICSS was not mediated through CB<sub>1</sub> or CB<sub>2</sub> receptors. It was interesting, as the magnitude of effect was near that produced by JZL184, though not mediated through cannabinoid receptors. There are a few possible explanations for the observed effects of PF-3845. This high dose of PF-3845 could simply be producing a non-specific effect, given the fact that 30.0 mg/kg is a 3-fold higher dose than the highest reported in the literature (Ahn, et al., 2009; Booker, et al., 2012; Ramesh et al., 2011; Wiskerke, et al., 2012). PF-3845 (30.0 mg/kg) could also be producing its effects through a substrate other than AEA. FAAH is implicated in the metabolism of fatty acid amides other than AEA, including PEA, and OEA. It has been shown that AEA, OEA, PEA, and other fatty acid amides have been shown to bind to peroxisome proliferator-activated alpha (PPAR $\alpha$ ) receptors, at TRPV1 receptors and other non-cannabinoid receptors (Jhaveri, et al., 2008; Ross, 2003; Zygmunt, et al., 1999). These receptors are also found throughout brain regions in the mesolimbic system (Luchicchi et al., 2010; Melis et al., 2010; Melis, et al., 2006). We will be pursuing this line of research in the future by

assessing antagonism of these receptors known to interact with AEA and other substrates increased during FAAH inhibition.

**SA-57.** The combined FAAH/MAGL inhibitor SA-57 (3.0, 10.0, and 17.8 mg/kg) also produced an attenuation, or rightward shift of ICSS, indicating it may be producing a reduction of brain-reward functioning in the mesolimbic pathway. The magnitude of ICSS reduction produced by SA-57 was more similar to that of THC than JZL84 or PF-3845. SA-57 produced its effects on brain-reward functioning within 1 h after administration and returned to normal stimulation rates within 24 h. Similar to THC and JZL184, but in contrast to PF-3845, SA-57-induced attenuation of ICSS was mediated through CB<sub>1</sub> receptors, and was also not affected by CB<sub>2</sub> antagonism. Interestingly, a higher dose of the CB<sub>1</sub> antagonist RIM (10.0 mg/kg) was required to block the effects produced by SA-57 than was used to block THC or JZL184 (3.0 mg/kg). This may indicate the involvement of a greater number of CB<sub>1</sub> receptors following administration of SA-57 (10.0 mg/kg) than with attenuation caused with THC and JZL184, since more antagonist was required to block the effect. SA-57 (10.0 mg/kg) reduced locomotor activity of naïve mice, similar to JZL184.

### **Endocannabinoids in Brain**

High dose JZL184 (40.0 mg/kg) increased 2-AG in all brain regions ranging between 4-fold and 13-fold of VEH levels, while low dose JZL184 (4.0 mg/kg) significantly increased 2-AG in all regions except prefrontal cortex. JZL184 (40.0 mg/kg) also produced a decrease in AEA in the prefrontal cortex and nucleus accumbens and trended toward a reduction in the amygdala. Not surprisingly, JZL184 (40.0 mg/kg) also significantly reduced AA levels in almost all regions.

High-dose SA-57 (10.0 mg/kg) increased 2-AG in all brain regions, while low-dose SA-57 (1.0 mg/kg) did not affect 2-AG levels as shown on Fig. 31. There was also a significant increase in AEA with 1.0 and 10.0 mg/kg SA-57 in nearly all brain regions, and reduced AA levels in the PFC, amygdala, and cerebellum only with high-dose SA-57 (10.0 mg/kg).

It was notable that 2-AG levels were increased across all brain regions with JZL184 (4.0 and 40.0 mg/kg); 4.0 mg/kg only failed to increase 2-AG in the prefrontal cortex, although there was a trend toward significance ( $p = 0.09$ ). If the reduction ICSS via MAGL inhibition is a 2-AG-mediated effect, which it seems to be since it is CB<sub>1</sub> mediated, the biochemical data could mean that the effect was driven by 2-AG elevation in the PFC. This is conceivable, since the PFC is responsible for executive functioning and the control of goal-directed behavior, and it has been reported that cannabinoid receptors, as well as diacylglycerol lipase- $\alpha$ , a 2-AG biosynthetic enzyme, are localized on glutamatergic neurons in the PFC (Lafourcade et al., 2007). Disrupting cannabinoid signaling in the PFC could interfere with motivated behavior by reducing dopamine output.

When assessing the brain level endocannabinoid data after SA-57, it was apparent that 2-AG elevation was necessary for disruption of ICSS through CB<sub>1</sub> receptors. Low-dose SA-57 (1.0 mg/kg) significantly increased AEA in all brain regions, but failed to alter ICSS behavior. However, the combined FAAH/MAGL inhibitor SA-57 produced a higher degree of attenuation than either FAAH or MAGL inhibition alone, so it seems that AEA played a role in this behavior. There are a few different reasons for why that might be. FAAH and MAGL inhibition combined may simply increase the amount of free endocannabinoids in the brain, producing an additive effect of AEA and 2-AG. AEA and 2-AG could also be producing their effects in distinct synapses within these brain regions. Although this study has shown increases in all brain



regions measured, we are not able to detect disparities between AEA and 2-AG at the synaptic level using these measures. That would be better assessed using *in vivo* microdialysis. As was noted in the introduction, AEA and 2-AG have distinct biosynthetic and metabolic pathways, which may underlie their differences in behavioral effects. For example, MAGL is located pre-synaptically, while FAAH is located in the post-synaptic terminal. It appears that AEA and 2-AG are producing cooperative effects in this assay. Dual FAAH and MAGL inhibition has also been shown to produce more THC-like effects than inhibiting either independently, including memory deficits, and substituting for the discriminative stimulus of THC (J. Z. Long, Nomura, et al., 2009; L. E. Wise, et al., 2012).

Additionally, we showed that the reduction in response rates produced by PF-3845 was CB<sub>1</sub> receptor independent, suggesting that it is suppressing ICSS through a completely different mechanism than SA-57, JZL184, and THC. It may be producing its effects through increased levels of AEA, as demonstrated with the neurochemical data. If this is the case, these effects are mediated through non-cannabinoid receptors activated by AEA (e.g., PPAR $\alpha$ , TrpV1), or via a non-specific mechanism. It would have been helpful for the interpretation of endocannabinoid level results to assess intermediate doses of JZL184 and SA-57. Moderate doses of JZL184 (16.0 mg/kg) and SA-57 (3.0 mg/kg) both produced effects in ICSS, but not in distance travelled or operant responding for food in the case of JZL184.

## **General Discussion**

Given the decreases in ICSS following treatment with direct and indirect cannabinoid agonists, it was important to test whether these compounds produced hypolocomotion, a well-documented cannabinoid behavioral effect. These experiments were intended to help delineate potential reward-attenuating effects from non-selective decreases in motor function. Both

JZL184 and SA-57 reduced spontaneous locomotor activity, but only with the highest dose tested (40.0 and 10.0 mg/kg, respectively). They also both decreased responding for ICSS, but at slightly lower doses (16.0 and 3.0 mg/kg, respectively). This is evidence that ICSS is more sensitive to the behaviorally disruptive effects of cannabinoids than spontaneous locomotor activity. An alternative explanation could be that spontaneous locomotor behavior is less physically demanding than lever pressing, and is an innate behavior, and therefore higher doses of CB<sub>1</sub> agonists are required to disrupt the behavior. Furthermore, spontaneous locomotor activity may be less goal-directed than operant responding for reinforcement, which could help differentiate motor suppression from effects on motivated behavior.

Further evidence that ICSS is more sensitive to the disruptive effects of cannabinoids was that only high dose JZL184 (40.0 mg/kg) disrupted operant-responding for food pellets. However, it should be noted that there were a number of procedural differences between operant responding for food and brain stimulation. First, and most obvious, the reinforcer is different (food vs. electrical stimulation). Second, the operant response is different, as lever-pressing was required for ICSS experiments and nose-poking was the operant in the food-responding experiments. Third, the test session length varied between the two tasks, operant responding for food sessions were 15 min, while ICSS test sessions were 24 min. Finally, in the operant responding tasks, subjects were food-deprived, while ICSS subjects were maintained at free-feeding weights. The differences in response topography between nose-poking and lever pressing have to be considered; nose-poking is a more innate response than lever pressing. It would be logical that the effort required for a given behavior would correlate with disruption of behavior. For example, as nose-poking is a more innate behavior than lever pressing, all other things being equal, lever-pressing should be disrupted before nose-poking. This has been demonstrated by

(Gerhardt & Liebman, 1981) who have shown that nose-poking is less susceptible to drug-induced gross motor effects than lever pressing behavior. ICSS is known to produce robust reinforcement, so it is intriguing that it is disrupted before a more innate response like nose-poking. Certainly it seems that ICSS is more sensitive to disruption by cannabinoids than spontaneous locomotor activity or operant responding for food. Food-deprivation could also play a role in the drug effects produced as some drug effects are enhanced, and some are reduced during food-deprivation (D'Cunha, Sedki, Macri, Casola, & Shalev, 2013; Shalev, 2012). Of course, the caveat with interpreting these results is that cannabinoids can have both stimulatory and suppressive effects on food intake and food-motivated behavior.

One potential explanation for the actions of endocannabinoids on reward-related measures is that these neurotransmitters/neuromodulators are involved in the environmental cue-evoked dopamine release associated with drug use and other rewards. In other words, cannabinoids are involved more in the signaling of reward-relevant stimuli, rather than being a substrate for reward. Many studies show that cannabinoid antagonism disrupts behavior for drug rewards including nicotine (Cohen, Perrault, Voltz, Steinberg, & Soubrie, 2002; Le Foll & Goldberg, 2004), methamphetamine (Vinklerova, Novakova, & Sulcova, 2002), opioids (Caille & Parsons, 2003), alcohol (Arnone et al., 1997; Economidou et al., 2006), and cocaine (Xi, et al., 2008). The evidence with cocaine may be the most revealing of cannabinoid effects on dopamine-evoked events. CB<sub>1</sub> receptor blockade interferes with progressive ratio responding to receive cocaine, and prevents drug-induced and cue-induced reinstatement to drug-seeking behavior (De Vries et al., 2001; Xi, et al., 2008; Xi et al., 2007). However, CB<sub>1</sub> antagonism is less efficacious in reducing ongoing self-administration (De Vries, et al., 2001; Filip et al., 2006; Tanda, et al., 2000). This is especially relevant to ICSS, as it directly stimulates the mesolimbic

system, which is thought to indirectly activate dopaminergic neurons in the VTA projecting to the NAc. However, there are cues present with ICSS as there are with almost all behaviors, so cannabinoids could be affecting cues associated with ICSS responding. Though this is not likely the case, as ICSS is a well-trained behavior with extremely salient reinforcement.

This cue-evoking role of endocannabinoids fits with the data from these studies. If the role of endocannabinoids is to signal reward-relevant stimuli, then disruption of cannabinoid signaling through either blockade or over-activation of CB<sub>1</sub> receptors could disrupt reward-related behavior. This would help explain why both CB<sub>1</sub> agonists (Arnold, et al., 2001; Vlachou, et al., 2005, 2006; Vlachou, et al., 2007) and antagonists (Arnold, et al., 2001; Deroche-Gamonet, et al., 2001; Trujillo-Pisanty, et al., 2011; Xi, et al., 2008) have been shown to reduce ICSS behavior. This is particularly interesting given the fact that high doses of CB<sub>1</sub> antagonists are typically required to disrupt ICSS, presumably by disrupting endocannabinoid tone, while relatively low doses of exogenous agonists typically disrupt ICSS, presumably by disrupting normal CB<sub>1</sub> signaling. Although RIM (10.0 mg/kg) did not attenuate ICSS in the current study, this may be a species-specific effect in the mouse. Thus, even though endocannabinoid tone may be necessary for cue-evoked dopamine release that shapes behavior, enhancing endocannabinoid tone without the ability of metabolic clearance may cause endocannabinoids to affect receptors similar to exogenous agonists and disrupt reward-motivated behavior rather than facilitate it.

It is important to consider that with ICSS we are assessing drugs by how they affect the brain during activation of the mesolimbic dopamine system. There is much evidence that this system is part of a common reward pathway, but of course it is not the only reward-mediating area of the brain. Cannabinoids may be exerting their rewarding effects in humans through activation of cannabinoid receptors in other areas of the brain that interact with the mesolimbic

system, with areas completely independent of that system. For example, cannabinoids have effects on stress and anxiety (Hill, Hunter, & McEwen, 2009; Hill & McEwen, 2010; Hill et al., 2009). Marijuana use has been reported to produce anxiolytic and mood-elevating effects that reduces stress and promotes relaxation, which likely contributes to its recreational use (Green, Kavanagh, & Young, 2003). The presence of endocannabinoids and cannabinoid receptors in stress-responsive areas of the brain including the amygdala and hypothalamus, and responsiveness of the endocannabinoid system to stress manipulations, suggests that they are involved in altering stress responses. Endocannabinoids have been shown to both activate and deactivate the hypothalamic-pituitary-adrenal (HPA) axis, which mediates stress responses (Hill et al., 2010). Stress causes an increase in glutamate release in the HPA axis, and specifically the basolateral amygdala. Endocannabinoids are located within this region, and can modulate glutamate release, which provides a mechanism for exogenous cannabinoids to be used for the management of stress responses.

Clearly, more effort will need to be devoted to understanding the discrepancies underlying this and other preclinical models of reward (CPP) and reinforcement (self-administration), as cannabinoid effects in these models may not accurately reflect the abuse-liability seen with marijuana in humans. It is also important to note that we are modeling marijuana use with THC in animals. THC can produce different effects than marijuana in humans, specifically it has been shown to sometimes produce aversive effects (Calhoun, et al., 1998) and produces only mood-reinforcing effects in experienced marijuana users (Hart, et al., 2005; Vandrey, et al., 2013). In light of these studies, perhaps we are accurately representing abuse-liability of THC in humans, as it is not typically used by humans for recreational purposes (Calhoun, et al., 1998). Thus, the failure of cannabinoids to produce effects in preclinical models

may be due to the fact that we are assessing THC rather than marijuana. Future studies assessing inhaled marijuana or synthetic cannabinoids in similar assays would provide an important contribution to this literature.

In conclusion, THC and endocannabinoid catabolic enzyme inhibitors do not produce facilitation of ICSS in C57BL/6 mice. In fact, at higher doses, they tend to produce the opposite effect, attenuation. This reduction of ICSS after cannabinoids is correlated with reduction of other behaviors including operant responding (nose-poking) for food and spontaneous locomotor activity. It does appear that ICSS is more sensitive to attenuation produced by cannabinoids than these other behaviors are. ICSS was consistently shown to be affected by lower doses of THC or cannabinoid enzyme inhibitors than other behaviors. With the exception of PF-3845, these effects were found to be mediated through CB<sub>1</sub>, but not CB<sub>2</sub> receptors. Interestingly, PF-3845-induced attenuation of ICSS was not reversed by either CB<sub>1</sub> or CB<sub>2</sub> antagonism. All time-course evaluations of the drugs tested were in line with previous determinations of their duration of action. 2-AG was shown to be important for the attenuation of ICSS with endocannabinoid inhibitors JZL184 and SA-57, as JZL184 produced an attenuation of ICSS, and principally elevated 2-AG levels. AEA does play a role also, though, as combined FAAH/MAGL inhibition produces a higher degree of ICSS attenuation than either enzyme inhibitor assessed alone and more similar to THC or other exogenous agonists. It seems that AEA and 2-AG produce a cooperative effect when elevated at the same time. Overall, it is unclear how exogenous and endocannabinoids modulate rewarding effects in the human brain. Based on the results from this and other studies, endocannabinoid modulation of reward may not result entirely from activity in the mesolimbic system.

## **List of References**

## List of References

- Ahn, K., Johnson, D. S., Fitzgerald, L. R., Liimatta, M., Arendse, A., Stevenson, T., . . . Cravatt, B. F. (2007). Novel mechanistic class of fatty acid amide hydrolase inhibitors with remarkable selectivity. [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't]. *Biochemistry*, 46(45), 13019-13030. doi: 10.1021/bi701378g
- Ahn, K., Johnson, D. S., Mileni, M., Beidler, D., Long, J. Z., McKinney, M. K., . . . Cravatt, B. F. (2009). Discovery and characterization of a highly selective FAAH inhibitor that reduces inflammatory pain. [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't]. *Chemistry & biology*, 16(4), 411-420. doi: 10.1016/j.chembiol.2009.02.013
- Ahn, K., McKinney, M. K., & Cravatt, B. F. (2008). Enzymatic pathways that regulate endocannabinoid signaling in the nervous system. [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't Review]. *Chemical reviews*, 108(5), 1687-1707. doi: 10.1021/cr0782067
- Alhouayek, M., Lambert, D. M., Delzenne, N. M., Cani, P. D., & Muccioli, G. G. (2011). Increasing endogenous 2-arachidonoylglycerol levels counteracts colitis and related systemic inflammation. [Research Support, Non-U.S. Gov't]. *FASEB journal : official publication of the Federation of American Societies for Experimental Biology*, 25(8), 2711-2721. doi: 10.1096/fj.10-176602
- Aliczki, M., Balogh, Z., Tulogdi, A., & Haller, J. (2012). The temporal dynamics of the effects of monoacylglycerol lipase blockade on locomotion, anxiety, and body temperature. [Research Support, Non-U.S. Gov't]. *Behavioural pharmacology*, 23(4), 348-357. doi: 10.1097/FBP.0b013e3283564dfa
- Antoniou, K., Galanopoulos, A., Vlachou, S., Kourouli, T., Nahmias, V., Thermos, K., . . . Spyraiki, C. (2005). Behavioral pharmacological properties of a novel cannabinoid 1',1'-dithiolane delta8-THC analog, AMG-3. [Comparative Study Research Support, Non-U.S. Gov't]. *Behavioural pharmacology*, 16(5-6), 499-510.
- Arnold, J. C., Hunt, G. E., & McGregor, I. S. (2001). Effects of the cannabinoid receptor agonist CP 55,940 and the cannabinoid receptor antagonist SR 141716 on intracranial self-stimulation in Lewis rats. [Research Support, Non-U.S. Gov't]. *Life sciences*, 70(1), 97-108.
- Arnone, M., Maruani, J., Chaperon, F., Thiebot, M. H., Poncelet, M., Soubrie, P., & Le Fur, G. (1997). Selective inhibition of sucrose and ethanol intake by SR 141716, an antagonist of central cannabinoid (CB1) receptors. [Comparative Study]. *Psychopharmacology*, 132(1), 104-106.



- Ashton, J. C., Friberg, D., Darlington, C. L., & Smith, P. F. (2006). Expression of the cannabinoid CB2 receptor in the rat cerebellum: an immunohistochemical study. *Neuroscience letters*, 396(2), 113-116. doi: 10.1016/j.neulet.2005.11.038
- Baek, J. H., Zheng, Y., Darlington, C. L., & Smith, P. F. (2008). Cannabinoid CB2 receptor expression in the rat brainstem cochlear and vestibular nuclei. [Research Support, Non-U.S. Gov't]. *Acta oto-laryngologica*, 128(9), 961-967. doi: 10.1080/00016480701796944
- Belfrage, P., Jergil, B., Stralfors, P., & Tornqvist, H. (1977). Hormone-sensitive lipase of rat adipose tissue: identification and some properties of the enzyme protein. *FEBS letters*, 75(1), 259-264.
- Bewernick, B. H., Kayser, S., Sturm, V., & Schlaepfer, T. E. (2012). Long-term effects of nucleus accumbens deep brain stimulation in treatment-resistant depression: evidence for sustained efficacy. *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology*, 37(9), 1975-1985. doi: 10.1038/npp.2012.44
- Bielajew, C., & Shizgal, P. (1986). Evidence implicating descending fibers in self-stimulation of the medial forebrain bundle. [Research Support, Non-U.S. Gov't]. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, 6(4), 919-929.
- Bisogno, T., Melck, D., Bobrov, M., Gretskaya, N. M., Bezuglov, V. V., De Petrocellis, L., & Di Marzo, V. (2000). N-acyl-dopamines: novel synthetic CB(1) cannabinoid-receptor ligands and inhibitors of anandamide inactivation with cannabimimetic activity in vitro and in vivo. [Research Support, Non-U.S. Gov't]. *The Biochemical journal*, 351 Pt 3, 817-824.
- Blankman, J. L., Simon, G. M., & Cravatt, B. F. (2007). A comprehensive profile of brain enzymes that hydrolyze the endocannabinoid 2-arachidonoylglycerol. [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't]. *Chemistry & biology*, 14(12), 1347-1356. doi: 10.1016/j.chembiol.2007.11.006
- Boger, D. L., Miyauchi, H., Du, W., Hardouin, C., Fecik, R. A., Cheng, H., . . . Cravatt, B. F. (2005). Discovery of a potent, selective, and efficacious class of reversible alpha-ketoheterocycle inhibitors of fatty acid amide hydrolase effective as analgesics. [Research Support, Non-U.S. Gov't Research Support, U.S. Gov't, P.H.S.]. *Journal of medicinal chemistry*, 48(6), 1849-1856. doi: 10.1021/jm049614v
- Booker, L., Kinsey, S. G., Abdullah, R. A., Blankman, J. L., Long, J. Z., Ezzili, C., . . . Lichtman, A. H. (2012). The fatty acid amide hydrolase (FAAH) inhibitor PF-3845 acts in the nervous system to reverse LPS-induced tactile allodynia in mice. [Research Support, N.I.H., Extramural]. *British journal of pharmacology*, 165(8), 2485-2496. doi: 10.1111/j.1476-5381.2011.01445.x
- Braida, D., Pozzi, M., Cavallini, R., & Sala, M. (2001). Conditioned place preference induced by the cannabinoid agonist CP 55,940: interaction with the opioid system. [Research Support, Non-U.S. Gov't]. *Neuroscience*, 104(4), 923-926.

- Brents, L. K., & Prather, P. L. (2013). The K2/Spice Phenomenon: emergence, identification, legislation and metabolic characterization of synthetic cannabinoids in herbal incense products. *Drug metabolism reviews*. doi: 10.3109/03602532.2013.839700
- Cadogan, A. K., Alexander, S. P., Boyd, E. A., & Kendall, D. A. (1997). Influence of cannabinoids on electrically evoked dopamine release and cyclic AMP generation in the rat striatum. [In Vitro Research Support, Non-U.S. Gov't]. *Journal of neurochemistry*, 69(3), 1131-1137.
- Caille, S., & Parsons, L. H. (2003). SR141716A reduces the reinforcing properties of heroin but not heroin-induced increases in nucleus accumbens dopamine in rats. [Comparative Study Research Support, Non-U.S. Gov't]. *The European journal of neuroscience*, 18(11), 3145-3149.
- Calhoun, S. R., Galloway, G. P., & Smith, D. E. (1998). Abuse potential of dronabinol (Marinol). [Research Support, Non-U.S. Gov't]. *Journal of psychoactive drugs*, 30(2), 187-196.
- Caprioli, A., Coccurello, R., Rapino, C., Di Serio, S., Di Tommaso, M., Vertechy, M., . . . Borsini, F. (2012). The novel reversible fatty acid amide hydrolase inhibitor ST4070 increases endocannabinoid brain levels and counteracts neuropathic pain in different animal models. [Research Support, Non-U.S. Gov't]. *The Journal of pharmacology and experimental therapeutics*, 342(1), 188-195. doi: 10.1124/jpet.111.191403
- Carlezon, W. A., Jr., & Chartoff, E. H. (2007). Intracranial self-stimulation (ICSS) in rodents to study the neurobiology of motivation. [Research Support, N.I.H., Extramural]. *Nature protocols*, 2(11), 2987-2995. doi: 10.1038/nprot.2007.441
- Carlson, R. H., & Lydic, R. (1976). The effects of ethanol upon threshold and response rate for self-stimulation. *Psychopharmacology*, 50(1), 61-64.
- Cazala, P., & Cardo, B. (1972). [Preliminary study on self stimulation in the mouse]. *Physiology & behavior*, 9(2), 255-257.
- Cheer, J. F., Aragona, B. J., Heien, M. L., Seipel, A. T., Carelli, R. M., & Wightman, R. M. (2007). Coordinated accumbal dopamine release and neural activity drive goal-directed behavior. [Research Support, N.I.H., Extramural]. *Neuron*, 54(2), 237-244. doi: 10.1016/j.neuron.2007.03.021
- Cheer, J. F., Kendall, D. A., & Marsden, C. A. (2000). Cannabinoid receptors and reward in the rat: a conditioned place preference study. *Psychopharmacology*, 151(1), 25-30.
- Cheer, J. F., Wassum, K. M., Heien, M. L., Phillips, P. E., & Wightman, R. M. (2004). Cannabinoids enhance subsecond dopamine release in the nucleus accumbens of awake rats. [Research Support, U.S. Gov't, P.H.S.]. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, 24(18), 4393-4400. doi: 10.1523/JNEUROSCI.0529-04.2004

- Cheer, J. F., Wassum, K. M., Sombers, L. A., Heien, M. L., Ariansen, J. L., Aragona, B. J., . . . Wightman, R. M. (2007). Phasic dopamine release evoked by abused substances requires cannabinoid receptor activation. [Comparative Study Research Support, N.I.H., Extramural]. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, 27(4), 791-795. doi: 10.1523/JNEUROSCI.4152-06.2007
- Chen, J., Marmur, R., Pulles, A., Paredes, W., & Gardner, E. L. (1993). Ventral tegmental microinjection of delta 9-tetrahydrocannabinol enhances ventral tegmental somatodendritic dopamine levels but not forebrain dopamine levels: evidence for local neural action by marijuana's psychoactive ingredient. [Comparative Study Research Support, Non-U.S. Gov't Research Support, U.S. Gov't, Non-P.H.S. Research Support, U.S. Gov't, P.H.S.]. *Brain research*, 621(1), 65-70.
- Chen, J., Paredes, W., Lowinson, J. H., & Gardner, E. L. (1990). Delta 9-tetrahydrocannabinol enhances presynaptic dopamine efflux in medial prefrontal cortex. [Research Support, U.S. Gov't, Non-P.H.S. Research Support, U.S. Gov't, P.H.S.]. *European journal of pharmacology*, 190(1-2), 259-262.
- Chen, J. P., Paredes, W., Li, J., Smith, D., Lowinson, J., & Gardner, E. L. (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. [Research Support, Non-U.S. Gov't Research Support, U.S. Gov't, Non-P.H.S. Research Support, U.S. Gov't, P.H.S.]. *Psychopharmacology*, 102(2), 156-162.
- Chen, J. P., Paredes, W., Lowinson, J. H., & Gardner, E. L. (1991). Strain-specific facilitation of dopamine efflux by delta 9-tetrahydrocannabinol in the nucleus accumbens of rat: an in vivo microdialysis study. [Research Support, Non-U.S. Gov't Research Support, U.S. Gov't, P.H.S.]. *Neuroscience letters*, 129(1), 136-180.
- Chen, S. A., O'Dell, L. E., Hofer, M. E., Greenwell, T. N., Zorrilla, E. P., & Koob, G. F. (2006). Unlimited access to heroin self-administration: independent motivational markers of opiate dependence. [Research Support, N.I.H., Extramural Validation Studies]. *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology*, 31(12), 2692-2707. doi: 10.1038/sj.npp.1301008
- Cohen, C., Perrault, G., Voltz, C., Steinberg, R., & Soubrie, P. (2002). SR141716, a central cannabinoid (CB(1)) receptor antagonist, blocks the motivational and dopamine-releasing effects of nicotine in rats. *Behavioural pharmacology*, 13(5-6), 451-463.
- Compton, D. R., Aceto, M. D., Lowe, J., & Martin, B. R. (1996). In vivo characterization of a specific cannabinoid receptor antagonist (SR141716A): inhibition of delta 9-tetrahydrocannabinol-induced responses and apparent agonist activity. [Research Support, U.S. Gov't, P.H.S.]. *The Journal of pharmacology and experimental therapeutics*, 277(2), 586-594.
- Corbett, D. (1991). Cocaine enhances the reward value of medial prefrontal cortex self-stimulation. [Research Support, Non-U.S. Gov't]. *Neuroreport*, 2(12), 805-808.

- Cravatt, B. F., Prospero-Garcia, O., Siuzdak, G., Gilula, N. B., Henriksen, S. J., Boger, D. L., & Lerner, R. A. (1995). Chemical characterization of a family of brain lipids that induce sleep. [Research Support, Non-U.S. Gov't Research Support, U.S. Gov't, Non-P.H.S. Research Support, U.S. Gov't, P.H.S.]. *Science*, 268(5216), 1506-1509.
- Cryan, J. F., Hoyer, D., & Markou, A. (2003). Withdrawal from chronic amphetamine induces depressive-like behavioral effects in rodents. [Research Support, Non-U.S. Gov't Research Support, U.S. Gov't, P.H.S.]. *Biological psychiatry*, 54(1), 49-58.
- D'Angio, M., Serrano, A., Rivy, J. P., & Scatton, B. (1987). Tail-pinch stress increases extracellular DOPAC levels (as measured by in vivo voltammetry) in the rat nucleus accumbens but not frontal cortex: antagonism by diazepam and zolpidem. [Comparative Study]. *Brain research*, 409(1), 169-174.
- D'Cunha, T. M., Sedki, F., Macri, J., Casola, C., & Shalev, U. (2013). The effects of chronic food restriction on cue-induced heroin seeking in abstinent male rats. [Research Support, Non-U.S. Gov't]. *Psychopharmacology*, 225(1), 241-250. doi: 10.1007/s00213-012-2810-1
- De Vries, T. J., Shaham, Y., Homberg, J. R., Crombag, H., Schuurman, K., Dieben, J., . . . Schoffelmeer, A. N. (2001). A cannabinoid mechanism in relapse to cocaine seeking. [Research Support, U.S. Gov't, P.H.S.]. *Nature medicine*, 7(10), 1151-1154. doi: 10.1038/nm1001-1151
- De Vry, J., Schreiber, R., Eckel, G., & Jentsch, K. R. (2004). Behavioral mechanisms underlying inhibition of food-maintained responding by the cannabinoid receptor antagonist/inverse agonist SR141716A. *European journal of pharmacology*, 483(1), 55-63.
- Deroche-Gamonet, V., Le Moal, M., Piazza, P. V., & Soubrie, P. (2001). SR141716, a CB1 receptor antagonist, decreases the sensitivity to the reinforcing effects of electrical brain stimulation in rats. *Psychopharmacology*, 157(3), 254-259. doi: 10.1007/s002130100804
- Devane, W. A., Hanus, L., Breuer, A., Pertwee, R. G., Stevenson, L. A., Griffin, G., . . . Mechoulam, R. (1992). Isolation and structure of a brain constituent that binds to the cannabinoid receptor. [Research Support, Non-U.S. Gov't Research Support, U.S. Gov't, P.H.S.]. *Science*, 258(5090), 1946-1949.
- Dinh, T. P., Freund, T. F., & Piomelli, D. (2002). A role for monoglyceride lipase in 2-arachidonoylglycerol inactivation. [Research Support, Non-U.S. Gov't Research Support, U.S. Gov't, P.H.S. Review]. *Chemistry and physics of lipids*, 121(1-2), 149-158.
- Economidou, D., Mattioli, L., Cifani, C., Perfumi, M., Massi, M., Cuomo, V., . . . Ciccocioppo, R. (2006). Effect of the cannabinoid CB1 receptor antagonist SR-141716A on ethanol self-administration and ethanol-seeking behaviour in rats. [Research Support, Non-U.S. Gov't]. *Psychopharmacology*, 183(4), 394-403. doi: 10.1007/s00213-005-0199-9

- Elmer, G. I., Meisch, R. A., & George, F. R. (1987). Mouse strain differences in operant self-administration of ethanol. [Comparative Study Research Support, U.S. Gov't, P.H.S.]. *Behavior genetics*, 17(5), 439-451.
- Falenski, K. W., Thorpe, A. J., Schlosburg, J. E., Cravatt, B. F., Abdullah, R. A., Smith, T. H., . . . Sim-Selley, L. J. (2010). FAAH-/- mice display differential tolerance, dependence, and cannabinoid receptor adaptation after delta 9-tetrahydrocannabinol and anandamide administration. [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't]. *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology*, 35(8), 1775-1787. doi: 10.1038/npp.2010.44
- Fattore, L., Cossu, G., Martellotta, C. M., & Fratta, W. (2001). Intravenous self-administration of the cannabinoid CB1 receptor agonist WIN 55,212-2 in rats. *Psychopharmacology*, 156(4), 410-416.
- Fernandez-Ruiz, J., Hernandez, M., & Ramos, J. A. (2010). Cannabinoid-dopamine interaction in the pathophysiology and treatment of CNS disorders. [Research Support, Non-U.S. Gov't Review]. *CNS neuroscience & therapeutics*, 16(3), e72-91. doi: 10.1111/j.1755-5949.2010.00144.x
- Filip, M., Golda, A., Zaniowska, M., McCreary, A. C., Nowak, E., Kolasiewicz, W., & Przegalinski, E. (2006). Involvement of cannabinoid CB1 receptors in drug addiction: effects of rimonabant on behavioral responses induced by cocaine. [Research Support, Non-U.S. Gov't]. *Pharmacological reports : PR*, 58(6), 806-819.
- Fish, E. W., Riday, T. T., McGuigan, M. M., Faccidomo, S., Hodge, C. W., & Malanga, C. J. (2010). Alcohol, cocaine, and brain stimulation-reward in C57Bl6/J and DBA2/J mice. [Comparative Study Research Support, N.I.H., Extramural]. *Alcoholism, clinical and experimental research*, 34(1), 81-89. doi: 10.1111/j.1530-0277.2009.01069.x
- Fish, E. W., Robinson, J. E., Krouse, M. C., Hodge, C. W., Reed, C., Phillips, T. J., & Malanga, C. J. (2012). Intracranial self-stimulation in FAST and SLOW mice: effects of alcohol and cocaine. [Comparative Study, Research Support, N.I.H., Extramural, Research Support, U.S. Gov't, Non-P.H.S.]. *Psychopharmacology*, 220(4), 719-730. doi: 10.1007/s00213-011-2523-x
- Flores, A., Maldonado, R., & Berrendero, F. (2013). The Hypocretin/Orexin Receptor-1 as a Novel Target to Modulate Cannabinoid Reward. *Biological psychiatry*. doi: 10.1016/j.biopsych.2013.06.012
- Fokos, S., & Panagis, G. (2010). Effects of delta9-tetrahydrocannabinol on reward and anxiety in rats exposed to chronic unpredictable stress. [Research Support, Non-U.S. Gov't]. *Journal of psychopharmacology*, 24(5), 767-777. doi: 10.1177/0269881109104904
- French, E. D. (1997). delta9-Tetrahydrocannabinol excites rat VTA dopamine neurons through activation of cannabinoid CB1 but not opioid receptors. [Comparative Study Research Support, U.S. Gov't, P.H.S.]. *Neuroscience letters*, 226(3), 159-162.

- French, E. D., Dillon, K., & Wu, X. (1997). Cannabinoids excite dopamine neurons in the ventral tegmentum and substantia nigra. [Research Support, U.S. Gov't, P.H.S.]. *Neuroreport*, 8(3), 649-652.
- Gao, Y., Vasilyev, D. V., Goncalves, M. B., Howell, F. V., Hobbs, C., Reisenberg, M., . . . Doherty, P. (2010). Loss of retrograde endocannabinoid signaling and reduced adult neurogenesis in diacylglycerol lipase knock-out mice. [Research Support, Non-U.S. Gov't]. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, 30(6), 2017-2024. doi: 10.1523/JNEUROSCI.5693-09.2010
- Gardner, E. L., Paredes, W., Smith, D., Donner, A., Milling, C., Cohen, D., & Morrison, D. (1988). Facilitation of brain stimulation reward by delta 9-tetrahydrocannabinol. [Research Support, Non-U.S. Gov't Research Support, U.S. Gov't, P.H.S.]. *Psychopharmacology*, 96(1), 142-144.
- Gerard, C. M., Mollereau, C., Vassart, G., & Parmentier, M. (1991). Molecular cloning of a human cannabinoid receptor which is also expressed in testis. [Research Support, Non-U.S. Gov't]. *The Biochemical journal*, 279 ( Pt 1), 129-134.
- Gerhardt, S., & Liebman, J. M. (1981). Differential effects of drug treatments on nose-poke and bar-press self-stimulation. *Pharmacology, biochemistry, and behavior*, 15(5), 767-771.
- Gessa, G. L., Melis, M., Muntoni, A. L., & Diana, M. (1998). Cannabinoids activate mesolimbic dopamine neurons by an action on cannabinoid CB1 receptors. [Comparative Study]. *European journal of pharmacology*, 341(1), 39-44.
- Ghosh, S., Wise, L. E., Chen, Y., Gujjar, R., Mahadevan, A., Cravatt, B. F., & Lichtman, A. H. (2012). The monoacylglycerol lipase inhibitor JZL184 suppresses inflammatory pain in the mouse carrageenan model. *Life sciences*. doi: 10.1016/j.lfs.2012.06.020
- Ghozland, S., Matthes, H. W., Simonin, F., Filliol, D., Kieffer, B. L., & Maldonado, R. (2002). Motivational effects of cannabinoids are mediated by mu-opioid and kappa-opioid receptors. [Research Support, Non-U.S. Gov't]. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, 22(3), 1146-1154.
- Gobbi, G., Bambico, F. R., Mangieri, R., Bortolato, M., Campolongo, P., Solinas, M., . . . Piomelli, D. (2005). Antidepressant-like activity and modulation of brain monoaminergic transmission by blockade of anandamide hydrolysis. [Research Support, N.I.H., Extramural Research Support, N.I.H., Intramural Research Support, Non-U.S. Gov't]. *Proceedings of the National Academy of Sciences of the United States of America*, 102(51), 18620-18625. doi: 10.1073/pnas.0509591102
- Goldman, M., Szucs-Reed, R. P., Jagannathan, K., Ehrman, R. N., Wang, Z., Li, Y., . . . Franklin, T. R. (2013). Reward-related brain response and craving correlates of marijuana cue exposure: a preliminary study in treatment-seeking marijuana-dependent subjects. [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't Research Support, U.S. Gov't, Non-P.H.S.]. *Journal of addiction medicine*, 7(1), 8-16. doi: 10.1097/ADM.0b013e318273863a

- Gong, J. P., Onaivi, E. S., Ishiguro, H., Liu, Q. R., Tagliaferro, P. A., Brusco, A., & Uhl, G. R. (2006). Cannabinoid CB2 receptors: immunohistochemical localization in rat brain. [Comparative Study In Vitro Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't]. *Brain research*, 1071(1), 10-23. doi: 10.1016/j.brainres.2005.11.035
- Gonsiorek, W., Lunn, C., Fan, X., Narula, S., Lundell, D., & Hipkin, R. W. (2000). Endocannabinoid 2-arachidonyl glycerol is a full agonist through human type 2 cannabinoid receptor: antagonism by anandamide. *Molecular pharmacology*, 57(5), 1045-1050.
- Goparaju, S. K., Ueda, N., Yamaguchi, H., & Yamamoto, S. (1998). Anandamide amidohydrolase reacting with 2-arachidonoylglycerol, another cannabinoid receptor ligand. [Research Support, Non-U.S. Gov't]. *FEBS letters*, 422(1), 69-73.
- Green, B., Kavanagh, D., & Young, R. (2003). Being stoned: a review of self-reported cannabis effects. [Review]. *Drug and alcohol review*, 22(4), 453-460. doi: 10.1080/09595230310001613976
- Guindon, J., Guijarro, A., Piomelli, D., & Hohmann, A. G. (2011). Peripheral antinociceptive effects of inhibitors of monoacylglycerol lipase in a rat model of inflammatory pain. [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't]. *British journal of pharmacology*, 163(7), 1464-1478. doi: 10.1111/j.1476-5381.2010.01192.x
- Hajos, N., & Freund, T. F. (2002a). Distinct cannabinoid sensitive receptors regulate hippocampal excitation and inhibition. [Research Support, Non-U.S. Gov't Research Support, U.S. Gov't, P.H.S. Review]. *Chemistry and physics of lipids*, 121(1-2), 73-82.
- Hajos, N., & Freund, T. F. (2002b). Pharmacological separation of cannabinoid sensitive receptors on hippocampal excitatory and inhibitory fibers. [Research Support, Non-U.S. Gov't Research Support, U.S. Gov't, P.H.S.]. *Neuropharmacology*, 43(4), 503-510.
- Haller, J., Goldberg, S. R., Pelczer, K. G., Aliczki, M., & Panlilio, L. V. (2013). The effects of anandamide signaling enhanced by the FAAH inhibitor URB597 on coping styles in rats. *Psychopharmacology*. doi: 10.1007/s00213-013-3161-2
- Hanus, L., Abu-Lafi, S., Frider, E., Breuer, A., Vogel, Z., Shalev, D. E., . . . Mechoulam, R. (2001). 2-arachidonyl glyceryl ether, an endogenous agonist of the cannabinoid CB1 receptor. [Research Support, Non-U.S. Gov't Research Support, U.S. Gov't, P.H.S.]. *Proceedings of the National Academy of Sciences of the United States of America*, 98(7), 3662-3665. doi: 10.1073/pnas.061029898
- Hart, C. L., Haney, M., Vosburg, S. K., Comer, S. D., & Foltin, R. W. (2005). Reinforcing effects of oral Delta9-THC in male marijuana smokers in a laboratory choice procedure. [Controlled Clinical Trial Research Support, N.I.H., Extramural Research Support, U.S. Gov't, P.H.S.]. *Psychopharmacology*, 181(2), 237-243. doi: 10.1007/s00213-005-2234-2
- Hart, C. L., Ilan, A. B., Gevins, A., Gunderson, E. W., Role, K., Colley, J., & Foltin, R. W. (2010). Neurophysiological and cognitive effects of smoked marijuana in frequent users.

- [Research Support, N.I.H., Extramural]. *Pharmacology, biochemistry, and behavior*, 96(3), 333-341. doi: 10.1016/j.pbb.2010.06.003
- Hart, C. L., van Gorp, W., Haney, M., Foltin, R. W., & Fischman, M. W. (2001). Effects of acute smoked marijuana on complex cognitive performance. [Clinical Trial Research Support, U.S. Gov't, P.H.S.]. *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology*, 25(5), 757-765. doi: 10.1016/S0893-133X(01)00273-1
- Hill, M. N., Hunter, R. G., & McEwen, B. S. (2009). Chronic stress differentially regulates cannabinoid CB1 receptor binding in distinct hippocampal subfields. [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't]. *European journal of pharmacology*, 614(1-3), 66-69. doi: 10.1016/j.ejphar.2009.04.048
- Hill, M. N., Kumar, S. A., Filipowski, S. B., Iverson, M., Stuhr, K. L., Keith, J. M., . . . McEwen, B. S. (2012). Disruption of fatty acid amide hydrolase activity prevents the effects of chronic stress on anxiety and amygdalar microstructure. *Molecular psychiatry*. doi: 10.1038/mp.2012.90
- Hill, M. N., & McEwen, B. S. (2010). Involvement of the endocannabinoid system in the neurobehavioural effects of stress and glucocorticoids. [Review]. *Progress in neuro-psychopharmacology & biological psychiatry*, 34(5), 791-797. doi: 10.1016/j.pnpbp.2009.11.001
- Hill, M. N., McLaughlin, R. J., Morrish, A. C., Viau, V., Floresco, S. B., Hillard, C. J., & Gorzalka, B. B. (2009). Suppression of amygdalar endocannabinoid signaling by stress contributes to activation of the hypothalamic-pituitary-adrenal axis. [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't]. *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology*, 34(13), 2733-2745. doi: 10.1038/npp.2009.114
- Hill, M. N., Patel, S., Campolongo, P., Tasker, J. G., Wotjak, C. T., & Bains, J. S. (2010). Functional interactions between stress and the endocannabinoid system: from synaptic signaling to behavioral output. [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't Review]. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, 30(45), 14980-14986. doi: 10.1523/JNEUROSCI.4283-10.2010
- Howlett, A. C., Bidaut-Russell, M., Devane, W. A., Melvin, L. S., Johnson, M. R., & Herkenham, M. (1990). The cannabinoid receptor: biochemical, anatomical and behavioral characterization. [Review]. *Trends in neurosciences*, 13(10), 420-423.
- Hrabovszky, E., Wittmann, G., Kallo, I., Fuzesi, T., Fekete, C., & Liposits, Z. (2012). Distribution of type 1 cannabinoid receptor-expressing neurons in the septal-hypothalamic region of the mouse: colocalization with GABAergic and glutamatergic markers. [Comparative Study Research Support, Non-U.S. Gov't]. *The Journal of comparative neurology*, 520(5), 1005-1020. doi: 10.1002/cne.22766
- Huggins, J. P., Smart, T. S., Langman, S., Taylor, L., & Young, T. (2012). An efficient randomised, placebo-controlled clinical trial with the irreversible fatty acid amide



- hydrolase-1 inhibitor PF-04457845, which modulates endocannabinoids but fails to induce effective analgesia in patients with pain due to osteoarthritis of the knee. [Randomized Controlled Trial Research Support, Non-U.S. Gov't]. *Pain*, 153(9), 1837-1846. doi: 10.1016/j.pain.2012.04.020
- Ignatowska-Jankowska, B. M., Ghosh, S., Crowe, M. S., Kinsey, S. G., Niphakis, M. J., Abdullah, R. A., . . . Lichtman, A. H. (2013). In vivo characterization of the highly selective monoacylglycerol lipase inhibitor KML29: Antinociceptive activity without cannabimimetic side effects. *British journal of pharmacology*. doi: 10.1111/bph.12298
- Ignatowska-Jankowska, B. M., Muldoon, P. P., Lichtman, A. H., & Damaj, M. I. (2013). The cannabinoid CB receptor is necessary for nicotine-conditioned place preference, but not other behavioral effects of nicotine in mice. *Psychopharmacology*. doi: 10.1007/s00213-013-3117-6
- Jhaveri, M. D., Richardson, D., Robinson, I., Garle, M. J., Patel, A., Sun, Y., . . . Chapman, V. (2008). Inhibition of fatty acid amide hydrolase and cyclooxygenase-2 increases levels of endocannabinoid related molecules and produces analgesia via peroxisome proliferator-activated receptor-alpha in a model of inflammatory pain. [Research Support, Non-U.S. Gov't]. *Neuropharmacology*, 55(1), 85-93. doi: 10.1016/j.neuropharm.2008.04.018
- Justinova, Z., Solinas, M., Tanda, G., Redhi, G. H., & Goldberg, S. R. (2005). The endogenous cannabinoid anandamide and its synthetic analog R(+)-methanandamide are intravenously self-administered by squirrel monkeys. [Research Support, N.I.H., Intramural Research Support, U.S. Gov't, P.H.S.]. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, 25(23), 5645-5650. doi: 10.1523/JNEUROSCI.0951-05.2005
- Justinova, Z., Tanda, G., Redhi, G. H., & Goldberg, S. R. (2003). Self-administration of delta9-tetrahydrocannabinol (THC) by drug naive squirrel monkeys. [Research Support, U.S. Gov't, P.H.S.]. *Psychopharmacology*, 169(2), 135-140. doi: 10.1007/s00213-003-1484-0
- Justinova, Z., Yasar, S., Redhi, G. H., & Goldberg, S. R. (2011). The endogenous cannabinoid 2-arachidonoylglycerol is intravenously self-administered by squirrel monkeys. [Research Support, N.I.H., Intramural]. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, 31(19), 7043-7048. doi: 10.1523/JNEUROSCI.6058-10.2011
- Kathuria, S., Gaetani, S., Fegley, D., Valino, F., Duranti, A., Tontini, A., . . . Piomelli, D. (2003). Modulation of anxiety through blockade of anandamide hydrolysis. [Research Support, Non-U.S. Gov't Research Support, U.S. Gov't, P.H.S.]. *Nature medicine*, 9(1), 76-81. doi: 10.1038/nm803
- Katona, I., Sperlagh, B., Magloczky, Z., Santha, E., Kofalvi, A., Czirjak, S., . . . Freund, T. F. (2000). GABAergic interneurons are the targets of cannabinoid actions in the human hippocampus. [In Vitro Research Support, Non-U.S. Gov't Research Support, U.S. Gov't, P.H.S.]. *Neuroscience*, 100(4), 797-804.
- Katsidoni, V., Kastellakis, A., & Panagis, G. (2013). Biphasic effects of Delta9-tetrahydrocannabinol on brain stimulation reward and motor activity. *The international*

- Kim, J., & Alger, B. E. (2004). Inhibition of cyclooxygenase-2 potentiates retrograde endocannabinoid effects in hippocampus. [Comparative Study In Vitro Research Support, U.S. Gov't, P.H.S.]. *Nature neuroscience*, 7(7), 697-698. doi: 10.1038/nm1262
- Kinsey, S. G., Long, J. Z., O'Neal, S. T., Abdullah, R. A., Poklis, J. L., Boger, D. L., . . . Lichtman, A. H. (2009). Blockade of endocannabinoid-degrading enzymes attenuates neuropathic pain. [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't]. *The Journal of pharmacology and experimental therapeutics*, 330(3), 902-910. doi: 10.1124/jpet.109.155465
- Kinsey, S. G., Naidu, P. S., Cravatt, B. F., Dudley, D. T., & Lichtman, A. H. (2011). Fatty acid amide hydrolase blockade attenuates the development of collagen-induced arthritis and related thermal hyperalgesia in mice. [Research Support, N.I.H., Extramural]. *Pharmacology, biochemistry, and behavior*, 99(4), 718-725. doi: 10.1016/j.pbb.2011.06.022
- Kinsey, S. G., Nomura, D. K., O'Neal, S. T., Long, J. Z., Mahadevan, A., Cravatt, B. F., . . . Lichtman, A. H. (2011). Inhibition of monoacylglycerol lipase attenuates nonsteroidal anti-inflammatory drug-induced gastric hemorrhages in mice. [Research Support, N.I.H., Extramural]. *The Journal of pharmacology and experimental therapeutics*, 338(3), 795-802. doi: 10.1124/jpet.110.175778
- Kinsey, S. G., O'Neal, S. T., Long, J. Z., Cravatt, B. F., & Lichtman, A. H. (2011). Inhibition of endocannabinoid catabolic enzymes elicits anxiolytic-like effects in the marble burying assay. [Research Support, N.I.H., Extramural]. *Pharmacology, biochemistry, and behavior*, 98(1), 21-27. doi: 10.1016/j.pbb.2010.12.002
- Kinsey, S. G., Wise, L. E., Ramesh, D., Abdullah, R., Selley, D. E., Cravatt, B. F., & Lichtman, A. H. (2013). Repeated low-dose administration of the monoacylglycerol lipase inhibitor JZL184 retains cannabinoid receptor type 1-mediated antinociceptive and gastroprotective effects. [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't]. *The Journal of pharmacology and experimental therapeutics*, 345(3), 492-501. doi: 10.1124/jpet.112.201426
- Kornetsky, C. (2004). Brain-stimulation reward, morphine-induced oral stereotypy, and sensitization: implications for abuse. [Review]. *Neuroscience and biobehavioral reviews*, 27(8), 777-786. doi: 10.1016/j.neubiorev.2003.11.014
- Kortleven, C., Fasano, C., Thibault, D., Lacaille, J. C., & Trudeau, L. E. (2011). The endocannabinoid 2-arachidonoylglycerol inhibits long-term potentiation of glutamatergic synapses onto ventral tegmental area dopamine neurons in mice. [Research Support, Non-U.S. Gov't]. *The European journal of neuroscience*, 33(10), 1751-1760. doi: 10.1111/j.1460-9568.2011.07648.x

- Kucharski, L. T., Williams, J. E., & Kornetsky, C. (1983). The effects of levonantradol on rewarding brain stimulation thresholds in the rat. [Research Support, U.S. Gov't, P.H.S.]. *Pharmacology, biochemistry, and behavior*, 19(1), 149-151.
- Kwilasz, A. J., & Negus, S. S. (2012). Dissociable effects of the cannabinoid receptor agonists Delta9-tetrahydrocannabinol and CP55940 on pain-stimulated versus pain-depressed behavior in rats. [Research Support, N.I.H., Extramural]. *The Journal of pharmacology and experimental therapeutics*, 343(2), 389-400. doi: 10.1124/jpet.112.197780
- Lafourcade, M., Elezgarai, I., Mato, S., Bakiri, Y., Grandes, P., & Manzoni, O. J. (2007). Molecular components and functions of the endocannabinoid system in mouse prefrontal cortex. [Research Support, Non-U.S. Gov't]. *PloS one*, 2(8), e709. doi: 10.1371/journal.pone.0000709
- Le Foll, B., & Goldberg, S. R. (2004). Rimobabant, a CB1 antagonist, blocks nicotine-conditioned place preferences. [Comparative Study Research Support, Non-U.S. Gov't Research Support, U.S. Gov't, P.H.S.]. *Neuroreport*, 15(13), 2139-2143.
- Lepore, M., Liu, X., Savage, V., Matalon, D., & Gardner, E. L. (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. [Research Support, Non-U.S. Gov't Research Support, U.S. Gov't, P.H.S.]. *Life sciences*, 58(25), PL365-372.
- Lepore, M., Vorel, S. R., Lowinson, J., & Gardner, E. L. (1995). Conditioned place preference induced by delta 9-tetrahydrocannabinol: comparison with cocaine, morphine, and food reward. [Comparative Study Research Support, Non-U.S. Gov't Research Support, U.S. Gov't, P.H.S.]. *Life sciences*, 56(23-24), 2073-2080.
- Leung, D., Saghatelian, A., Simon, G. M., & Cravatt, B. F. (2006). Inactivation of N-acyl phosphatidylethanolamine phospholipase D reveals multiple mechanisms for the biosynthesis of endocannabinoids. [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't]. *Biochemistry*, 45(15), 4720-4726. doi: 10.1021/bi060163l
- Li, G. L., Winter, H., Arends, R., Jay, G. W., Le, V., Young, T., & Huggins, J. P. (2012). Assessment of the pharmacology and tolerability of PF-04457845, an irreversible inhibitor of fatty acid amide hydrolase-1, in healthy subjects. [Randomized Controlled Trial Research Support, Non-U.S. Gov't]. *British journal of clinical pharmacology*, 73(5), 706-716. doi: 10.1111/j.1365-2125.2011.04137.x
- Liu, J., Wang, L., Harvey-White, J., Osei-Hyiaman, D., Razdan, R., Gong, Q., . . . Kunos, G. (2006). A biosynthetic pathway for anandamide. [In Vitro Research Support, N.I.H., Intramural]. *Proceedings of the National Academy of Sciences of the United States of America*, 103(36), 13345-13350. doi: 10.1073/pnas.0601832103
- Long, J. Z., Li, W., Booker, L., Burston, J. J., Kinsey, S. G., Schlosburg, J. E., . . . Cravatt, B. F. (2009). Selective blockade of 2-arachidonoylglycerol hydrolysis produces cannabinoid behavioral effects. [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't]. *Nature chemical biology*, 5(1), 37-44. doi: 10.1038/nchembio.129

- Long, J. Z., Nomura, D. K., Vann, R. E., Walentiny, D. M., Booker, L., Jin, X., . . . Cravatt, B. F. (2009). Dual blockade of FAAH and MAGL identifies behavioral processes regulated by endocannabinoid crosstalk in vivo. [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't]. *Proceedings of the National Academy of Sciences of the United States of America*, 106(48), 20270-20275. doi: 10.1073/pnas.0909411106
- Long, L. E., Chesworth, R., Huang, X. F., McGregor, I. S., Arnold, J. C., & Karl, T. (2010). A behavioural comparison of acute and chronic Delta9-tetrahydrocannabinol and cannabidiol in C57BL/6JArc mice. [Comparative Study Research Support, Non-U.S. Gov't]. *The international journal of neuropsychopharmacology / official scientific journal of the Collegium Internationale Neuropsychopharmacologicum*, 13(7), 861-876. doi: 10.1017/S1461145709990605
- Lu, X. R., Ong, W. Y., & Mackie, K. (1999). A light and electron microscopic study of the CB1 cannabinoid receptor in monkey basal forebrain. [Research Support, Non-U.S. Gov't Research Support, U.S. Gov't, P.H.S.]. *Journal of neurocytology*, 28(12), 1045-1051.
- Luchicchi, A., Lecca, S., Carta, S., Pillolla, G., Muntoni, A. L., Yasar, S., . . . Pistis, M. (2010). Effects of fatty acid amide hydrolase inhibition on neuronal responses to nicotine, cocaine and morphine in the nucleus accumbens shell and ventral tegmental area: involvement of PPAR-alpha nuclear receptors. [Research Support, N.I.H., Intramural Research Support, Non-U.S. Gov't]. *Addiction biology*, 15(3), 277-288. doi: 10.1111/j.1369-1600.2010.00222.x
- Lupica, C. R., & Riegel, A. C. (2005). Endocannabinoid release from midbrain dopamine neurons: a potential substrate for cannabinoid receptor antagonist treatment of addiction. [Review]. *Neuropharmacology*, 48(8), 1105-1116. doi: 10.1016/j.neuropharm.2005.03.016
- Malone, D. T., & Taylor, D. A. (1999). Modulation by fluoxetine of striatal dopamine release following Delta9-tetrahydrocannabinol: a microdialysis study in conscious rats. *British journal of pharmacology*, 128(1), 21-26. doi: 10.1038/sj.bjp.0702753
- Markou, A., & Koob, G. F. (1991). Postcocaine anhedonia. An animal model of cocaine withdrawal. [Research Support, U.S. Gov't, P.H.S.]. *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology*, 4(1), 17-26.
- Marsicano, G., & Lutz, B. (1999). Expression of the cannabinoid receptor CB1 in distinct neuronal subpopulations in the adult mouse forebrain. *The European journal of neuroscience*, 11(12), 4213-4225.
- Martellotta, M. C., Cossu, G., Fattore, L., Gessa, G. L., & Fratta, W. (1998). Self-administration of the cannabinoid receptor agonist WIN 55,212-2 in drug-naïve mice. [Research Support, Non-U.S. Gov't]. *Neuroscience*, 85(2), 327-330.
- Marusich, J. A., & Wiley, J. L. (2012). Rimonabant abolishes sensitivity to workload changes in a progressive ratio procedure. [Research Support, N.I.H., Extramural]. *Pharmacology, biochemistry, and behavior*, 101(4), 575-580. doi: 10.1016/j.pbb.2012.03.004

- Matsuda, L. A., Lolait, S. J., Brownstein, M. J., Young, A. C., & Bonner, T. I. (1990). Structure of a cannabinoid receptor and functional expression of the cloned cDNA. [Research Support, Non-U.S. Gov't Research Support, U.S. Gov't, P.H.S.]. *Nature*, 346(6284), 561-564. doi: 10.1038/346561a0
- Matyas, F., Urban, G. M., Watanabe, M., Mackie, K., Zimmer, A., Freund, T. F., & Katona, I. (2008). Identification of the sites of 2-arachidonoylglycerol synthesis and action imply retrograde endocannabinoid signaling at both GABAergic and glutamatergic synapses in the ventral tegmental area. [In Vitro Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't]. *Neuropharmacology*, 54(1), 95-107. doi: 10.1016/j.neuropharm.2007.05.028
- Mavrikaki, M., Markaki, E., Nomikos, G. G., & Panagis, G. (2010). Chronic WIN55,212-2 elicits sustained and conditioned increases in intracranial self-stimulation thresholds in the rat. [Research Support, Non-U.S. Gov't]. *Behavioural Brain Research*, 209(1), 114-118. doi: 10.1016/j.bbr.2010.01.024
- McGregor, I. S., Issakidis, C. N., & Prior, G. (1996). Aversive effects of the synthetic cannabinoid CP 55,940 in rats. [Research Support, Non-U.S. Gov't]. *Pharmacology, biochemistry, and behavior*, 53(3), 657-664.
- Mechoulam, R., Ben-Shabat, S., Hanus, L., Ligumsky, M., Kaminski, N. E., Schatz, A. R., . . . et al. (1995). Identification of an endogenous 2-monoglyceride, present in canine gut, that binds to cannabinoid receptors. [Research Support, Non-U.S. Gov't Research Support, U.S. Gov't, P.H.S.]. *Biochemical pharmacology*, 50(1), 83-90.
- Melis, M., Carta, S., Fattore, L., Tolu, S., Yasar, S., Goldberg, S. R., . . . Pistis, M. (2010). Peroxisome proliferator-activated receptors- $\alpha$  modulate dopamine cell activity through nicotinic receptors. [Research Support, N.I.H., Intramural Research Support, Non-U.S. Gov't]. *Biological psychiatry*, 68(3), 256-264. doi: 10.1016/j.biopsych.2010.04.016
- Melis, M., Pillolla, G., Bisogno, T., Minassi, A., Petrosino, S., Perra, S., . . . Pistis, M. (2006). Protective activation of the endocannabinoid system during ischemia in dopamine neurons. [In Vitro Research Support, Non-U.S. Gov't]. *Neurobiology of disease*, 24(1), 15-27. doi: 10.1016/j.nbd.2006.04.010
- Mendizabal, V., Zimmer, A., & Maldonado, R. (2006). Involvement of kappa/dynorphin system in WIN 55,212-2 self-administration in mice. [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't]. *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology*, 31(9), 1957-1966. doi: 10.1038/sj.npp.1300957
- Milner, P. M. (1989). The discovery of self-stimulation and other stories. [Historical Article]. *Neuroscience and biobehavioral reviews*, 13(2-3), 61-67.
- Mittleman, G., Van Brunt, C. L., & Matthews, D. B. (2003). Schedule-induced ethanol self-administration in DBA/2J and C57BL/6J mice. [Comparative Study Research Support,

- U.S. Gov't, P.H.S.]. *Alcoholism, clinical and experimental research*, 27(6), 918-925. doi: 10.1097/01.ALC.0000071930.48632.AE
- Naidu, P. S., Varvel, S. A., Ahn, K., Cravatt, B. F., Martin, B. R., & Lichtman, A. H. (2007). Evaluation of fatty acid amide hydrolase inhibition in murine models of emotionality. [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't]. *Psychopharmacology*, 192(1), 61-70. doi: 10.1007/s00213-006-0689-4
- Negus, S. S., Morrissey, E. M., Rosenberg, M., Cheng, K., & Rice, K. C. (2010). Effects of kappa opioids in an assay of pain-depressed intracranial self-stimulation in rats. [Research Support, N.I.H., Extramural Research Support, N.I.H., Intramural]. *Psychopharmacology*, 210(2), 149-159. doi: 10.1007/s00213-009-1770-6
- Neill, D. B., Fenton, H., & Justice, J. B., Jr. (2002). Increase in accumbal dopaminergic transmission correlates with response cost not reward of hypothalamic stimulation. [Research Support, U.S. Gov't, Non-P.H.S. Research Support, U.S. Gov't, P.H.S.]. *Behavioural Brain Research*, 137(1-2), 129-138.
- Ng Cheong Ton, J. M., Gerhardt, G. A., Friedemann, M., Etgen, A. M., Rose, G. M., Sharpless, N. S., & Gardner, E. L. (1988). The effects of delta 9-tetrahydrocannabinol on potassium-evoked release of dopamine in the rat caudate nucleus: an in vivo electrochemical and in vivo microdialysis study. [Research Support, Non-U.S. Gov't Research Support, U.S. Gov't, Non-P.H.S. Research Support, U.S. Gov't, P.H.S.]. *Brain research*, 451(1-2), 59-68.
- Niphakis, M. J., Cognetta, A. B., 3rd, Chang, J. W., Buczynski, M. W., Parsons, L. H., Byrne, F., . . . Cravatt, B. F. (2013). Evaluation of NHS Carbamates as a Potent and Selective Class of Endocannabinoid Hydrolase Inhibitors. *ACS chemical neuroscience*. doi: 10.1021/cn400116z
- Niphakis, M. J., Johnson, D. S., Ballard, T. E., Stiff, C., & Cravatt, B. F. (2012). O-hydroxyacetamide carbamates as a highly potent and selective class of endocannabinoid hydrolase inhibitors. *ACS chemical neuroscience*, 3(5), 418-426. doi: 10.1021/cn200089j
- Niyuhire, F., Varvel, S. A., Martin, B. R., & Lichtman, A. H. (2007). Exposure to marijuana smoke impairs memory retrieval in mice. [Research Support, N.I.H., Extramural]. *The Journal of pharmacology and experimental therapeutics*, 322(3), 1067-1075. doi: 10.1124/jpet.107.119594
- Nomura, D. K., Morrison, B. E., Blankman, J. L., Long, J. Z., Kinsey, S. G., Marcondes, M. C., . . . Cravatt, B. F. (2011). Endocannabinoid hydrolysis generates brain prostaglandins that promote neuroinflammation. [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't]. *Science*, 334(6057), 809-813. doi: 10.1126/science.1209200
- Ohno-Shosaku, T., Tsubokawa, H., Mizushima, I., Yoneda, N., Zimmer, A., & Kano, M. (2002). Presynaptic cannabinoid sensitivity is a major determinant of depolarization-induced retrograde suppression at hippocampal synapses. [In Vitro Research Support, Non-U.S. Gov't]. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, 22(10), 3864-3872. doi: 20026387

- Olds, J., & Milner, P. (1954). Positive reinforcement produced by electrical stimulation of septal area and other regions of rat brain. *Journal of comparative and physiological psychology*, 47(6), 419-427.
- Onaivi, E. S. (2006). Neuropsychobiological evidence for the functional presence and expression of cannabinoid CB2 receptors in the brain. *Neuropsychobiology*, 54(4), 231-246. doi: 10.1159/000100778
- Onaivi, E. S., Ishiguro, H., Gong, J. P., Patel, S., Perchuk, A., Meozzi, P. A., . . . Uhl, G. R. (2006). Discovery of the presence and functional expression of cannabinoid CB2 receptors in brain. [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't Review]. *Annals of the New York Academy of Sciences*, 1074, 514-536. doi: 10.1196/annals.1369.052
- Ong, W. Y., & Mackie, K. (1999). A light and electron microscopic study of the CB1 cannabinoid receptor in primate brain. [Research Support, Non-U.S. Gov't Research Support, U.S. Gov't, P.H.S.]. *Neuroscience*, 92(4), 1177-1191.
- Pan, B., Wang, W., Long, J. Z., Sun, D., Hillard, C. J., Cravatt, B. F., & Liu, Q. S. (2009). Blockade of 2-arachidonoylglycerol hydrolysis by selective monoacylglycerol lipase inhibitor 4-nitrophenyl 4-(dibenzo[d][1,3]dioxol-5-yl(hydroxy)methyl)piperidine-1-carboxylate (JZL184) Enhances retrograde endocannabinoid signaling. [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't]. *The Journal of pharmacology and experimental therapeutics*, 331(2), 591-597. doi: 10.1124/jpet.109.158162
- Panagis, G., Vlachou, S., & Nomikos, G. G. (2008). Behavioral pharmacology of cannabinoids with a focus on preclinical models for studying reinforcing and dependence-producing properties. [Research Support, Non-U.S. Gov't Review]. *Current drug abuse reviews*, 1(3), 350-374.
- Panlilio, L. V., & Goldberg, S. R. (2007). Self-administration of drugs in animals and humans as a model and an investigative tool. [Research Support, N.I.H., Intramural Review]. *Addiction*, 102(12), 1863-1870. doi: 10.1111/j.1360-0443.2007.02011.x
- Panos, J. J., & Baker, L. E. (2010). An in vivo microdialysis assessment of concurrent MDMA and cocaine administration in Sprague-Dawley rats. [Research Support, N.I.H., Extramural]. *Psychopharmacology*, 209(1), 95-102. doi: 10.1007/s00213-009-1774-2
- Patel, S., & Hillard, C. J. (2006). Pharmacological evaluation of cannabinoid receptor ligands in a mouse model of anxiety: further evidence for an anxiolytic role for endogenous cannabinoid signaling. [Comparative Study Research Support, N.I.H., Extramural]. *The Journal of pharmacology and experimental therapeutics*, 318(1), 304-311. doi: 10.1124/jpet.106.101287
- Pertwee, R. G. (2012). Targeting the endocannabinoid system with cannabinoid receptor agonists: pharmacological strategies and therapeutic possibilities. [Review]. *Philosophical transactions of the Royal Society of London. Series B, Biological sciences*, 367(1607), 3353-3363. doi: 10.1098/rstb.2011.0381

- Pertwee, R. G., Howlett, A. C., Abood, M. E., Alexander, S. P., Di Marzo, V., Elphick, M. R., . . . Ross, R. A. (2010). International Union of Basic and Clinical Pharmacology. LXXIX. Cannabinoid receptors and their ligands: beyond CB(1) and CB(2). [Research Support, N.I.H., Extramural Research Support, N.I.H., Intramural Research Support, Non-U.S. Gov't Review]. *Pharmacological reviews*, 62(4), 588-631. doi: 10.1124/pr.110.003004
- Piomelli, D., Tarzia, G., Duranti, A., Tontini, A., Mor, M., Compton, T. R., . . . Putman, D. (2006). Pharmacological profile of the selective FAAH inhibitor KDS-4103 (URB597). [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't Review]. *CNS drug reviews*, 12(1), 21-38. doi: 10.1111/j.1527-3458.2006.00021.x
- Porter, A. C., Sauer, J. M., Knierman, M. D., Becker, G. W., Berna, M. J., Bao, J., . . . Felder, C. C. (2002). Characterization of a novel endocannabinoid, virodhamine, with antagonist activity at the CB1 receptor. [Research Support, U.S. Gov't, P.H.S.]. *The Journal of pharmacology and experimental therapeutics*, 301(3), 1020-1024.
- Pryce, G., Cabranes, A., Fernandez-Ruiz, J., Bisogno, T., Di Marzo, V., Long, J., . . . Baker, D. (2013). Control of experimental spasticity by targeting the degradation of endocannabinoids using selective fatty acid amide hydrolase inhibitors. *Multiple sclerosis*. doi: 10.1177/1352458513485982
- Ramesh, D., Gamage, T. F., Vanuytsel, T., Owens, R. A., Abdullah, R. A., Niphakis, M. J., . . . Lichtman, A. H. (2013). Dual Inhibition of Endocannabinoid Catabolic Enzymes Produces Enhanced Antiwithdrawal Effects in Morphine-Dependent Mice. *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology*. doi: 10.1038/npp.2012.269
- Ramesh, D., Ross, G. R., Schlosburg, J. E., Owens, R. A., Abdullah, R. A., Kinsey, S. G., . . . Lichtman, A. H. (2011). Blockade of endocannabinoid hydrolytic enzymes attenuates precipitated opioid withdrawal symptoms in mice. [Research Support, N.I.H., Extramural]. *The Journal of pharmacology and experimental therapeutics*, 339(1), 173-185. doi: 10.1124/jpet.111.181370
- Randt, C. T., & Quartermain, D. (1972). Motivation for electrical stimulation of the brain and for natural reinforcement in mice. *Experimental neurology*, 35(2), 366-377.
- Rinaldi-Carmona, M., Barth, F., Heaulme, M., Shire, D., Calandra, B., Congy, C., . . . et al. (1994). SR141716A, a potent and selective antagonist of the brain cannabinoid receptor. [In Vitro]. *FEBS letters*, 350(2-3), 240-244.
- Robbe, D., Alonso, G., Duchamp, F., Bockaert, J., & Manzoni, O. J. (2001). Localization and mechanisms of action of cannabinoid receptors at the glutamatergic synapses of the mouse nucleus accumbens. [In Vitro Research Support, Non-U.S. Gov't]. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, 21(1), 109-116.
- Robinson, J. E., Agoglia, A. E., Fish, E. W., Krouse, M. C., & Malanga, C. J. (2012). Mephedrone (4-methylmethcathinone) and intracranial self-stimulation in C57BL/6J mice: comparison to cocaine. [Comparative Study Research Support, N.I.H., Extramural]. *Behavioural Brain Research*, 234(1), 76-81. doi: 10.1016/j.bbr.2012.06.012



- Robledo, P., Trigo, J. M., Panayi, F., de la Torre, R., & Maldonado, R. (2007). Behavioural and neurochemical effects of combined MDMA and THC administration in mice. [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't]. *Psychopharmacology*, 195(2), 255-264. doi: 10.1007/s00213-007-0879-8
- Rolls, E. T., Burton, M. J., & Mora, F. (1980). Neurophysiological analysis of brain-stimulation reward in the monkey. *Brain research*, 194(2), 339-357. doi: [http://dx.doi.org/10.1016/0006-8993\(80\)91216-0](http://dx.doi.org/10.1016/0006-8993(80)91216-0)
- Ross, R. A. (2003). Anandamide and vanilloid TRPV1 receptors. [Review]. *British journal of pharmacology*, 140(5), 790-801. doi: 10.1038/sj.bjp.0705467
- Sagar, D. R., Staniaszek, L. E., Okine, B. N., Woodhams, S., Norris, L. M., Pearson, R. G., . . . Chapman, V. (2010). Tonic modulation of spinal hyperexcitability by the endocannabinoid receptor system in a rat model of osteoarthritis pain. [Research Support, Non-U.S. Gov't]. *Arthritis and rheumatism*, 62(12), 3666-3676. doi: 10.1002/art.27698
- Sanudo-Pena, M. C., Tsou, K., Delay, E. R., Hohman, A. G., Force, M., & Walker, J. M. (1997). Endogenous cannabinoids as an aversive or counter-rewarding system in the rat. [Research Support, U.S. Gov't, P.H.S.]. *Neuroscience letters*, 223(2), 125-128.
- Schaefer, G. J., & Michael, R. P. (1992). Interactions between alcohol and nicotine on intracranial self-stimulation and locomotor activity in rats. [Research Support, Non-U.S. Gov't]. *Drug and alcohol dependence*, 30(1), 37-47.
- Schaefer, G. J., Richardson, W. R., Bonsall, R. W., & Michael, R. P. (1988). Brain self-stimulation, locomotor activity and tissue concentrations of ethanol in male rats. [Research Support, Non-U.S. Gov't]. *Drug and alcohol dependence*, 21(1), 67-75.
- Schlosburg, J. E., Blankman, J. L., Long, J. Z., Nomura, D. K., Pan, B., Kinsey, S. G., . . . Cravatt, B. F. (2010). Chronic monoacylglycerol lipase blockade causes functional antagonism of the endocannabinoid system. [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't]. *Nature neuroscience*, 13(9), 1113-1119. doi: 10.1038/nn.2616
- Schulteis, G., Markou, A., Cole, M., & Koob, G. F. (1995). Decreased brain reward produced by ethanol withdrawal. [Comparative Study Research Support, U.S. Gov't, P.H.S.]. *Proceedings of the National Academy of Sciences of the United States of America*, 92(13), 5880-5884.
- Sciolino, N. R., Zhou, W., & Hohmann, A. G. (2011). Enhancement of endocannabinoid signaling with JZL184, an inhibitor of the 2-arachidonoylglycerol hydrolyzing enzyme monoacylglycerol lipase, produces anxiolytic effects under conditions of high environmental aversiveness in rats. [Research Support, N.I.H., Extramural]. *Pharmacological research : the official journal of the Italian Pharmacological Society*, 64(3), 226-234. doi: 10.1016/j.phrs.2011.04.010

- Shalev, U. (2012). Chronic food restriction augments the reinstatement of extinguished heroin-seeking behavior in rats. [Research Support, Non-U.S. Gov't]. *Addiction biology*, 17(4), 691-693. doi: 10.1111/j.1369-1600.2010.00303.x
- Shizgal, P., Schindler, D., & Rompre, P. P. (1989). Forebrain neurons driven by rewarding stimulation of the medial forebrain bundle in the rat: comparison of psychophysical and electrophysiological estimates of refractory periods. [Research Support, Non-U.S. Gov't]. *Brain research*, 499(2), 234-248.
- Stark, P., & Dews, P. B. (1980). Cannabinoids. I. Behavioral effects. *The Journal of pharmacology and experimental therapeutics*, 214(1), 124-130.
- Stella, N. (2010). Cannabinoid and cannabinoid-like receptors in microglia, astrocytes, and astrocytomas. [Research Support, N.I.H., Extramural Review]. *Glia*, 58(9), 1017-1030. doi: 10.1002/glia.20983
- Straub, C. J., Carlezon, W. A., Jr., & Rudolph, U. (2010). Diazepam and cocaine potentiate brain stimulation reward in C57BL/6J mice. [Research Support, Non-U.S. Gov't]. *Behavioural Brain Research*, 206(1), 17-20. doi: 10.1016/j.bbr.2009.08.025
- Sugiura, T., Kondo, S., Sukagawa, A., Nakane, S., Shinoda, A., Itoh, K., . . . Waku, K. (1995). 2-Arachidonoylglycerol: a possible endogenous cannabinoid receptor ligand in brain. *Biochemical and biophysical research communications*, 215(1), 89-97.
- Takahashi, R. N., & Singer, G. (1979). Self-administration of delta 9-tetrahydrocannabinol by rats. [Research Support, U.S. Gov't, P.H.S.]. *Pharmacology, biochemistry, and behavior*, 11(6), 737-740.
- Takahashi, R. N., & Singer, G. (1980). Effects of body weight levels on cannabis self-injection. [Research Support, Non-U.S. Gov't]. *Pharmacology, biochemistry, and behavior*, 13(6), 877-881.
- Tanda, G., & Goldberg, S. R. (2003). Cannabinoids: reward, dependence, and underlying neurochemical mechanisms--a review of recent preclinical data. [Research Support, U.S. Gov't, P.H.S. Review]. *Psychopharmacology*, 169(2), 115-134. doi: 10.1007/s00213-003-1485-z
- Tanda, G., Munzar, P., & Goldberg, S. R. (2000). Self-administration behavior is maintained by the psychoactive ingredient of marijuana in squirrel monkeys. *Nature neuroscience*, 3(11), 1073-1074. doi: 10.1038/80577
- Tanda, G., Pontieri, F. E., & Di Chiara, G. (1997). Cannabinoid and heroin activation of mesolimbic dopamine transmission by a common mu1 opioid receptor mechanism. [Comparative Study Research Support, Non-U.S. Gov't]. *Science*, 276(5321), 2048-2050.
- Tanimura, A., Yamazaki, M., Hashimotodani, Y., Uchigashima, M., Kawata, S., Abe, M., . . . Kano, M. (2010). The endocannabinoid 2-arachidonoylglycerol produced by diacylglycerol lipase alpha mediates retrograde suppression of synaptic transmission. [In

- Vitro Research Support, Non-U.S. Gov't]. *Neuron*, 65(3), 320-327. doi: 10.1016/j.neuron.2010.01.021
- Trujillo-Pisanty, I., Hernandez, G., Moreau-Debord, I., Cossette, M. P., Conover, K., Cheer, J. F., & Shizgal, P. (2011). Cannabinoid receptor blockade reduces the opportunity cost at which rats maintain operant performance for rewarding brain stimulation. [Research Support, Non-U.S. Gov't]. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, 31(14), 5426-5435. doi: 10.1523/JNEUROSCI.0079-11.2011
- Tsuboi, K., Sun, Y. X., Okamoto, Y., Araki, N., Tonai, T., & Ueda, N. (2005). Molecular characterization of N-acylethanolamine-hydrolyzing acid amidase, a novel member of the cholesteryl glycerophosphorylcholine hydrolase family with structural and functional similarity to acid ceramidase. [Research Support, Non-U.S. Gov't]. *The Journal of biological chemistry*, 280(12), 11082-11092. doi: 10.1074/jbc.M413473200
- Ueda, N., Yamanaka, K., & Yamamoto, S. (2001). Purification and characterization of an acid amidase selective for N-palmitoylethanolamine, a putative endogenous anti-inflammatory substance. [Research Support, Non-U.S. Gov't]. *The Journal of biological chemistry*, 276(38), 35552-35557. doi: 10.1074/jbc.M106261200
- Van Sickle, M. D., Duncan, M., Kingsley, P. J., Mouihate, A., Urbani, P., Mackie, K., . . . Sharkey, K. A. (2005). Identification and functional characterization of brainstem cannabinoid CB2 receptors. [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't Research Support, U.S. Gov't, P.H.S.]. *Science*, 310(5746), 329-332. doi: 10.1126/science.1115740
- van Tienhoven, M., Atkins, J., Li, Y., & Glynn, P. (2002). Human neuropathy target esterase catalyzes hydrolysis of membrane lipids. [Research Support, Non-U.S. Gov't]. *The Journal of biological chemistry*, 277(23), 20942-20948. doi: 10.1074/jbc.M200330200
- Vandrey, R., Stitzer, M. L., Mintzer, M. Z., Huestis, M. A., Murray, J. A., & Lee, D. (2013). The dose effects of short-term dronabinol (oral THC) maintenance in daily cannabis users. [Clinical Trial Research Support, N.I.H., Extramural]. *Drug and alcohol dependence*, 128(1-2), 64-70. doi: 10.1016/j.drugalcdep.2012.08.001
- Varvel, S. A., Wiley, J. L., Yang, R., Bridgen, D. T., Long, K., Lichtman, A. H., & Martin, B. R. (2006). Interactions between THC and cannabidiol in mouse models of cannabinoid activity. [Research Support, N.I.H., Extramural]. *Psychopharmacology*, 186(2), 226-234. doi: 10.1007/s00213-006-0356-9
- Varvel, S. A., Wise, L. E., Niyuhire, F., Cravatt, B. F., & Lichtman, A. H. (2007). Inhibition of fatty-acid amide hydrolase accelerates acquisition and extinction rates in a spatial memory task. [Research Support, N.I.H., Extramural]. *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology*, 32(5), 1032-1041. doi: 10.1038/sj.npp.1301224
- Vinklerova, J., Novakova, J., & Sulcova, A. (2002). Inhibition of methamphetamine self-administration in rats by cannabinoid receptor antagonist AM 251. [Research Support, Non-U.S. Gov't]. *Journal of psychopharmacology*, 16(2), 139-143.

- Vlachou, S., Nomikos, G. G., & Panagis, G. (2003). WIN 55,212-2 decreases the reinforcing actions of cocaine through CB1 cannabinoid receptor stimulation. *Behavioural Brain Research*, 141(2), 215-222. doi: 10.1016/s0166-4328(02)00370-4
- Vlachou, S., Nomikos, G. G., & Panagis, G. (2005). CB1 cannabinoid receptor agonists increase intracranial self-stimulation thresholds in the rat. [Research Support, Non-U.S. Gov't]. *Psychopharmacology*, 179(2), 498-508. doi: 10.1007/s00213-004-2050-0
- Vlachou, S., Nomikos, G. G., & Panagis, G. (2006). Effects of endocannabinoid neurotransmission modulators on brain stimulation reward. [Research Support, Non-U.S. Gov't]. *Psychopharmacology*, 188(3), 293-305. doi: 10.1007/s00213-006-0506-0
- Vlachou, S., Nomikos, G. G., Stephens, D. N., & 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. [Research Support, Non-U.S. Gov't]. *Behavioural pharmacology*, 18(4), 311-319. doi: 10.1097/FBP.0b013e3282186cf2
- Vlachou, S., Stamatopoulou, F., Nomikos, G. G., & Panagis, G. (2008). Enhancement of endocannabinoid neurotransmission through CB1 cannabinoid receptors counteracts the reinforcing and psychostimulant effects of cocaine. [Research Support, Non-U.S. Gov't]. *The international journal of neuropsychopharmacology / official scientific journal of the Collegium Internationale Neuropsychopharmacologicum*, 11(7), 905-923. doi: 10.1017/S1461145708008717
- Wei, B. Q., Mikkelsen, T. S., McKinney, M. K., Lander, E. S., & Cravatt, B. F. (2006). A second fatty acid amide hydrolase with variable distribution among placental mammals. [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't]. *The Journal of biological chemistry*, 281(48), 36569-36578. doi: 10.1074/jbc.M606646200
- Wiebelhaus, J. M., Poklis, J. L., Poklis, A., Vann, R. E., Lichtman, A. H., & Wise, L. E. (2012). Inhalation exposure to smoke from synthetic "marijuana" produces potent cannabimimetic effects in mice. [Research Support, N.I.H., Extramural]. *Drug and alcohol dependence*, 126(3), 316-323. doi: 10.1016/j.drugalcdep.2012.05.034
- Wiley, J. L., Burston, J. J., Leggett, D. C., Alekseeva, O. O., Razdan, R. K., Mahadevan, A., & Martin, B. R. (2005). CB1 cannabinoid receptor-mediated modulation of food intake in mice. [Comparative Study Research Support, N.I.H., Extramural Research Support, U.S. Gov't, P.H.S.]. *British journal of pharmacology*, 145(3), 293-300. doi: 10.1038/sj.bjp.0706157
- Wiley, J. L., Lowe, J. A., Balster, R. L., & Martin, B. R. (1995). Antagonism of the discriminative stimulus effects of delta 9-tetrahydrocannabinol in rats and rhesus monkeys. [Research Support, U.S. Gov't, P.H.S.]. *The Journal of pharmacology and experimental therapeutics*, 275(1), 1-6.
- Wiley, J. L., Marusich, J. A., Lefever, T. W., Grabenauer, M., Moore, K. N., & Thomas, B. F. (2013). Cannabinoids in disguise: Delta-Tetrahydrocannabinol-like effects of tetramethylcyclopropyl ketone indoles. *Neuropharmacology*, 75C, 145-154. doi: 10.1016/j.neuropharm.2013.07.022

- Wise, L. E., Harloe, J. P., & Lichtman, A. H. (2009). Fatty acid amide hydrolase (FAAH) knockout mice exhibit enhanced acquisition of an aversive, but not of an appetitive, Barnes maze task. [Comparative Study Research Support, N.I.H., Extramural]. *Neurobiology of learning and memory*, 92(4), 597-601. doi: 10.1016/j.nlm.2009.06.001
- Wise, L. E., Long, K. A., Abdullah, R. A., Long, J. Z., Cravatt, B. F., & Lichtman, A. H. (2012). Dual Fatty Acid Amide Hydrolase and Monoacylglycerol Lipase Blockade Produces THC-Like Morris Water Maze Deficits in Mice. *ACS chemical neuroscience*, 3(5), 369-378. doi: 10.1021/cn200130s
- Wise, R. A. (2002). Brain Reward Circuitry: Insights from Unsensed Incentives. *Neuron*, 36(2), 229-240. doi: [http://dx.doi.org/10.1016/S0896-6273\(02\)00965-0](http://dx.doi.org/10.1016/S0896-6273(02)00965-0)
- Wise, R. A. (2005). Forebrain substrates of reward and motivation. [Review]. *The Journal of comparative neurology*, 493(1), 115-121. doi: 10.1002/cne.20689
- Wiskerke, J., Irimia, C., Cravatt, B. F., De Vries, T. J., Schoffelmeer, A. N., Pattij, T., & Parsons, L. H. (2012). Characterization of the effects of reuptake and hydrolysis inhibition on interstitial endocannabinoid levels in the brain: an in vivo microdialysis study. *ACS chemical neuroscience*, 3(5), 407-417. doi: 10.1021/cn300036b
- Xi, Z. X., Peng, X. Q., Li, X., Song, R., Zhang, H. Y., Liu, Q. R., . . . Gardner, E. L. (2011). Brain cannabinoid CB(2) receptors modulate cocaine's actions in mice. [Research Support, N.I.H., Intramural]. *Nature neuroscience*, 14(9), 1160-1166. doi: 10.1038/nn.2874
- Xi, Z. X., Spiller, K., Pak, A. C., Gilbert, J., Dillon, C., Li, X., . . . Gardner, E. L. (2008). Cannabinoid CB1 receptor antagonists attenuate cocaine's rewarding effects: experiments with self-administration and brain-stimulation reward in rats. [Research Support, N.I.H., Intramural]. *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology*, 33(7), 1735-1745. doi: 10.1038/sj.npp.1301552
- Xi, Z. X., Yang, Z., Li, S. J., Li, X., Dillon, C., Peng, X. Q., . . . Gardner, E. L. (2007). Levo-tetrahydropalmatine inhibits cocaine's rewarding effects: experiments with self-administration and brain-stimulation reward in rats. [Research Support, N.I.H., Intramural Research Support, Non-U.S. Gov't]. *Neuropharmacology*, 53(6), 771-782. doi: 10.1016/j.neuropharm.2007.08.004
- Yoshida, T., Fukaya, M., Uchigashima, M., Miura, E., Kamiya, H., Kano, M., & Watanabe, M. (2006). Localization of diacylglycerol lipase-alpha around postsynaptic spine suggests close proximity between production site of an endocannabinoid, 2-arachidonoyl-glycerol, and presynaptic cannabinoid CB1 receptor. [Comparative Study Research Support, Non-U.S. Gov't]. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, 26(18), 4740-4751. doi: 10.1523/JNEUROSCI.0054-06.2006
- Zygmunt, P. M., Petersson, J., Andersson, D. A., Chuang, H., Sorgard, M., Di Marzo, V., . . . Hogestatt, E. D. (1999). Vanilloid receptors on sensory nerves mediate the vasodilator action of anandamide. [In Vitro Research Support, Non-U.S. Gov't Research Support, U.S. Gov't, P.H.S.]. *Nature*, 400(6743), 452-457. doi: 10.1038/22761

## Vita

Jason Michael Wiebelhaus was born September 8, 1982 in Whittier, CA. He grew up in Austin, MN. He received his Bachelor of Science degree in psychology from the University of WI-Eau Claire in 2006. Jason entered the department of psychology (biopsychology) at Virginia Commonwealth University as a graduate student in 2007, where he earned his Master of Science degree while working under Dr. Joseph H. Porter studying the discriminative stimulus properties of antipsychotic drugs in 2009.