The Therapeutic Potential of Serotonin 1B Receptor Agonists for Treating Psychostimulant Use Disorders

by

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ABSTRACT

Serotonin 1B receptor (5-HT_{1B}R) agonists enhance cocaine intake in rats during daily self-administration (SA) sessions, yet decrease cocaine intake after prolonged abstinence. The goal of my dissertation was to examine if 5-HT_{1B}Rs are suitable targets for treatment development to attenuate psychostimulant intake. I first investigated if 5-HT_{1B}R agonist effects that had been observed with cocaine generalize across psychostimulants, i.e., methamphetamine. Rats trained to self-administer methamphetamine received either CP 94,253 or the clinically-available but less selective 5-$HT_{1D/1B}$R agonist, zolmitriptan, prior to tests for effects on SA both before and after a 21-day abstinence period. I found that CP 94,253 and zolmitriptan decreased the reinforcing and incentive motivational effects of methamphetamine, regardless of abstinence, unlike the pre-abstinence increase in cocaine SA observed previously with 5-HT_{1B}R agonists. The attenuating effects of CP 94,253 on methamphetamine were antagonized in a 5-HT_{1B}R-mediated manner. Subsequently, I investigated the efficacy and mechanism involved in effects of zolmitriptan on cocaine SA in male and female rats. Rats trained to self-administer cocaine received zolmitriptan prior to tests for effects on SA before a 21-day abstinence period. I found that zolmitriptan decreased cocaine intake in both sexes regardless of abstinence and without altering sucrose intake. I further demonstrated that the zolmitriptan effects on cocaine SA were mediated by both 5-HT_{1B}Rs and 5-HT_{1D}Rs. Finally, I examined if the abstinence-induced decrease in cocaine intake observed with the selective 5-HT_{1B}R agonist, CP 94,253, persists during relapse after abstinence or reverts to enhancing cocaine intake, similar to effects observed without an abstinence period. Rats trained to self-administer cocaine resumed daily cocaine SA sessions after the forced abstinence period to investigate

the effects of CP 94,253 on cocaine relapse. I found that CP 94,253 attenuated cocaine intake in relapse tests, suggesting that the abstinence-dependent attenuation of CP 94,253 on cocaine SA remains even after resumption of daily cocaine intake. The findings suggest that 5-HT_{1B}R agonists like CP 94,253 and zolmitriptan have clinical potential as treatments for psychostimulant use disorders.

DEDICATION

To all my mentors over the years,

for inspiring me, challenging me,

and guiding me on the path to success.

To my entire family and dear friends,

for providing unrelenting love and support along this journey.

To my mom, dad, and brothers

for always being proud of me and encouraging me to accomplish my goals.

Finally, I would like to dedicate this dissertation

to the underrepresented and underprivileged community

that raised me and taught me the value of hard work

and the importance of resilience.

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CHAPTER 1

GENERAL OVERVIEW

Substance use disorders

Substance use disorders (SUDs) remain a prevalent problem worldwide with many individual and social consequences comprising an estimated cost of more than $700 billion related to health care, crime, and loss of productivity (NDIC, 2011; NIDA, 2020). SUDs are classified as a continuum of symptoms ranging from mild to severe. The diagnostic criteria consists of an individual having two or more of the following symptoms: social/interpersonal problems related to use, neglected impaired control over drug use, increased time and energy spent seeking and using drugs, intense craving to use drugs, inability to abstain or reduce drug use, hazardous drug use, undergoing withdrawal, developing tolerance, compulsive drug use, activities given up due to drug use, and physical/psychological problems related to drug use. Overall severity diagnosis depends on total criteria count where two to three symptoms indicate mild, four to five symptoms indicate moderate, and six or more symptoms indicate the most severe form of SUDs (APA, 2013; NIDA, 2018).

Significant advances have been made in developing pharmacotherapeutics to treat some SUDs (e.g. methadone for opioid and varenicline for nicotine abuse); however, there is still no clinically-available treatment for psychostimulant abuse. In the United States, the use of psychostimulants including cocaine and methamphetamine is on the rise (Karila et al., 2014; Hughes, Williams, Lipari, & Horn, 2016; SAMHSA, 2017) and reports indicate a 37% increase in deaths from psychostimulant overdose from 2016 to 2017 (CDC, 2017;

Kariisa et al., 2019). These recent reports stress the importance for finding effective pharmacotherapeutics to treat psychostimulant abuse.

Evaluation of several pharmacological candidates for treating psychostimulant use disorders have produced limited results. Modafinil is a stimulant used to treat sleep disorders and initially was found to block the euphoric effects of cocaine in humans (Dackis et al., 2003; Malcolm et al., 2006). However, large clinical trials have produced negative results indicating that modafinil does not alter reported craving for cocaine in cocaine-dependent patients relative to control groups (Dackis et al., 2012). Additionally, modafinil administration resulted in low retention success with only 10% of subjects completing treatment (Nuijten et al., 2015). Evaluation of selective 5-HT reuptake inhibitors (SSRIs) such as fluoxetine and paroxetine, demonstrated either limited or no efficacy for reducing methamphetamine use in clinical trials (Batki et al., 2000; Piasecki et al., 2002). Similarly, other candidates like topiramate and vigabatrin have generated negative or mixed results for cocaine or methamphetamine (Elkashef et al., 2012; Somoza et al., 2013; Singh et al., 2016). This lack of success highlights the importance for comprehensive preclinical and clinical evaluation of treatments for psychostimulant use disorders.

Self-administration model

The drug self-administration (SA) model is highly utilized in the laboratory setting in animals to examine drug-taking and drug-seeking behavior under a controlled environment. The drug SA model has face, construct, and predictive validity as well as species generality (Panlilio & Goldberg, 2007). Animals reliably self-administer most

drugs of abuse in a manner homologous to human drug taking and will reinstate drug-seeking following a period of abstinence, like humans, when exposed to external or interoceptive cues like those that put humans at risk for drug relapse. Additionally, the SA model characterizes the propensity of drugs to lead to SUDs since it demonstrates the reinforcing efficacy of known drugs of abuse and has been successful in predicting the abuse liability of novel compounds (Yokel, 1987; Panlilio & Goldberg, 2007; Haney & Spealman, 2008; O'Connor et al., 2011).

The SA model relies on operant conditioning procedures that require an organism to perform a response on an operandum resulting in reinforcer presentation. Typically, a rat must press a lever or poke an aperture with its nose to receive an intravenous drug infusion. This model assumes that drugs of abuse function as positive reinforcers, which increase the likelihood of the response that preceded the reinforcer. The availability of the reinforcer is contingent on performance requirements known as schedules of reinforcement. These schedules describe the number of responses required (ratio) or the total time needed to elapse (interval) before the reinforcer is available. For example, a rat on a fixed-ratio 5 (FR5) schedule of reinforcement would need to emit 5 lever responses for delivery of the reinforcer. Additionally, the number or responses or time needed to elapse can vary across succeeding trials (i.e. variable-ratio or variable-interval schedules). Each of these reinforcement schedules produce patterns of responding that can inform how treatments affect behavioral processes. Additionally, environmental cues present during drug delivery or presented with the drug reward, such as auditory or visual cues, become predictive of the drug's availability and/or the drug's effects. These previously neutral stimuli become associated with the drug reward and other effects, and through this

association they acquire incentive salience and can elicit incentive motivation to initiate and sustain drug-seeking and drug-taking behaviors (Markou et al., 1993; Robinson & Berridge, 1993).

The SA procedure can also model aspects of relapse following cessation of drug use. Relapse involves resumption of drug use following a period of forced or voluntary abstinence. The extinction/reinstatement and forced abstinence models are two common procedures utilized to study aspects of relapse in the preclinical setting. In the extinction/reinstatement model, conditioned operant responses no longer elicit drug availability resulting in overall decline or *extinguishing* of operant responding for the drug. Operant responding can then be reinstated following non-contingent exposure to either the drug or drug-paired stimuli (de Wit & Stewart, 1981; Stewart & de Wit, 1987; Fuchs et la., 1998). Re-exposure to the drug, drug-associated cues, or stressors have been demonstrated to reinstate drug craving in humans and drug-seeking behavior in laboratory animals (Sinha et al., 2000; Shaham & Miczek, 2003). In the forced abstinence model, the subject is removed from the drug-accessible environment and maintained in its home cage typically for a protracted withdrawal period before being reintroduced to the drug-accessible environment and tested with or without drug (Neisewander et al., 2000; Tran-Nguyen et al., 1998; Pentkowski et al., 2012). Under these testing conditions, drug-seeking has been shown to increase as a function of abstinence length (Neisewander et al., 2000; Tran-Nguyen et al., 1998, Grimm et al., 2001), which has become known as the incubation effect (Grimm et al., 2001). In summary, the SA paradigm has expanded our understanding of the neurobiological mechanisms involved in vulnerability to initiate drug-taking as well as the susceptibility to relapse following abstinence from the drug. More importantly, it has

helped identify pharmacological candidates for clinical evaluation (Haney & Spealman, 2008).

Psychostimulants

Psychostimulants, like other drugs of abuse, exert their effects by activating the mesolimbic "reward" pathway originating in the ventral tegmental area (VTA) that sends axonal projections to the nucleus accumbens (NAc) as well as other parts of the limbic system (Pierce & Kumaresan, 2006; Feltenstein & See, 2008; Wise, 2008; Ikemoto, 2010). Psychostimulants act as potent reinforcers due in part to their ability to increase synaptic monoamine (e.g. dopamine) levels by blocking presynaptic monoamine transporters (e.g. dopamine transporters, DAT) that recycle and terminate synaptic monoamine signaling (Howell & Kimmel, 2007). Additionally, methamphetamine and other amphetamine derivatives also interfere with the function of monoamine transporters by redistributing and reversing intracellular transport of monoamines through action at vesicular and plasma membrane monoamine transporters, resulting in overall increased extravesicular and extracellular monoamine levels (Sager & Torres, 2011; Panenka et al., 2013). Studies utilizing humans and animals have provided evidence for an enhancement of dopamine neurotransmitter levels in the striatum following psychostimulant administration (Koob, 1992; Volkow et al., 1999; Wise, 2008; Volkow, Koob, & McLellan, 2016). However, dopamine is not the only monoamine that psychostimulants affect. In addition to blocking DATs, psychostimulants also block serotonin and norepinephrine transporters (SERT, & NET, respectively; Rothman & Baumann, 2001, 2003; Feltenstein & See, 2008; Howell & Kimmel, 2008). Indeed, a plethora of evidence exists detailing the effects of psychostimulants on brain serotonin levels. Both cocaine and methamphetamine

administration have been shown to increase extracellular serotonin levels throughout sub- and neocortical structures, including those involved in the mesolimbic pathway (Parsons & Justice, 1993; Teneud et al., 1996; Kuczenski et al., 1995; Reith, Li, & Yan, 1997; Segal & Kuczenski, 1997; Műller et al., 2002; Pum, et al., 2007).

Serotonin

Serotonin (5-hydroxytryptamine; 5-HT) is an evolutionary conserved monoamine found across most living organisms including fungi, plants, and animals (Garattinin and Valzelli, 1965). In humans, 5-HT is found in all organs of the body including the skin, gut, lungs, kidneys, liver, and brain, as well as on linings of blood vessels (Lucki, 1998; Azmitia, 2007). Most of the 5-HT found in the nervous system is in enterochromaffin cells of the enteric nervous system; while ~10% of 5-HT is in a cluster of cell bodies in the hindbrain collectively known as the raphe nuclei (Tork, 1990). In the central nervous system, 5-HT has wide neuronal projections innervating multiple regions including the hypothalamus, cortex, hippocampus, amygdala, striatum, ventral tegmental area, and substantia nigra (Steinbusch, 1981). This pattern of innervation is ideally structured to influence the function of several brain regions and is involved in various physiological functions including cardiovascular regulation, respiration, thermoregulation, circadian rhythm, appetite, sexual behavior, and pain sensitivity (Eison & Eison, 1994; Schwartz et al., 1995; Lucki, 1998; Cote et al., 2004: Aleksandrin, Tarasova, & Tarakanov, 2005). In addition, 5-HT has been implicated in several psychiatric and behavioral disorders such as depression, anxiety disorders, obsessive-compulsive disorders, schizophrenia, eating

disorders, aggression, impulsivity, and substance use disorders (Naughton, Mulrooney, & Leonard, 2000; Ener et al., 2003; Filip & Bader, 2009; Nordquist & Oreland, 2010).

The effects of 5-HT are mediated by 14 receptor subtypes that are classified into 7 receptor families, each having a unique brain distribution and varying signal transduction mechanisms (Hoyer et al., 1994). The 5-HT_1 receptor family comprises the largest subclass of 5-HT receptors and consists of 5-HT receptors 1A, 1B, 1D, 1E, & 1F. These five receptor subtypes are characterized by their similar signaling properties ($G_{i/o}$-coupled signaling) and structural homology but can be distinguished based on their differential brain distribution and with selective ligands (Gerhardt & van Heerikhuizen, 1997; Albert & Tiberi, 2001). We and others have suggested that the 5-HT_{1B} receptor subtype (5-HT_{1B}R) is a potential target for psychostimulant abuse treatment development (Callahan & Cunningham, 1995; Rocha, et al., 1998; Miszkiel, Filip, & Przegalinski, 2011; Neisewander, Cheung, & Pentkowski, 2014; Garcia et al., 2017).

5-HT_{1B}Rs

5-HT_{1B}Rs are G-protein coupled receptors that are widely distributed throughout the brain and are expressed as either autoreceptors on 5-HT terminals or heteroreceptors on non-5-HT terminals (Bruinvels et al., 1994; Lanfumey and Hamon, 2004; Varnas et al., 2005; Clark et al., 2006). In both cases, 5-HT_{1B}R transduction is inhibitory and prevents neurotransmitter release through the following sequence of events: (1) negative coupling to adenylyl cyclase, (2) inhibiting production of cyclic adenosine monophosphate, and (3) activating G-protein inwardly rectifying potassium (GIRK) channels (Hen 1992; Ghavami, Baruscotti, & Robinson, 1997; Sari, 2004; McDevitt and Neumaier, 2011; Barnes and

Neumaier, 2011). The dorsal portion of the raphe nucleus (DRN) contains the highest levels of 5-HT_{1B} mRNA (Clark et al., 2006). The DRN sends axonal projections, containing 5-HT_{1B}-autoreceptors, primarily to the VTA, substantia nigra, globus pallidus, hypothalamus, hippocampus, striatum, nucleus accumbens, and frontal cortex (Morikawa et al., 2000; McDevitt & Neumaier, 2011). However, 5-HT_{1B} mRNA and protein levels are also detected within these brain areas in dopamine, glutamate, GABA, and cholinergic expressing neurons where they function as 5-HT_{1B}-heteroreceptors and inhibit release of these neurotransmitter types (Johnson, Mercuri, & North, 1992; Cassel, et al., 1995; Boeijinga & Boddeke, 1996; Yan & Yan, 2001; Miszkiel, Filip, & Przegalinski, 2011).

Advances in medicinal chemistry have yielded ligands with high selectivity for 5-HT_{1B}Rs in the central nervous system (Koe et al., 1992; Selkirk et al., 1998; Murray & Rees, 2009; Rodriguez et al., 2014). The agonist 5-propoxy-3-(1,2,3,6-tetrahydro-4-pyridinyl)-1*H*-pyrrolo[3,2-*b*]pyridine (**CP 94,253**) and the antagonist 1'-methyl-5-[[2'-methyl-4'-(5-methyl-1,2,4-oxadiazol-3-yl)biphenyl-4-yl]carbonyl]-2,3,6,7-tetrahydrospiro[furo[2,3-*f*]indole-3,4' piperidine (**SB 224,289**) have high affinities (K_i = 2 and 8.2 nM, respectively) for 5-HT_{1B}Rs (Koe et al., 1992; Selkirk et al., 1998). Another group of 5-HT_{1B}R agonists are tryptamine-based, including 311C90 (**zolmitriptan**, Zomig®), which is clinically used to treat migraine headaches. Although zolmitriptan is not as selective for 5-HT_{1B}Rs as CP 94,253, it has a high affinity (K_i = 5.01 nM) for 5-HT_{1B}Rs. The selectivity profiles (Table 1) of CP 94,253, SB 224,289, and zolmitriptan have established these drugs as useful tools for studying the role of 5-HT_{1B}Rs in psychostimulant addiction.

5-HT$_{1B}$Rs and psychostimulant abuse

5-HT$_{1B}$Rs modulate the effects of cocaine and other psychostimulants in rodents. Mice lacking the 5-HT$_{1B}$R gene exhibit a cocaine-induced increase in locomotor behavior and an increase in cocaine intake as compared to wild-type mice (Rocha et al., 1998), suggesting that 5-HT acting at 5-HT$_{1B}$Rs inhibits cocaine reinforcement. However, findings in wild-type rodents given 5-HT$_{1B}$Rs are somewhat discrepant with those in knock-out models. Systemic administration of a 5-HT$_{1B}$R antagonist in wild-type mice decreases, rather than increases, cocaine reward (Castanon et al., 2000; David et al., 2004) and systemic administration of 5-HT$_{1B}$R agonists in rats facilitate the rewarding effects of cocaine in a place conditioning procedure (Cervo et al., 2002; Barot, Ferguson, & Neumaier, 2007). Furthermore, 5-HT$_{1B}$R agonist treatment produces a leftward shift in the cocaine dose-effect function and increases responding on a progressive ratio (PR) reinforcement schedule, indicative of enhanced reinforcing value of cocaine. (Parsons, Koob, & Weiss, 1998; Pentkowski et al., 2014). Similarly, viral-mediated overexpression of 5-HT$_{1B}$Rs in the NAc of rats produces increases in cocaine intake, cocaine-induced hyperlocomotion, cocaine sensitization, and cocaine conditioned place preference (Neumaier et al., 2002; Barot, Ferguson, & Neumaier, 2007; Pentkowski, et al., 2012).

These experiments provide compelling evidence for counterindicative use of 5-HT$_{1B}$R agonists to treat cocaine abuse. However, we recently found that the effects of 5-HT$_{1B}$R agonists vary depending on whether or not animals have undergone abstinence. Specifically, the selective 5-HT$_{1B}$R agonist CP 94,253 shifts the cocaine dose-effect function leftward when given as a pretreatment prior to a daily SA session (pre-abstinence)

but produces a downward shift when given as a pretreatment prior to resumption of SA after 21 days of abstinence (post-abstinence; Pentkowski et al., 2009; Pentkowski et al., 2014). In addition, CP 94,253 pretreatment decreases cocaine intake and response rates on the progressive ratio reinforcement schedule. CP 94,253 also attenuates cocaine-seeking behavior in tests of both cue-induced and cocaine-primed reinstatement following a few weeks of extinction training during which rats were abstinent (Pentkowski et al., 2009). Cocaine-seeking behavior under these conditions reflect incentive motivational effects produced by the cues and cocaine priming injections (Markou et al., 1993). Collectively, these results suggest that pre-abstinence administration of 5-HT_{1B}R agonists facilitates the reinforcing and motivational effects of cocaine while post-abstinence 5-HT_{1B}R agonists attenuate these effects. These abstinence-dependent effects suggest that 5-HT_{1B}R agonists have therapeutic potential as anti-relapse medications.

5-HT_{1B}R activation also modulates amphetamine-related behaviors. Systemic or local administration of 5-HT_{1B}R agonists have been shown to enhance amphetamine-induced hyperlocomotion in mice and rats (Przegalinski et al., 2001; Papla, Filip, & Przegalinski, 2002) and 5-HT_{1B}R stimulation in mice facilitates development of amphetamine sensitization (Przegalinski et al., 2001). Pharmacological antagonism of 5-HT_{1B}Rs, on the other hand, block the acute MDMA-induced hyperlocomotor effects in rats, but has no effect on methamphetamine-induce locomotion (McCreary, Bankson, & Cunningham, 1999; Fletcher et al., 2002; Steed, Jones, & McCreary, 2011). Collectively, these studies implicate 5-HT_{1B}R involvement in enhancing the rewarding and reinforcing effects of psychostimulants. Further research is needed to investigate if 5-HT_{1B}R agonists

produce an abstinence-dependent effect on amphetamine SA similar to the effects on cocaine.

Aims of research

This dissertation aimed to investigation the therapeutic potential of 5-HT_{1B}R ligands to treat psychostimulant abuse. I investigated if the 5-HT_{1B}R agonist effects observed previously with cocaine in male rats generalize across sex and psychostimulants. In Chapter 2, I investigated how 5-HT_{1B}R agonists modulate the reinforcing and incentive motivational effects for self-administered methamphetamine across different periods of the drug-taking cycle. These experiments are the first to examine 5-HT_{1B}R agonist effects on methamphetamine-related behavior. In Chapter 3, I examined the effects of the clinically-available but less selective 5-HT_{1B}R agonist, zolmitriptan, to reduce cocaine reinforcement in male and female rats. The pharmacokinetic and pharmacodynamic properties of zolmitriptan for treating migraine headaches is well established. However, this is the first study to examined how treatment with zolmitriptan affects cocaine-related behaviors and to explore whether the mechanism involves actions at 5-HT_{1B}Rs and/or 5-HT_{1D}Rs. Finally, in Chapter 4, I examined if the selective 5-HT_{1B}R agonist, CP 94,253, had therapeutic potential using a model of relapse. Previous studies have investigated the effects of CP 94,253 following abstinence; however, this was the first study to investigate if the attenuation of cocaine SA after abstinence observed with CP 94,253 persists following resumption of daily cocaine SA.

CHAPTER 2

EFFECTS OF 5-HT$_{1B}$R STIMULATION ON REINFORCEMENT AND INCENTIVE MOTIVATION FOR METHAMPHETAMINE SELF-ADMINISTRATION

Garcia et al., (2017). International Journal of Neuropsychopharmacology, 20:8.

Little research has focused on how 5-HT$_{1B}$Rs modulate the reinforcing effects of methamphetamine. Similar to cocaine, methamphetamine is a psychostimulant that inhibits monoamine transport function and enhances 5-HT neurotransmission (Panenka et al., 2013). Furthermore, methamphetamine redistributes intracellular 5-HT by acting on vesicular monoamine transporters, and reverses 5-HT transport across the plasma membrane, resulting in increased release (Sager & Torres, 2011; Panenka et al., 2013). Recently, Igari and colleagues demonstrated that SERT knockout mice given repeated methamphetamine administration did not show the locomotor sensitization that was observed in wild-type controls. However, administration of a 5-HT$_{1B}$R antagonist, prior to methamphetamine administration, restored locomotor sensitization (Igari et al., 2015). Igari et al., further demonstrated that the 5-HT$_{1B}$R antagonist reduced locomotor sensitization in wild-type mice. This study suggests that 5-HT$_{1B}$Rs also modulate the effects of methamphetamine and that 5-HT$_{1B}$R's may be useful targets for developing medications to treat methamphetamine abuse.

In this chapter, we examined if CP 94,253 produced a similar abstinence-dependent decrease in methamphetamine intake. First, we examined CP 94,253 pretreatment effects on the methamphetamine self-administration (SA) dose-effect function using low ratio schedules of reinforcement (i.e. FR5 and VR5). Second, we examined CP 94,253 pretreatment effects on incentive motivation for methamphetamine using a PR schedule of

reinforcement as this more demanding schedule is particularly sensitive to changes in motivation for a drug. Third, we examined if the effects of CP 94,253 pretreatment on methamphetamine intake were 5-HT_{1B}R-mediated by administering the selective 5-HT_{1B}R antagonist SB 224,289 to reverse the agonist effects. Fourth, we examined if CP 94,253 and SB 224,289 affected locomotor activity. Finally, we examined if acute and intermittent repeated treament with zolmitriptan affected methamphetamine intake. Since both methamphetamine and cocaine enhance monoaminergic neurotransmission by an action at monoamine transporters, we hypothesized that 5-HT_{1B}R stimulation would increase methamphetamine intake pre-abstinence and decrease methamphetamine intake post-abstinence similar to that observed with cocaine intake in male rats.

Methods

Animals

Male Sprague Dawley rats (Charles River, San Diego, CA) weighing 225-250 g were single-housed in a climate-controlled environment on a 14:10 reverse light/dark cycle (lights off at 6 am). Rats had *ad libitum* access to food except for initial SA training when they were food-restricted to 85% of their *ad libitum* weights. The experiments proceeded in accordance with a protocol and standard operating procedures approved by the Arizona State University Institutional Animal Care and Use Committee.

Drugs

Methamphetamine hydrochloride (Sigma-Aldrich, USA) was dissolved in saline (Hospira Inc. Lake Forest, IL) and filtered with 0.2 μm membrane Acrodisc syringe filters (PALL Corporation, USA). CP 94,253 hydrochloride (Tocris Bioscience, USA) was dissolved in saline and SB 224,289 hydrochloride (Tocris Biosciences, USA) was

dissolved in 10% (2-hydroxypropyl)-β-cyclodextrin (Sigma-Aldrich, USA) in saline and sonicated for ~2 min. Zolmitriptan (Sigma-Aldrich, USA) was dissolved in 10% dimethyl sulfoxide in saline and sonicated for ~5 min. CP 94,253, SB 224,289, and zolmitriptan were prepared fresh daily. Vehicle refers to the respective solvent. All drug injections, with the exception of self-administered methamphetamine, were injected at a volume of 1 ml/kg body weight.

Surgery

Rats underwent surgery for implantation of chronic indwelling catheters into the jugular vein as detailed previously (Pockros et al., 2011). Rats had 6-7 days of recovery before commencing SA training. Catheters were flushed daily with 0.1 ml of either timentin (experiment 1; 66.67 mg/ml; GlaxoSmithKline, Research Triangle Park, NC, USA) or cefazolin (experiment 2, 3, 4, & 5; 10 mg/ml; WG Critical Care, LLC, NJ, USA) mixed with heparin/saline (70 U/ml; APP Pharmaceuticals, Schaumburg, IL, USA). In addition, catheter patency was tested periodically by administering 0.05 ml of methohexital sodium (16.7 mg/ml; Jones Pharma Inc., St. Louis, MO), a dose which produces brief loss of muscle tone when administered intravenously (i.v.).

Apparatus

The operant conditioning chambers (Med Associates, St. Albans, VT, USA) contained an active and inactive lever, a cue light, and a tone generator as previously described (Pentkowski et al., 2010). All chambers had infusion pumps (Med Associates) that connected to liquid swivels (Instech, Plymouth Meeting, PA) fastened to an outlet polyethylene tubing sheltered within a metal leash (PlasticsOne, Roanoke, VA) that

attached to the rat's catheter. All operant conditioning chambers were housed within sound attenuating boxes that contained a ventilation fan.

General Procedures

Experimental sessions commenced at approximately the same time of day, 6 days/week during the rats' dark cycle. Rats were first trained to self-administer 0.1 mg/kg (i.v.) methamphetamine on an FR1 schedule of reinforcement and they later progressed to either a FR5, VR5, or PR schedule based on individual performance. In all experiments, schedule advancement during training occurred once rats received at least 10 reinforcers/session for two consecutive sessions and testing commenced once rats reached a stability criterion of < 15% variability in the number of infusions obtained across three consecutive sessions. The training dose was choosen as it has been shown to be effective in producing methamphetamine acquisition (Kitamura et al., 2006; Clemens et al., 2006; Krasnova et al., 2010). The VR5 schedule was chosen for its tendency to sustain consistent steady response rates (Domjan, 2003). Session length was 2 h, except for training and testing on the PR schedule for which sessions were 4 h long in order to capture break points in most rats. In the PR schedule, the number of active lever responses required to obtain each subsequent infusion increased exponentially (e.g. 1, 2, 4, 6, 9, 12, 15, etc.), identical to the exponential equation from Richardson & Roberts (1996). The PR schedule was chosen because it is progressively more effortful to obtain reinforcement, reflecting in part how *motivated* a rat is to work for methamphetamine (Markou et al., 1993). These reinforcement schedules were chosen to help inform how our treatment affected behavioral processes across different effort requirements. Completion of any of the operant schedules activated the tone and light cue followed 1 s later by an infusion of 0.1 ml

methamphetamine delivered across 6 s (dose is given in the specific experiments section). The tone, light, and pump were then turned off, and simultaneously a house light was activated for 20 s to signal a timeout period during which additional lever responses were recorded but had no programmed consequences. After the timeout period, the house light turned off and methamphetamine was available again. Abstinence from methamphetamine occurred for 21 or more consecutive days, during which the rats were maintained in the home cage, handled, and weighed daily for i.v. administration of timentin or cefazolin to maintain catheter patency.

Experiment 1a: Effects of CP 94,253 on the methamphetamine self-administration dose-effect function pre- and post-abstinence.

Beginning two days prior to the start of training and continuing throughout the experiment, rats were food-restricted to 85% of their initial free-feeding body weight. During training, rats progressed from a FR1 to FR5 schedule (17-20 sessions) after reaching a criterion of ≥10 infusions for two consecutive sessions. Once reinforcement rates met the stability criterion on the FR5, the rats commenced training on a within-session dose-response procedure. For these sessions, each methamphetamine dose (0.003, 0.01, 0.03, 0.1, & 0.30 mg/kg, i.v.) was available in ascending order for 30 minutes with a 5-min time-out period between doses. After again meeting the stability criterion for reinforcement rates across the within-session dose-response training days (20-28 sessions), rats were randomly assigned to receive a subcutaneous (s.c.) injection of either CP 94,253 (5.6 mg/kg, s.c.) or vehicle 15 min prior to their daily dose-response session. The dose of CP 94,253 was selected based on our previous research demonstrating that it selectively reduces cocaine intake without affecting sucrose intake (Pentkowski et al., 2009). After

this first test session, additional training sessions were given until rats met the stability criterion. Then rats were tested again, receiving the treatment opposite from their first treatment (i.e. animals that received vehicle first, now received CP 94,253 and vice versa). After this test session, rats underwent abstinence for 21 days, during which they remained in their home cages, but continued to receive daily i.v. administration of heparin/antibiotic to maintain catheter patency.

For the post-abstinence tests, rats received CP 94,253 or vehicle, and 15 min later, they were given access to methamphetamine using the same within session dose-response procedure as used during pre-abstinence tests. Rats then remained abstinent in the home cage for three days to allow time for CP 94,253 to be eliminated and to reinstate an abstinence period prior to the second test. On the second test day, the rats received the treatment opposite from that given prior to the first post-abstinence test. A total of 10 rats ran through the entire experiment.

Experiment 1b: Effects of CP 94,253 on methamphetamine self-administration on a progressive ratio schedule pre- and post-abstinence.

A new cohort of experimentally-naïve rats were food-restricted and trained to self-administer methamphetamine, progressing from a FR1 to a VR5 schedule during 2 h sessions (11-13 sessions) using the same procedure as the previous experiment, with the exception that food restriction was gradually discontinued once rats were on the VR5 schedule. After meeting the stability criterion on the VR5 schedule, rats were trained on the PR schedule of methamphetamine reinforcement during 4 h sessions until again meeting the stability criterion (10-18 sessions). We increased session length to 4 h to ensure that CP 94,253 would remain effective throughout the test (Parsons, Koob, & Weiss, 1998).

We also reduced the methamphetamine dose to half the training dose (0.05 mg/kg, i.v.) with the intention that more rats would reach break point during the 4 h session. Breakpoint was defined as the last schedule of reinforcement completed prior to a 1 h period during which the next required ratio failed to be completed, or 4 h had elapsed, whichever came first. After rats met the stability criterion on the PR schedule, they were randomly assigned to either a CP 94,253 (10 mg/kg, s.c., n = 8) or a vehicle group (n = 7), counterbalanced for similar number of total drug infusions during training. The dose of CP 94,253 was higher than the previous experiment to sustain CP 94,253 levels throughout testing and based on previous research this dose selectively modulates cocaine intake while not affecting sucrose intake (Pentkowski et al., 2009). These groups received their respective treatments 15 min before testing on the PR schedule. After testing, both groups of rats were placed into abstinence for 21 days as described in experiment 1a. Post-abstinence, both groups received their injections, which were identical to those given pre-abstinence, 15 min prior to a test for resumption of methamphetamine SA on the PR schedule.

Experiment 1c: Reversing the attenuating effects of CP 94,253 on methamphetamine self-administration with the selective 5HT$_{1B}$R antagonist, SB 224,289.

A new cohort of experimentally-naïve rats were trained to self-administer methamphetamine, progressing from a FR1 to a VR5 schedule (12-17 sessions) of methamphetamine (0.1 mg/kg, i.v.) reinforcement. Rats were food-restricted only during acquisition of SA and all sessions lasted for 2 h. Once reinforcement rates stabilized under free feeding conditions (4-12 sessions), a subset of rats were randomly assigned to one of two groups (n = 14 and 17, respectively); Group 1 received an intraperitoneal (i.p.)

injection of either vehicle or SB 224,289 (10 mg/kg, i.p.) 30 min prior to the first test and the opposite treatment injection 30 min prior to the second test (i.e., rats that received vehicle on test 1 received SB 224,289 on test 2 and vice versa). Group 1 also received a vehicle injection 15 min prior to both tests. For Group 2, identical procedures were followed except that rats received vehicle followed by CP 94,253 (5.6 mg/kg, s.c.) on one test day and SB 224,289 (10 mg/kg, i.p.) followed by CP 94,253 (5.6 mg/kg, s.c.) on the other test day.

In addition to testing whether the antagonist would reverse the effects of the agonist in this experiment, we also verified that these rats showed a CP 94,253-induced decrease in methamphetamine intake post-abstinence. A subset of rats, randomly selected from both Group 1 and Group 2, underwent 21 days of abstinence as described above. They were then assigned to two groups, counterbalanced for the number of infusions obtained during training. One group received an injection of CP 94,253 (5.6 mg/kg, s.c.) while the other group received an injection of vehicle 15 min prior to a test for resumption of methamphetamine SA (0.1 mg/kg, i.v.).

Experiment 1d: Effects of 5-HT$_{1B}$R ligands on spontaneous locomotion.

Rats from experiment 1b were used and they had a history of methamphetamine SA (38 sessions) and had undergone an abstinence period (~21 days). The test chambers (45.72 x 25.4 x 20.32 cm) were similar to the home cages and had a camera mounted above to record horizontal movement with Topscan software (Clever Systems, Reston, VA). The rats were randomly assigned to one of two groups and tested twice for locomotor activity with three rest days intervening the two test days. They remained in their home cages during rest days. Thirty min prior to the first test, rats were pretreated with either vehicle

or SB 224,289 (10 mg/kg, i.p.) and 30 min prior to the second test the rats received the opposite treatment as that given on the first test (i.e., rats that received vehicle on test 1 received SB 224,289 on test 2 and vice versa). Group 1 (n = 7) received a vehicle injection 15 min prior to both tests and Group 2 (n = 7) received CP 94,253 (10 mg/kg, s.c.) 15 min prior to both tests. The tests began by placing the rat into the test chamber and distance traveled was measured for 2 h.

Experiment 1e: Effects of zolmitriptan on methamphetamine self-administration pre- and post-abstinence.

A subset of rats that were used in experiment 1c were tested for the effects of zolmitriptan on methamphetamine (0.1 mg/kg, i.v.) after achieving stable SA rates on a VR5 schedule (3-11 sessions) across 2 h training sessions. Rats were then randomly assigned to either a zolmitriptan (10 mg/kg, s.c.; n = *9*) or a vehicle treatment group (n = *6*), counterbalanced for similar number of total drug infusions. Rats received their assigned treatment 15 min prior to the start of a SA session. Then rats underwent a period of abstinence for a minimum of 21 days followed by a test phase. During the test phase, the rats again received their assigned treatment of either zolmitriptan or vehicle 15 min prior to each of three SA sessions with 2-3 SA sessions without treatment between each of the treatment test days.

Data Analysis

Statistical analyses were conducted with IBM SPSS Statistics® v. 23. Descriptive statistics are reported as the mean ± standard error of the mean. Self-administration data, including active and inactive lever responses, and infusions obtained were analyzed by either repeated measures or a mixed design ANOVA with drug pretreatment (s) and dose

of methamphetamine as between-subjects or within-subjects factors depending on the experimental design. In addition, breakpoint and total distance travelled were analyzed as described above for experiment 1b and 1d, respectively. All sources of significant effects were further analyzed by Tukey's post-hoc tests. There was some attrition in each experiment due to catheter failure or failure to acquire SA. Final n/group are reported in the methods and figure captions and the timeline for each experiment is outlined along with its respective figure.

Results

Experiment 1a: Methamphetamine produced an inverted U-shaped dose-effect function and CP 94,253 decreased methamphetamine infusions and active lever responses both pre- and post-abstinence (Fig. 1). For the pre-abstinence tests, there were main effects of methamphetamine dose for both infusions [F (4, 36) = 17.67, $p < 0.05$] and active lever responses [F (4, 36) = 8.40, $p < 0.05$]. Post-hoc tests indicated that the 0.01 and 0.03 mg/kg doses produced higher values than the lowest dose (0.003 mg/kg) and the highest dose (0.30 mg/kg) produced lower values than all doses except 0.10 mg/kg ($p < 0.05$; Fig. 1A-B). There were also main effects of treatment, which indicated that averaged across methamphetamine dose, rats exhibited lower infusion and active lever response rates when pretreated with CP 94,253 than when pretreated with vehicle (Fig. 1A-B insets). There were no significant effects for inactive lever responses during pre-abstinence tests (Fig. 1C).

Analysis of post-abstinence infusions showed main effects of treatment [F (1, 9) = 30.74, $p < 0.05$] and methamphetamine dose [F (4, 36) = 35.27, $p < 0.05$], as well as a treatment by methamphetamine dose interaction [F (4, 36) = 2.87, $p < 0.05$]. CP 94,253

pretreatment decreased infusions at the three lowest doses of methamphetamine compared to vehicle pretreatment ($p < 0.05$; Fig 1D). The analysis of post-abstinence active lever responses also revealed main effects for treatment [F (1, 9) = 10.38, $p < 0.05$] and methamphetamine dose [F (4, 36) = 22.20, $p < 0.05$], but no treatment by methamphetamine dose interaction. The main effect of methamphetamine dose was due to the inverted U-shaped dose-response function similar to pre-abstinence. The main effect of treatment shows that averaged across methamphetamine doses, rats exhibited lower infusions and active lever response rates when pretreated with CP 94,253 than when pretreated with vehicle (Fig. 1D-E insets). There were no significant effects for inactive lever responses during the post-abstinence tests (Fig. 1F).

Experiment 1b: CP 94,253 decreased methamphetamine infusions, breakpoint, and active lever responses (Fig. 2A-C). Most rats reached break point within the 4 h test during both the pre-abstinence (80%) and post-abstinence (73%) tests. The analyses showed main effects of pretreatment across pre- and post-abstinence tests for infusions [F (1, 13) = 9.86, $p < 0.05$], breakpoints [F (1, 13) = 6.46, $p < 0.05$], and active lever responses [F (1, 12) = 11.09, $p < 0.05$]. In each case, CP 94,253 pretreatment decreased these measures compared to the vehicle pretreatment ($p < 0.05$). There were no main effects for inactive lever responses (Fig. 2D).

Experiment 1c: CP 94,253 decreased methamphetamine infusions and this effect was blocked by SB 224,289 (Fig. 3). Data analysis showed a main effect of the first pretreatment (i.e., vehicle vs. SB 224,289) [F (1, 29) = 6.86, $p < 0.05$] and an interaction between the first and second (vehicle vs. CP 94,253) pretreatments [F (1, 29) = 12.52, $p < 0.05$]. Post-hoc tests showed that pretreatment with vehicle + CP 94,253 decreased

methamphetamine infusions compared to all other pretreatment conditions, including SB 224,289 + CP 94,253 ($p < 0.05$). This finding indicates that administration of the antagonist SB 224,289 blocked the attenuating effect of CP 94,253 on methamphetamine infusions, suggesting this effect was mediated by 5-HT_{1B}Rs. There were no effects for active or inactive lever responses (data not shown).

The rats in this experiment underwent 21 days of abstinence and were then tested for the effects of CP 94,253 (5.6 mg/kg, s.c.) on resumption of methamphetamine SA (0.1 mg/kg, i.v.). Similar to the results from experiment 1a, pretreatment with a 5.6 mg/kg dose of CP 94,253 decreased the number of infusions compared to pretreatment with vehicle during the post-abstinence tests (data not shown). The mean number of methamphetamine infusions obtained in the vehicle vs. CP 94,253 pretreatment groups was 12.40 ± 0.88 and 8.90 ± 0.71, respectively [t (9) = 7.72, $p < 0.05$]. Additionally, CP 94,253 decreased active lever responses (data not shown). The mean number of active lever responses in the vehicle vs. CP 94,253 pretreatment groups was 70.30 ± 20.81 and 49.20 ± 17.89, respectively [t (9) = 2.62, $p < 0.05$]. There was no group difference for inactive lever responses.

Experiment 1d: Neither pretreatment with CP 94,253, SB 224,289, or the combination of both 5-HT_{1B}R ligands altered locomotor activity (Fig. 4). There were no main or interaction effects between treatment and the total distance traveled by rats.

Experiment 1e: Acute zolmitriptan treatment decreased methamphetamine infusions and active lever responses during pre-abstinence tests (Fig. 5A-B). Comparisons between vehicle and zolmitriptan showed a difference in infusions [t (10) = 3.50, $p < 0.05$] and active lever responses [t (10) = 2.90, $p < 0.05$]. There were no effects on inactive lever

responses after vehicle or zolmitriptan treatment with means of 39.18 ± 16.37 and 16.55 ± 6.41, respectively.

Zolmitriptan pretreatment given intermittently across three post-abstinence tests consistently decreased infusions and active lever responses (Fig. 5C-D). The ANOVA showed a main effect of treatment group for infusions [F (1, 11) = 21.92, $p < 0.05$] but no effect of treatment day or treatment group by treatment day interaction. For active lever responses, there were significant main effects of treatment group [F (1, 10) = 21.17, $p < 0.05$] and treatment day [F (2, 20) = 6.08, $p < 0.05$], but no treatment group by treatment day interaction. Post-hoc tests for treatment day showed that zolmitriptan treatment produced lower active lever response rates on day two compared to treatment days one and three. There were no effects on inactive lever responses after vehicle or zolmitriptan treatment with means of 7.89 ± 3.68 and 3.56 ± 0.68, respectively.

Discussion

Unlike the abstinence-dependent modulatory role of 5-HT_{1B}R agonists on cocaine SA that we observed previously (Pentkowski et al., 2012; Pentkowski et al., 2014), this study found that 5-HT_{1B}R agonists attenuated methamphetamine SA when given either pre- or post-abstinence. Specifically, a moderate dose of CP 94,253 (5.6 mg/kg, s.c.) decreased methamphetamine infusion and active lever responses averaged across methamphetamine dose available both when administered during maintenance of daily SA sessions and following a period of abstinence (main effect of pretreatment, Fig. 1 insets). After abstinence, the effect of CP 94,253 was more pronounced at lower doses of methamphetamine primarily because intake appeared higher under the vehicle pretreatment condition post-abstinence compared to pre-abstinence. This enhancement of

methamphetamine intake post-abstinence is consistent with sensitized methamphetamine SA reported previously (Schenk & Partridge, 1997). Thus, the findings suggest that CP 94,253 attenuates expression of the abstinence-induced, enhanced sensitivity to methamphetamine observed with vehicle pretreatment. CP 94,253 (10 mg/kg, s.c.) also decreased methamphetamine intake (0.05 mg/kg, i.v.) under a PR schedule of reinforcement both pre- and post-abstinence (Fig. 2), further suggesting attenuation of methamphetamine reinforcing and/or motivational effects. Importantly, administration of the 5-HT_{1B}R antagonist, SB 224,289 (10 mg/kg, i.p.), blocked the attenuating effects of CP 94,253 (5.6 mg/kg, s.c.) on methamphetamine intake in rats tested during maintenance of SA on a VR5 schedule (Fig. 3), suggesting that the effects of the agonist are mediated by 5-HT_{1B}Rs. Finally, we report that zolmitriptan (10 mg/kg, s.c.) also attenuated methamphetamine intake on a VR5 schedule. Zolmitriptan treatment given acutely during maintenance, as well as given intermittently following abstinence, decreased methamphetamine intake and active lever responses (Fig. 5).

There are a number of possible reasons for the 5-HT_{1B}R agonist-induced decreases in methamphetamine SA, including an effect on methamphetamine reinforcement value and/or incentive motivation, an effect on anxiety, or an effect on motor capability. The decrease in methamphetamine intake is unlikely due to impairments in motor capability, as treatment with the 5-HT_{1B}R ligands did not alter spontaneous locomotion (Fig. 4) or inactive lever responses at the doses used in the present study. Furthermore, previous research from our laboratory has shown that CP 94,253 (0.3 - 10.0 mg/kg, s.c.) has no effect on sucrose reinforcement (Pentkowski et al., 2009). Although the antagonist SB 224,289 decreases cocaine-induced locomotion in drug naïve rats (Hoplight et al., 2005),

it has no effect on locomotion in rats with a history of cocaine SA (Pentkowski et al., 2009, Pentkowski et al., 2014). Thus, it seems unlikely that CP 94,253 or SB 224,289 altered methamphetamine intake by impairing motor capability.

We cannot rule out the possibility that CP 94,253 may have influenced methamphetamine intake nonspecifically by increasing anxiety-like behaviors rather than attenuating reinforcement *per se*. Indeed, previous studies have found that either 5-HT_{1B}R agonists or antagonists can increase baseline and cocaine-induced anxiety-like behaviors (Lin & Parsons, 2002; Hoplight et al., 2005; Pentkowski et al., 2009). It is important to note that the rats in these previous studies were naïve to the experimental procedures used to assess anxiety-like behaviors, which would likely maximize any potential drug effect. In contrast, rats tested for CP 94,253 effects on methamphetamine SA in the present study were well habituated to the testing environment, which would likely minimize potential anxiogenic effects. Furthermore, animals with increased exposure to stress, such as foot-shock, often increase rather than decrease drug intake (Goeders & Guerin, 1994; Ahmed & Koob, 1997; Piazza & Le Moal, 1998; Logrip, Zorrilla, & Koob, 2012), mitigating the idea that CP 94,253-induced anxiety-like states interfered with responding. Finally, it seems that potential anxiogenic effects of CP 94,253 on reinforcement would manifest as a decrease in both sucrose and psychostimulant intake, yet CP 94,253 has been shown to selectively decrease cocaine intake (Pentkowski et al., 2009).

We suggest that the most likely explanation for the agonist-induced decreases in methamphetamine intake in the present study is that 5-HT_{1B}Rs modulate psychostimulant reinforcement and/or incentive motivation (Pentkowski et al., 2012; Pentkowski et al., 2014). The decrease of methamphetamine intake in response to 5-HT_{1B}R stimulation

following abstinence may result from an attenuation on the expression of enhanced sensitivity to the reinforcing effects of methamphetamine. This explanation is consistent with our previous findings that 5-HT_{1B}R agonists or 5-HT_{1B}R over-expression by viral-mediated gene transfer attenuate cocaine SA following a period of abstinence, as well as reduce cocaine-seeking behavior (Pentkowski et al., 2009, 2012, 2014). Furthermore, these agonist effects are reversed by 5-HT_{1B}R antagonists, including SB 224,289, supporting a 5-HT_{1B}R mechanism.

We were surprised that CP 94,253 attenuated methamphetamine intake prior to any abstinence given that this same treatment enhances cocaine intake prior to abstinence (Pentkowski et al., 2009, 2014). However, others have shown that 5-HT_{1B}R agonists attenuate d-amphetamine intake without prolonged abstinence, as well as attenuate d-amphetamine-induced responding for conditioned reward (Fletcher & Korth, 1999; Fletcher et al., 2002; Miszkiel et al., 2012; Miszkiel & Przegalinski, 2013). Therefore, the 5-HT_{1B}R agonist enhancement of cocaine intake prior to abstinence may be due to differences in the pharmacological actions of cocaine versus amphetamines. While both amphetamines and cocaine inhibit and down-regulate 5-HT, dopamine, and norepinephrine transporters (Azzaro & Rutledge, 1973; Ritz, Cone, & Kuhar, 1990), they interact differently with the transporters. Amphetamines, including methamphetamine, not only inhibit monoamine transport, but also redistribute intracellular monoamines by acting at the vesicular monoamine transporter (VMAT) causing release of monoamines into the cytosol while at the same time reversing monoamine transport across the plasma membrane resulting in monoamine release (Sulzer et al., 1995; Sager & Torres, 2011; Panenka et al., 2013). Cocaine and amphetamines also interact at different sites on the dopamine

transporter and produce differential effects on the releasable vesicular pool and on regulation of VMAT (Thomsen et al., 2009). The latter effects may result in a larger releasable pool of dopamine after cocaine versus methamphetamine following acute or subchronic administration (Brown, Hanson, & Fleckenstein, 2001).

Although the specific mechanisms responsible for the attenuating effect of 5-HT_{1B}R agonists on cocaine and methamphetamine SA are unclear, we hypothesize that such mechanisms may involve a dysregulation of 5-HT_{1B}R functions (Neisewander et al., 2014). 5-HT_{1B}Rs are widely distributed in the brain (Bruinvels et al., 1994; Lanfumey & Hamon, 2004; Varnas, Hurd, & Hall, 2005; Clark, McDevitt, & Neumaier, 2006) and are expressed as either 5-HT terminal autoreceptors or heteroreceptors on terminals of non-5-HTergic cells. In both cases, these receptors negatively couple to adenylyl cyclase activity via G-proteins and function to inhibit neurotransmitter release (Hen 1992; Sari, 2004; McDevitt & Neumaier, 2011; Barnes & Neumaier, 2011). Several manipulations in the mesolimbic system have provided evidence for a modulatory role of 5-HT_{1B}Rs for psychostimulant addiction; specifically, overexpression of 5-HT_{1B}Rs in the nucleus accumbens of rats facilitates the rewarding and reinforcing effects of cocaine (Neumaier et al., 2002; Pentkowski et al., 2012). Furthermore, local activation of 5-HT_{1B}Rs in the ventral tegmental area alters cocaine-induced increases in dopamine levels in the nucleus accumbens and cocaine-induced decreases in gamma-aminobutyric acid levels (Parsons, Koob, & Weiss, 1999; O'Dell & Parsons, 2004). Similarily, activation of 5-HT_{1B}Rs in the nucleus accumbens dose-dependently decreases the rewarding and reinforcing effects of amphetamine (Fletcher & Korth, 1999; Fletcher, 2002).

The present findings suggest that 5-HT_{1B}Rs are potential targets for developing pharmacotherapies for psychostimulant addiction. Here we show that the clinically available anti-migraine medication zolmitriptan, which is a 5-$HT_{1D/1B}$R agonist, attenuated methamphetamine intake. The attenuation of methamphetamine intake was likely mediated, at least in part, by stimulation of 5-HT_{1B}Rs, although, it is possible that 5-HT_{1D}Rs may have also contributed to the attenuation effect. Zolmitriptan, unlike CP 94,253, has a higher affinity (Table 1) for 5-HT_{1D}Rs (Ki = 0.63 nM) than for 5-HT_{1B}Rs (Ki = 5.01 nM; Murray, et al., 2011). CP 94,253 also has affinity for 5-HT_{1D}Rs (Ki = 49 nM; Koe et al., 1992) and therefore 5-HT_{1D}Rs may also contribute to its effects on methamphetamine SA. The effects of zolmitriptan on methamphetamine SA were not likely due to a decrease in general activity as we did not observe any differences in inactive lever responses in our treatment groups. Furthermore, previous research found that zolmitriptan (1-30 mg/kg, i.p.) attenuates alcohol-induced aggression in mice but has no effect on locomotion (de Almeida et al., 2001).

In conclusion, this study provides evidence that the selective 5-HT_{1B}R agonist, CP 94,253, attenuates methamphetamine SA pre- and post-abstinence under several schedules of reinforcement and in an antagonist-reversible manner. These findings build upon previous research demonstrating similar effects of 5-HT_{1B}R agonists on d-amphetamine SA (Fletcher & Korth, 1999; Fletcher et al., 2002; Miszkiel et al., 2012; Miszkiel & Przegalinski, 2013), and together the effect of the agonists on SA of amphetamines contrasts with the enhanced cocaine SA that has been observed prior to any abstinence (Pentkowski et al., 2009, 2014). These results suggest that 5-HT_{1B}R agonists may differentially modulate cocaine and methamphetamine SA initially, but that after a period

of abstinence, the agonists inhibit the reinforcing effects of both psychostimulants. The latter findings suggest that 5-HT_{1B}R agonists may have potential therapeutic effects for psychostimulant abuse. In addition, we have provided evidence that the FDA-approved 5-$HT_{1D/1B}$R agonist, zolmitriptan, also attenuates methamphetamine SA both pre- and post-abstinence. Our findings suggest that 5-HT_{1B}R agonists warrant further investigation as putative treatments of psychostimulant use disorders. Important future directions include determining the neural circuitry involved in the agonist effects, whether the effects are also observed in female rats, and whether the effects are observed in rats given more extensive access to the stimulants and more extensive abstinence.

CHAPTER 3

POTENTIAL FOR REPURPOSING ZOLMITRIPTAN FOR TREATING COCAINE USE DISORDERS

Garcia et al., (in prep).

Recent increases in cocaine use and deaths related to cocaine overdose emphasize the clinical priority for developing effective pharmacotherapeutics. Unfortunately, no effective treatment for cocaine use disorders exists. Current treatment interventions focus on individual or group counseling, cognitive behavioral therapy, or contingency management (Kampman, 2019). Additionally, some treatments approved by the U.S. Food & Drug Administration (FDA) for other health disorders have been proposed and used as off-label medications to evaluate treatment efficacy for cocaine use disorders (Myrick, et al., 2001; Prince & Bowling, 2018). However, these psycho-behavioral and off-label pharmacotherapies have had low success rates for the following reasons: 1) some patients do not respond to these treatments, 2) there are undesirable side effects, or 3) the effects of intervention on abstinence are not long-lasting (Buchholz & Saxon, 2019; Paino, Aletraris, & Roman, 2019).

We previously suggested that zolmitriptan may have therapeutic efficacy for treating cocaine use disorders because this treatment was effective in attenuating methamphetamine SA regardless of whether rats underwent abstinence unlike effects observed previously with cocaine (Garcia et al., 2017). Zolmitriptan is a clinically-available 5-$HT_{1D/1B}$R agonist approved for treatment of migraine headaches with slightly higher affinity for 5-HT_{1D}Rs over 5-HT_{1B}Rs (Table 1; Spencer & Gunasekara, 1999; Lucas-Osma et al., 2019). Zolmitriptan is well tolerated via oral, intranasal, and

intravenous routes of administration and readily crosses the blood-brain barrier (Seaber et al., 1997; Peterlin & Rapoport, 2007; Varnäs et al., 2013). Furthermore, zolmitriptan is effective following long-term use in both adolescent and adult males and females (Spencer, Gunaekara, & Hills, 1999; Linder & Dowson, 2000; Dowson et al., 2003). These qualities of zolmitriptan make it a suitable candidate for clinical studies as a novel treatment for cocaine use disorders.

Previous research examining the role of 5-HT_{1B}Rs has focused on male rats, however, there are sex differences in the etiology and consequences of cocaine use disorders. Women tend to have a faster progression to cocaine abuse even after using smaller amounts of drug and they have higher rates of relapse following abstinence (Peters et al., 2019; NIDA, 2020). Preclinical animal models have provided similar evidence indicating that there are sex differences across all phases of cocaine use disorder development. Female rats acquire SA of drugs, including cocaine, at a faster rate (Lynch, Roth, & Carroll, 2002), females consume more cocaine under a FR1 and PR schedule (Roberts, Bennett, & Vickers, 1989; Lynch & Carroll, 1999; Lynch & Taylor, 2004), and females respond more under extinction and drug-primed reinstatement conditions compared to males (Lynch, 2006). Overall, evidence from human and animal studies suggest that females are more vulnerable to SUDs.

In this chapter, we examined the efficacy of zolmitriptan to reduce cocaine SA in male and female rats. First, we examined zolmitriptan pretreatment effects using a dose-effect function on maintenance of cocaine SA without a period of prolonged abstinence. Second, we examined if the zolmitriptan-induced effects observed were 5-HT_{1B}R-mediated and/or 5-HT_{1D}R-mediated using selective antagonist to reverse the agonist effects at the

respective receptors. Third, as a control for possible effects on general appetitive consummatory behvaviors, we examined zolmitriptan pretreatment effects on sucrose reinforcement. Finally, we examined if the antagonists affected locomotor activity as a control for potential motor confounds. Based on our previous findings with 5-HT_{1B}R agonists and cocaine, we hypothesized that zolmitriptan would increase cocaine intake pre-abstinence and decrease cocaine intake post-abstinence in male rats similar to that observed with pretreatment with CP 94,253 and that these effects would also extend to female rats.

Methods

Animals

Sprague Dawley male rats and free-cycling female rats (Charles River, San Diego, CA) weighing 200-300 g were single-housed in separate climate-controlled environments on a 14:10 reverse light/dark cycle (lights off at 6 am). Rats had *ad libitum* access to food and water except for initial cocaine and sucrose self-administration (SA) training when they were food-restricted to 85% of their *ad libitum* weights to facilitate acquisition of SA. Rats were handled for 5-6 days prior to the beginning of any procedure. The experiments proceeded in accordance with a protocol and standard operating procedures approved by the Arizona State University Institutional Animal Care and Use Committee.

Drugs

Cocaine hydrochloride (RTI International, Research Triangle Park, NC) was dissolved in saline and filtered with 0.2 µm membrane Acrodisc syringe filters (PALL Corporation, USA). Zolmitriptan, SB 224,289, and BRL 15,572 (Tocris Bioscience, USA) were dissolved with dimethyl sulfoxide (DMSO) and diluted with saline to a final concentration of 10% DMSO. Additionally, high doses of SB 224,289 and BRL 15,572 were sonicated

for ~2 mins with gentle warming in a bath sonicator (VWR B2500A-MTH). BRL 15,572 was chosen for its high affinity, displaying 60-fold selectivity for 5-HT_{1D}Rs over 5-HT_{1B}Rs (Table 1; Price et al., 1997). Additionally, BRL 15,572 crosses the blood-brain-barrier and has been shown to be effective at reversing the agonist-induced effects of sumatriptan, a triptan less selective but similar to zolmitriptan (Hagan et al., 1997; Hopwood et al., 2001). All drug injections, except for self-administered cocaine, were prepared fresh daily and injected at a volume of 1 ml/kg body weight. Vehicle refers to the respective solvent.

Surgery

Rats for the cocaine SA experiments underwent surgery for implanting a chronic indwelling catheter into the jugular vein as detailed in Neisewander et al. (2000). Rats had 5-6 days of recovery before commencing cocaine SA training. During the recovery period, catheters were flushed daily with 0.1 ml of cefazolin (10 mg/ml; WG Critical Care, LLC, NJ, USA) mixed with heparin/saline (70 U/ml; APP Pharmaceuticals, Schaumburg, IL, USA). After recovery, catheters were flushed only with 0.1 ml of heparin/saline. Catheter patency was tested periodically (0.05 ml; i.v.) with the short-acting barbiturate methohexital sodium (16.7 mg/ml; Jones Pharma Inc., St. Louis, MO) at a dose that produces brief loss of muscle tone when administered intravenously.

Apparatus

The operant conditioning chambers (Med Associates, St. Albans, VT, USA) contained an active and inactive lever, a cue light, and a tone generator as previously described in Pentkowski et al. (2009). Operant chambers contained either an infusion pump (Med Associates) connected to a liquid swivel (Instech, Plymouth Meeting, PA) and attached to a polyethylene tubing sheltered within a metal leash (PlasticsOne, Roanoke,

VA) or contained pellet dispensers (Med Associates). All operant conditioning chambers were housed within sound attenuating boxes that contained a ventilation fan. Male and female rats were separated in different rooms to avoid any overlap and potential confounding hormonal influences.

General Procedures

Experimental sessions commenced at approximately the same time of day, 6-7 days/week during the rats' dark cycle. Rats were first trained to lever press on a fixed ratio (FR) 1 schedule of reinforcement for an infusion of cocaine (0.75 mg/kg/0.1 ml infusion, i.v.) or a single sucrose pellet (45 mg, Bio-Serv). Rats progressed from a FR1 to FR5 if >10 reinforcers were obtained at the end of each session for two consecutive sessions. Rats on cocaine SA were gradually placed on *ad libitum* feeding after reaching the FR5 schedule. All testing procedures occurred once rats met a stability criterion of <15% variance in total number of reinforcers obtained on the FR5 schedule across three consecutive sessions. The beginning of the session was signaled by extending both levers. Completion of the operant schedule activated the tone and light cue followed 1 s later by either an infusion of cocaine delivered across 6 s or delivery of a sucrose pellet. The tone, light, and pump were then turned off, and simultaneously a house light was activated for 20 s to signal a timeout period during which additional lever responses were recorded but had no programmed consequences. After the timeout period, the house light turned off and the reinforcer was available again. All rats were acclimated to subcutaneous (s.c.) injections with saline prior to the first test.

Several studies indicate that ovarian hormones mediate sex differences for cocaine-related behaviors (Lynch, Arizzi, & Carroll, 2000; Lynch, Roth, & Carroll, 2002; Hu et al.,

2004). However, previous research in our lab has found no estrous-cycle differences under a chronic cocaine SA procedure similar to the experimental design used for these experiments. Additionally, we found that pretreatment with a selective 5-HT_{1B}R agonist had the same effect for cocaine SA in female rats regardless of the estrous-cycle (Scott et al., *in prep*). Therefore, the estrous-cycle was not monitored in this study.

Experiment 2a: Effects of zolmitriptan treatment on maintenance of cocaine self-administration.

Experimentally naïve rats were food restricted and trained to self-administer cocaine (0.75 mg/kg, i.v.) progressing from a FR1 to FR5 schedule of reinforcement during 2 h sessions (males: 10-25 sessions; females: 14-35 sessions). The rats were then placed on *ad-libitum* feeding and continued training until they reached the stability criterion as described above. Using a within-subjects design, the rats (n = 30 males and 20 females) then received treatments of zolmitriptan (3.0, 5.6, and 10 mg/kg, s.c.) in descending order across tests. Rats also received a vehicle treatment test in between each of the three zolmitriptan doses, with order of vehicle and zolmitriptan treatments counterbalanced across rats for similar number of infusions to mitigate confounding effects of treatment order and potential effects of repeated administration with DMSO as a solvent. Rats underwent cocaine SA on the training dose between test sessions until the stability criteria were re-established. All doses of zolmitriptan were chosen based on previous research indicating the effectiveness and safety profile of this drug in rodents and human subjects (Ferrari, 1997; Spencer, Gunasekara, & Hills, 1999).

During test sessions, rats had access to the training dose (0.75 mg/kg, i.v.) for the first hour and a low dose of cocaine (0.075 mg/kg, i.v.) for the second hour. These doses

were selected based on previous experiments in our lab investigating the effects of the more selective 5-HT_{1B}R agonist, CP 94,253, on cocaine SA (Pentkoswki et al., 2014). Specifically, the low (0.075 mg/kg) and training (0.75 mg/kg) doses of cocaine are on the ascending and descending limb of the dose-response function, respectively. Treatment-induced changes with these doses can be interpreted as changes in the reinforcing value of cocaine (Mello & Negus, 1996). For example, a leftward shift of the dose-response curve is indicated by an increase in cocaine intake at the low dose and a decrease in intake at the high dose and is interpreted as an enhancement in cocaine reinforcement. Similarly, a downward shift of the dose-response curve is indicated by a decrease in cocaine intake at both doses and is interpreted as an attenuation in cocaine reinforcement. The training dose was in effect for the first hour because rats are slow to stabilize on the low dose of cocaine when it is presented first.

Experiment 2b: Effects of zolmitriptan on maintenance of sucrose self-administration.

A new group of experimentally naïve rats were trained to self-administer sucrose across 30-min sessions progressing from a FR1 to FR5 (males: 10-24 sessions; females: 12-27 sessions), as described above. Treatment assignment and testing procedures for experiment 2b were similar to experiment 2a. Briefly, rats (*n = 10* males and *14* females), were assigned to received s.c. injections of either vehicle or 10 mg/kg zolmitriptan on test day 1 followed by the opposite treatment on test day 2, i.e. rats that received vehicle first now received 10 mg/kg zolmitriptan and vice versa. Rats then received 3.0 and 5.6 mg/kg zolmitriptan, in descending order, using a within-subjects design. All testing procedures

occurred once rats met a baseline stability criterion, as described above, prior to each test with zolmitriptan.

Experiment 2c: Effects of 5-HT$_{1B}$R and 5-HT$_{1D}$R antagonists on zolmitriptan-induced attenuation of cocaine self-administration.

Experimentally naïve rats (*n* = 20 males and 10 females) were trained to self-administer cocaine identical to the procedures described in experiment 2a (males: 9-25 sessions; females: 10-43 sessions). Additionally, a subset of rats (*n* = 14 males and 14 females) from experiment 2a were used for the following procedures. After meeting the stability criterion, rats were randomly assigned to receive vehicle, SB 224,289 (3.2, 5.6, or 10 mg/kg, s.c.), or BRL 15,572 (0.3, 1, or 3 mg/kg, i.p.) alone or in combination with zolmitriptan (5.6 mg/kg, s.c.). Rats were separated into two groups where the first group tested with all doses of SB 224,289 first followed by all doses of BRL 15,572 and the second group tested with BRL 15,572 first followed by SB 224,289. Prior to each test, rats received two injections 15 min apart of their assigned antagonist dose first followed by zolmitriptan or vehicle. Testing began 15 min after the latter injection. All other testing procedures were identical to experiment 2a.

Experiment 2d: Effects of 5-HT$_{1B}$R and 5-HT$_{1D}$R ligands on spontaneous locomotion.

A subset of rats (*n = 6* males and *7* females) from experiment 2b were placed into abstinence for a minimum of 10 days following the last treatment with the 5-HT$_{1B}$R and 5-HT$_{1D}$R antagonists on cocaine SA. Rats received an injection of vehicle, zolmitriptan (5.6 mg/kg, s.c.) or SB 224, 289 (10 mg/kg, s.c) using a within-subjects design to assess the effects of these drugs on spontaneous locomotor activity. After completing these tests, rats underwent 5 rest days in their home cage and then began tests for the effect of vehicle or a

5-HT_{1D}R antagonist, BRL 15,572 (0.3, 1.0, & 3.0 mg/kg, i.p.) on spontaneous locomotor activity using a within-subjects design. SB 224,289 and BRL 15,572 were injected 30 mins prior to locomotor activity testing. In addition, rats tested on SB 224,289 received an injection 15 mins later of either vehicle or zolmitriptan (5.6 mg/kg, s.c.). Rats were then placed in testing chambers as described in experiment 1d and spontaneous locomotor activity was recorded for 60 min. The dose of SB 224,289 was chosen based on the effectiveness of reversing the SA attenuating effects of zolmitriptan. Limited evidence exists regarding the role of 5-HT_{1D}R antagonists on locomotor ability, and therefore, multiple doses of BRL 15,572 were examined in both males and females. Rats underwent two - three rest days in between test sessions where they remained in their home cage. All treatments were randomly assigned and counter-balanced to mitigate confounding treatment order effects.

Data Analysis

Statistical analyses were conducted with IBM SPSS Statistics® v. 25. Descriptive statistics are reported as the mean ± standard error of the mean. Self-administration data, including active and inactive lever responses, reinforcers obtained, and total consumption (mg/kg), as well as total distance traveled for locomotor assessment were analyzed by either repeated measures or a mixed design ANOVA depending on the experimental design. All sources of significant effects were further analyzed by Tukey's post-hoc tests or t-tests. Final n/group are reported in the methods and figure captions along with its respective figure.

Results

Experiment 2a: Zolmitriptan decreased the number of cocaine infusions obtained, total cocaine intake (mg/kg, i.v.), and active lever responding (Fig. 6). Male and female rats had similar response rates during acquisition (data not shown) although there were sex differences in the cocaine infusions obtained. Average daily reinforcement rates during acquisition were higher in males than females with mean infusions/2 h of 21.20 ± 0.57 and 16.82 ± 0.58, respectively ($p < 0.05$). During the final days of training when reinforcement rates were stable, this sex difference was no longer evident and all baseline reinforcement rates prior to each test with zolmitriptan showed no effects of sex, day, nor interaction (Table 2).

All omnibus analyses indicated no main effect of sex nor interactions, and therefore data were collapsed across males and females for subsequent analyses. Because there were no effects of vehicle pretreatment across each of the three vehicle tests occurring between each of the zolmitriptan dose pretreatments on any of the measures, the average of all three vehicle tests was used in further analyses to achieve the most representative value. Analyses of cocaine infusions, intake, and active lever responses all revealed main effects of cocaine dose [$F(1, 27) = 81.61$, 617.65, and 69.45, respectively, $p's < 0.05$], zolmitriptan dose [F (3, 81) = 7.46, 7.76, 5.91, respectively, $p's < 0.05$], and a cocaine dose by zolmitriptan dose interaction [F (3, 81) = 6.33, 6.31, 4.84, respectively, $p's < 0.05$]. Rats received a higher number of cocaine infusions (Fig. 6A) and pressed the active lever more (Fig. 6C) while the low cocaine dose was available, but their consumption of cocaine was higher when the high cocaine dose was available (Fig. 6B; $p's < 0.05$) as expected. More importantly, zolmitriptan at 3.0 and 5.6 mg/kg decreased cocaine infusions (Fig. 6A),

intake (Fig. 6B), and active lever responding (Fig. 6C) when 0.075 mg/kg cocaine was available, and all doses of zolmitriptan (3.0 – 10 mg/kg, s.c.) decreased cocaine infusions, intake, and active lever responding when 0.75 mg/kg cocaine was available (Fig. 6A-C; *p's* < 0.05). Neither cocaine dose nor zolmitriptan influenced inactive lever responding (Fig. 6D).

Experiment 2b: Male and female rats had similar sucrose reinforcement and response rates during acquisition (data not shown). However, sex differences emerged during the final days of training in which sucrose reinforcement rates, averaged across three consecutive sessions, were higher in males than females with mean reinforcers obtained/30 min of 28.33 ± 0.60 and 22.71 ± 0.18, respectively ($p < 0.05$). This sex difference was likely due to differences in body weight because males gained more weight than females (baseline weights by sex interaction [F (3, 66) = 17.68, $p < 0.05$]; Fig. 7A). Similarly, baseline sucrose reinforcement rates increased across time [F (3, 66) = 25.94, $p < 0.05$] and were higher in males than females [F (1, 22) = 5.00, $p < 0.05$] (Fig. 7B).

Since sucrose reinforcement baselines varied across sessions, subsequent analyses were performed on the difference between the zolmitriptan dose on test day minus the appropriate baseline to correct for changing baseline values. Analyses indicated no main effects nor interaction effects of zolmitriptan on the total sucrose reinforcers obtained (Fig. 7C) nor on active (Fig. 7D) or inactive (Fig. 7E) lever responding. Therefore, zolmitriptan (0-10 mg/kg, s.c.) had no effect on sucrose reinforcement.

Experiment 2c: The effects of the selective 5-HT_{1B}R and 5-HT_{1D}R antagonists on cocaine SA were assessed both alone and in combination with zolmitriptan. Initial analyses of baseline reinforcement rates (Table 3) and test day measures showed no effects of sex

and therefore all data are collapsed across males and females for subsequent analyses. When effects of SB 224,289 alone were examined (Fig. 8), analysis of cocaine infusions and active lever responses revealed only main effects of cocaine dose [F (1, 88) = 97.52 and 87.01, respectively, p's < 0.05]. However, analysis of cocaine intake (mg/kg, i.v.) revealed main effects of cocaine dose [F (1, 88) = 1003.21, $p < 0.05$], SB 224,289 dose [F (3, 88) = 3.25, $p < 0.05$], and a cocaine dose by SB 224,289 dose interaction [F (3, 88) = 4.59, $p < 0.05$]. While rats received a higher number of cocaine infusions (Fig. 8A) and pressed the active lever more (Fig. 8C) during availability of the low cocaine dose, they consumed more cocaine when the high cocaine dose was available (Fig. 8B; $p < 0.05$) as expected. Further analyses of the cocaine dose by SB 224,289 interaction on intake revealed that SB 224,289 reduced cocaine intake at 5.6 mg/kg relative to 3.2 mg/kg when the high dose of cocaine was available ($p < 0.05$). However, neither of these two SB 224,289 doses were different from vehicle. There were no effects on inactive lever responding (Fig. 8D). Overall, treatment with SB 224,289 alone did not alter cocaine SA.

SB 224,289 blocked the zolmitriptan-induced attenuation of cocaine-related behaviors (Fig. 9). Analyses of cocaine infusions, total intake (mg/kg, i.v.), and active lever responses all revealed main effects of cocaine dose [F (1, 111) = 104.90, 1206.31, and 99.95, respectively, p's < 0.05], 5-HT_{1B}R dose group [F (4, 111) = 4.02, 3.35, 3.52, respectively, p's < 0.05] and cocaine dose by 5-HT_{1B}R dose group interactions [F (4, 111) = 3.04, 1.08, 2.99, respectively, p's < 0.05]. While rats received a higher number of cocaine infusions (Fig. 9A) and pressed the active lever more (Fig. 9C) during availability of the low cocaine dose, they consumed more cocaine when the high cocaine dose was available (Fig. 9B; $p < 0.05$) as expected. Zolmitriptan alone attenuated infusions (Fig. 9A), intake

(Fig. 9B), and active lever responses (Fig. 9C, *p's* < 0.05) as expected. Importantly, SB 224,289 dose-dependently reversed the attenuating effects of zolmitriptan, with no differences from vehicle pretreatment noted at the two highest doses of SB 224,289 across all of these measures. There were no effects on inactive lever responding (Fig. 9D). Collectively, these results indicate that zolmitriptan decreases cocaine intake in a 5-HT_{1B}R-mediated fashion.

The selective 5-HT_{1D}R antagonist, BRL 15,572 given alone had no effect on cocaine infusions (Fig. 10A), cocaine intake (Fig. 10B), nor active (Fig. 10C) or inactive lever (Fig. 10D) responding. There were only main effects of cocaine dose for these respective measures [F (1, 87) = 142.71, 919.75, and 138.23, $p's < 0.05$], replicating the expected effects observed in the previous experiments. There were no effects or interactions in the analysis of inactive lever responding. These results indicate that treatment with BRL 15,572 alone does not alter cocaine SA relative to vehicle treatment.

BRL 15,572 partially reversed the zolmitriptan-induced attenuation of cocaine-related behaviors (Fig. 11-13). For infusions, unlike all other analyses in this study, there was a main effect of sex [F (1, 111) = 8.09, $p < 0.05$] and a cocaine dose by sex interaction [F (1, 111) = 7.511, $p < 0.05$] where female rats took fewer infusions than male rats only when the low dose of cocaine was available (Fig. 11A, $p < 0.05$). Additionally, there were main effects of cocaine dose [F (1, 111) = 134.86, $p < 0.05$], BRL 15,572 dose group [F (4, 111) = 4.22, $p < 0.05$], and a cocaine dose by BRL 15,572 dose group interaction [F (4, 111) = 3.32, $p < 0.05$]. Zolmitriptan alone significantly decreased cocaine infusions ($p < 0.05$) and the lowest dose of BRL 15,572 was ineffective in altering the effect of zolmitriptan when the high dose of cocaine was available (Fig. 11B). However, BRL

15,572 at 1.0 and 3.0 mg/kg seemed to partially reverse the effects of zolmitriptan when the high dose of cocaine was available since these dosage groups were not different from vehicle (Fig. 11B).

Analysis of intake revealed main effects of cocaine dose [F (1, 111) = 1069.95, $p <$ 0.05], BRL 15,572 dose group [F (4, 111) = 4.58, $p < 0.05$], and a cocaine dose by BRL 15,572 dose group interaction [F (1, 111) = 3.79, $p < 0.05$]. Similar to infusions, zolmitriptan alone decreased cocaine intake (Fig. 12, $p < 0.05$) and the lowest dose of BRL 15,572 was ineffective in altering the effect of zolmitriptan when the high dose of cocaine was available. BRL 15,572 at 1.0 and 3.0 mg/kg reversed the effects of zolmitriptan when the high dose of cocaine was available (Fig. 12) similar to the effects on infusions.

Analysis of active lever responses revealed a main effect of sex [F (1, 109) = 5.84, $p < 0.05$] and a cocaine dose by sex interaction [F (1, 109) = 6.59, $p < 0.05$] where female rats pressed less on the active lever than male rats only when the low dose of cocaine was available (Fig. 13A, $p < 0.05$). Additionally, there were main effects of cocaine dose [F (1, 109) = 114.97, $p < 0.05$], BRL 15,572 dose group [F (4, 109) = 3.00, $p < 0.05$], and a cocaine dose by BRL 15,572 dose group interaction [F (4, 109) = 2.52, $p < 0.05$]. Similar to the effects on infusions, zolmitriptan alone significantly decreased active lever responding (Fig. 13B, $p < 0.05$) and the lowest dose of BRL 15,572 was ineffective in altering the effect of zolmitriptan when the high dose of cocaine was available, However, but the 1.0 and 3.0 mg/kg doses partially reversed the effects of zolmitriptan since these dosage groups were not different from vehicle (Fig. 13B). There were no effects on inactive lever responding (data not shown). In summary, BRL 15,572 was not as efficacious at blocking the effects of zolmitriptan when the low dose of cocaine was available, in part,

because these effects were less pronounced in female rats at the low cocaine dose (Figs. 11, 13; $p < 0.05$). However, BRL 15,572 at 1.0 and 3.0 mg/kg (i.p.) blocked the attenuating effects of zolmitriptan when the high cocaine dose was available (Fig. 11-13) suggesting that zolmitriptan's effects also involve 5-HT_{1D} receptors.

Experiment 2d: Assessment of 5-HT_{1B}R (Fig. 14) and 5-HT_{1D}R (Fig. 15) antagonist pretreatment effects on locomotor activity revealed no sex differences and therefore locomotor data was collapsed across male and female rats. Analyses of total distance traveled revealed no effects of zolmitriptan, SB 224,289, or the combination of both 5-HT_{1B}R drugs at the doses that were effective in altering cocaine SA measures. BRL 15,572 also had no effect total distance traveled. Therefore, 5-HT_{1B}R and 5-HT_{1D}R activation fails to alter locomotor activity.

Discussion

The results of this study are exciting because zolmitriptan attenuated cocaine intake in rats during maintenance of SA, in contrast to our prediction that it would enhance cocaine intake as previously observed with more selective 5-HT_{1B}R agonists. Specifically, zolmitriptan (3.0, 5.6, & 10 mg/kg, s.c.) decreased cocaine intake, as well as infusions obtained, and active lever response rates when a low (0.075 mg/kg) or a high (0.75 mg/kg) cocaine dose was available in both male and female rats (Fig. 6). The decrease in cocaine intake at low and high doses is indicative of a downward shift in the dose-effect curve and is interpreted as an attenuation in cocaine reinforcement. Importantly, the doses of zolmitriptan effective at reducing cocaine intake had no effect on sucrose reinforcement (Fig. 7). In addition, studies have found that systemic administration with zolmitriptan at doses ranging from 3.0 – 30 mg/kg fails to alter locomotor measures including duration of

walking, rearing, and grooming (de Almeida, 2001). Similarly, we found that zolmitriptan does not alter locomotor activity (Fig. 14-15). The lack of zolmitriptan effects on sucrose reinforcement and locomotor activity mitigate the possibility that zolmitriptan nonspecifically alters motor ability or other processes involved in operant behavior. These findings support the interpretation that zolmitripan reduces the reinforcing effects of cocaine and suggest that zolmitriptan has potential to be repurposed as a medication for cocaine use disorders.

The findings also suggest that the mechanism of zolmitriptan's effects involves both 5-HT_{1B} and 5-HT_{1D} receptors. When administered alone, neither the 5-HT_{1B}R or 5-HT_{1D}R antagonist affected cocaine SA (Figs. 8, 10). However, the selective 5-HT_{1B}R antagonist, SB 224,289, dose-dependently reversed zolmitriptan's effects, which was evident at a lower dose of SB 224,289 when the high dose of cocaine was available (Fig. 9). The selective 5-HT_{1D}R antagonist, BRL 15,572, also reversed zolmitriptan effects although less efficaciously than SB 224,289 (Fig. 11-13). The effects of BRL 15,572 were more variable when the low cocaine dose was available. Both the 1.0 and 3.0 mg/kg doses of BRL 15,572 blocked the zolmitriptan-induced attenuation when the high cocaine dose was available.

We were surprised to find that zolmitriptan attenuated cocaine intake since under similar procedures, treatment with more selective 5-HT_{1B}R agonists enhance cocaine intake (Parsons, Koob, & Weiss, 1998, Pentkowski et al., 2012; 2014). However, we recently discovered that zolmitriptan attenuates the reinforcing effects of methamphetamine regardless of whether the rats undergo a period of abstinence (Garcia et al., 2017). Furthermore, zolmtriptan attenuates psychostimulant reinforcement without

altering sucrose reinforcement and the zolmitriptan-induced effects on cocaine are antagonized in a 5-HT_{1B} and 5-HT_{1D}R-mediated fashion. Therefore, the zolmitriptan-induced attenuation of cocaine intake prior to abstinence may be due to differences in the pharmacological actions at 5-HT_{1D}Rs relative to other selective 5-HT_{1B}R agonists. Collectively, these studies provide support for the selective effects of zolmitriptan on psychostimulant reinforcement.

5-HT_{1B}R and 5-HT_{1D}Rs have been implicated in modulating locomotion. 5-HT_{1B}R agonists either increase (Koe et al., 1992) or produce no change in spontaneous locomotor activity (Pentkowski et al., 2009; Clissold, Choi, & Pratt, 2013; Garcia et al., 2017). Less evidence exists documenting the role 5-HT_{1D}Rs in ambulation in rodents. More recent reports suggest that zolmitriptan and other 5-HT_{1D}R agonists inhibit the monosynaptic stretch reflex (Lucas-Osma et al., 2019). However, it is important to point out that these studies were conducted *in vitro* on spinal column transections. Saracheva and colleagues have examined the effects of the 5-$HT_{1D/1B}$R agonists, almotriptan, frovatriptan, and eletriptan, on locomotion in male and female rats. They found that these triptans increased horizontal and vertical movement (Saracheva & Getova, 2014; Saracheva, Vasileva, & Getova, 2019). These three triptans have relatively high affinity for 5-HT_{1B} and 5-HT_{1D}Rs (Tfelt-Hansen, De Vries, & Saxena, 2000) and therefore the increase in locomotion may be attributed to 5-HT_{1B}R activation as 5-$HT_{1B/1D}$R agonists increase psychostimulant-induced locomotion, although some studies failed to observe this effect (Chaouloff et al., 1999; Przegalinski et al., 2001; Borycz et la., 2008; Miszkiel, Filip, & Przegalinski, 2011; Shahidi et al., 2018). The zolmitriptan-induced attenuation of cocaine intake in this study

is unlikely a result of an effect on locomotor capability because there was no effect on locomotion.

There is a possibility that zolmitriptan decreased cocaine intake as a result of actions at, or interactions with, receptors other than 5-HT_{1B}Rs. Zolmitriptan, like other triptans, displays affinity for both 5-HT_{1D}Rs and 5-HT_{1B}Rs (K_i = 0.63 and 5.01 nM, respectively) and lower affinity at 5-HT_{1F}Rs (K_i = 63.09 nM; Spencer & Gunasekara, 1999; Lucas-Osma et al., 2019). Recently, Shahidi and colleagues investigated the role of 5-HT_{1D}R and 5-HT_{1F}R agonists on methamphetamine reward. Administration of either the 5-HT_{1D}R agonist PNU142633 or 5-HT_{1F}R agonist LY344864 has no effect on expression of methamphetamine conditioned place preference but both receptor agonists attenuate reinstatement of methamphetamine reward (Shahidi et al., 2018[a,b]). However, to my knowledge, no other evidence exists investigating the effects of 5-HT_{1D}R or 5-HT_{1F}R agonists on cocaine effects. We cannot completely rule out that 5-HT_{1D}Rs are involved in modulating cocaine reward/reinforcement since we found that BRL 15,572 mildly attenuated the effects of zolmitriptan. However, this effect did not appear dose-dependent and may therefore be nonspecific. Future research will need to further assess if 5-HT_{1D}R activation attenuates cocaine intake.

Some reports suggest that concomitant treatment of triptans and 5-HT reuptake inhibitors may precipitate the 5-HT syndrome, which can be life-threatening. Serotonin syndrome is characterized by excessive 5-HT availability in the central nervous system and consists of autonomic hyperactivity, neuromuscular abnormalities, and changes in mental status like restlessness and hallucinations (Sternbach, 1991; Bodner, Lynch, & Kahn, 1995; Volpi et al., 2013). In 2006, the FDA issued a warning indicating that triptans co-

administered with either selective monoamine reuptake or monoamine oxidase inhibitors could precipitate the fatal 5-HT syndrome (Evans, 2007; Shapiro et al., 2007). However, this warning is based on only a few case studies from ~20 years ago, while in the intervening time there are no reports indicating that zolmitriptan treatment either alone or with selective monoamine reuptake inhibitors induces 5-HT syndrome (Shapiro et al., 2007; Rolan, 2012; Sclar et al., 2012). More recent evidence implicates the 5-HT_{2A} receptor in 5-HT syndrome (Gillman, 2010, Francescangeli et al., 2019; Scotton et al., 2019). Zolmitriptan, like all triptans, binds with high affinity to 5-$HT_{1B/1D}$Rs and to a lesser extent 5-HT_{1F}Rs but has no agonist action at 5-HT_{2A} receptors (Gillman, 2010). Studies investigating the interaction between other triptans (e.g. sumatriptan) and antidepressants on 5-HT syndrome have produced negative results (Blier & Bergeron, 1995; Putnam et al., 1999). Despite the FDA report, the concomitant use of triptans with selective monoamine reuptake inhibitors is widespread without indication of serious health consequences (Shapiro & Tepper, 2007; Robblee et al., 2019). Future studies will need to investigate the relation between chronic exposure to cocaine, a 5-HT reuptake inhibitor, cardiovascular disease, and zolmitriptan treatment. Cocaine use has been implicated in cardiovascular complications like myocardial infarctions and arrhythmias (Mouhaffel et al., 1995; Vasica & Tennant, 2002) and zolmitriptan is contra-indicated for use in patients with cardiovascular disorders. However, no serious cardiovascular events have been associated with zolmitriptan treatment (Spencer, Gunasekara, & Hills, 1999; Peterlin & Rapport, 2007; Marmura, 2009), and zolmitriptan has been shown to transiently increase arterial pressure only at doses that are 5-10 times greater than the recommended therapeutic dose (Spencer, Gunasekara, & Hills, 1999; Peterlin & Rapport, 2007). Nevertheless, further

investigation of the effects of zolmitriptan treatment in models of cardiovascular disease risk profiles is needed to better understand zolmitriptan's therapeutic potential to treat cocaine use disorders.

To our knowledge, we are the first group to screen for the clinical efficacy of zolmitriptan on cocaine reinforcement. On the positive side, zolmitriptan has good bioavailability and tolerability via oral and intravenous routes of administration (Seaber et al., 1997) and readily crosses the blood-brain barrier (Varnäs et al., 2013). Furthermore, zolmitriptan is effective in male and female adolescent and adults and shows efficacy following long-term use (Spencer, Gunaekara, & Hills, 1999; Linder & Dowson, 2000; Dowson et al., 2003). These qualities of zolmitriptan along with our positive results suggest that zolmitriptan is a suitable candidate for clinical studies as a novel treatment for psychostimulant dependence.

CHAPTER 4

5-HT_{1B}R AGONIST ATTENUATION OF COCAINE SELF-ADMINISTRATION PERSISTS AFTER A PERIOD OF RELAPSE

Garcia et al., (in prep).

Drug addiction is a relapsing disorder characterized by persistent drug use despite negative consequences. Unfortunately, cocaine and other psychostimulants produce changes in the brain that can induce intense desire for the drug, resulting in relapse even after a protracted period of abstinence (McKay et al., 1995; McLellan et al., 2000; Farrell, Schoch, & Mahler, 2018). Indeed, ~ 85% of individuals with substance use disorders relapse within the first year after drug cessation (Brandon et al., 2007; Sinha, 2011; NIDA, 2019) and it is not uncommon for recovering individuals to cycle between drug use and abstinence (McLellan et al., 2000). Therefore, effective pharmacological treatments need to reduce the risk of relapse.

Relapse to SUDs is defined as the resumption of substance use after a period of either forced or voluntary abstinence. Unfortunately, there are multiple precipitating factors that can trigger relapse including drug craving, re-exposure to the drug or drug-paired stimuli, and stress (Sinha et al., 2000; Shaham et al., 2002; Burmeister et al., 2004). Stimuli repeatedly paired with drug-taking can acquire incentive motivational value that elicits an intense desire and/or expectation for the drug (Markou et al., 1993; Robinson & Berridge, 1993). These conditioned responses to drug-conditioned stimuli, referred to as cue reactivity, have been frequently observed and reported in drug-dependent patients and are believed to be involved in the desire to use drugs (Childress et al., 1993; Ingmar et al., 1999; Robbins et al., 1999; Tiffany, Carter, & Singleton, 2000; LeCocq et al., 2020).

In the preclinical setting, the forced abstinence procedure can be used to model aspects of human relapse including incentive motivation and resumption of drug-taking (Neisewander, Lucki, & McGonigle, 1993; Fuchs, Branham, & See, 2006; Reichel & Bevins, 2009: Pentkowski et al., 2014). In this procedure, rats are removed entirely from the drug-accessible environment and housed in an animal facility for different periods of abstinence. This situation is analogous to human incarceration or patient treatment programs in which an individual is forced to undergo a protracted period without drug. After, rats are re-introduced to the drug-accessible environment and tested for drug-seeking or drug-taking behaviors. This forced abstinence model allows for preclinical evaluation of pharmacotherapies in a situation similar to where an abstinent individual may come across drug-conditioned stimuli such as drug paraphernalia or come to sample the drug resulting in recidivism.

We and others have suggested targeting 5-HT_{1B}Rs for developing pharmacotherapies to prevent relapse. Initial studies using agonists to target 5-HT_{1B}Rs indicate that 5-HT_{1B}R stimulation during maintenance of cocaine SA enhance the rewarding effects of cocaine (Parsons, Koob, & Weiss, 1998). However, we found that 5-HT_{1B}R stimulation modulates cocaine SA differently depending on the stage of the drug-taking cycle. Following a period of abstinence from cocaine, stimulation of 5-HT_{1B}Rs during a lapse challenge test (i.e., a single priming injection of cocaine), produces the opposite effect, i.e., attenuates cocaine intake (Pentkowski, et al., 2014), compared to that observed during daily SA. Similarly, both over-expression of 5-HT_{1B}Rs and systemic treatment with 5-HT_{1B}R agonists, dose-dependently decrease cocaine-seeking (Acosta et al., 2005; Pentkowski et al, 2009, 2012). These results suggest that there is a "switch" in

the functional effects of 5-HT_{1B}R agonists on cocaine reinforcement/motivation from facilitation during maintenance of SA to inhibition after abstinence. However, it remains unclear if the 5-HT_{1B}R agonist-induced attenuation of cocaine reinforcement/motivation is persistent. Since activation of 5-HT_{1B}Rs during maintenance of cocaine SA increases cocaine intake, we wanted to investigate if the abstinence-induced decrease in cocaine intake was long-lasting following abstinence or if these effects would revert to enhancing cocaine intake similar to the maintenance phase of SA.

In this chapter, we first replicated the finding that the selective 5-HT_{1B}R agonist, CP 94,253, decreases cocaine intake following a period of forced abstinence. Second, we investigated if the 5-HT_{1B}R agonist-induced attenuation of cocaine intake persisted following resumption of cocaine SA, similar to a full-blown relapse. Third, we investigated if 5-HT_{1B}R stimulation following abstinence would attenuate incentive motivation as measured by cocaine-seeking elicited by drug-conditioned stimuli during a cue reactivity test. We hypothesized that once the "switch" from facilitation to inhibition of cocaine SA occurs with 5-HT_{1B}R agonists treatment, it persists even in rats that resume daily cocaine SA.

Methods

Animals and surgeries

Sprague Dawley male rats (Charles River, San Diego, CA) weighing 250 – 300 g at the start of the experiment, were single-housed in a climate-controlled environment on a 14:10 reverse light/dark cycle (lights off at 6 am). Rats had *ad libitum* access to food and water except for initial cocaine SA training when they were food-restricted to 85% of their *ad libitum* weights to facilitate acquisition of SA. Rats were handled for 5-6 days prior to

the beginning of the experiment. Rats underwent surgery for implanting a chronic indwelling catheter into the jugular vein as detailed above. Rats had 5 days of recovery before commencing cocaine SA training. During recovery, catheters were flushed daily with cefazolin (10 mg/0.1 ml; WG Critical Care, LLC, NJ, USA) and heparin/saline (7 units/0.1 ml; APP Pharmaceuticals, Schaumburg, IL, USA). After recovery, catheters were flushed only with heparin/saline. Catheter patency was tested periodically with methohexital sodium (0.835 mg/0.05 ml; Jones Pharma Inc., St. Louis, MO) at a dose that produces brief loss of muscle tone when administered intravenously. All experimental procedures proceeded in accordance with a protocol and standard operating procedures approved by the Arizona State University Institutional Animal Care and Use Committee.

Drugs

Cocaine hydrochloride (RTI International, Research Triangle Park, NC) was dissolved in saline and filtered with 0.2 μm membrane Acrodisc syringe filters (PALL Corporation, USA). CP 94,253 (donated by Dr. Benjamin Blass, Temple University) was dissolved in distilled water (vehicle). All drug injections, except for self-administered cocaine, were prepared fresh daily and injected at a volume of 1 ml/kg body weight.

Apparatus

Operant conditioning chambers (Med Associates, St. Albans, VT, USA) contained an active and inactive lever, a cue light, and a tone generator as detailed above. Additionally, the chambers contained an infusion pump (Med Associates) connected to a liquid swivel (Instech, Plymouth Meeting, PA) attached to a polyethylene tubing sheltered within a metal leash (PlasticsOne, Roanoke, VA) that had a plastic screw connector that

was connected to the outer casing of the catheter during sessions. All operant conditioning chambers were housed within sound attenuating boxes that contained a ventilation fan.

General Procedures

SA sessions lasted for 2 h and commenced at approximately the same time of day, 6 days/week during the rats' dark cycle. Rats were initially trained to lever press on a fixed ratio 1 (FR1) schedule of cocaine (0.75 mg/kg/0.1 ml) reinforcement. They progressed to an FR5 schedule once they had >10 infusions at the end of the SA session for two consecutive sessions. Completing of the operant schedule activated a tone and light cue followed 1 s later by the cocaine infusion, which was delivered across 6 s. The tone, light, and pump were then turned off, and simultaneously a house light was activated for 20 s to signal a timeout period during which additional lever responses were recorded but had no programmed consequences. After the timeout period, the house light turned off and the reinforcer was available again. Rats were gradually placed on ad-libitum feeding after reaching the FR5 schedule and all testing procedures occurred once rats met a stability criterion of <15% variance in total number of reinforcers obtained across three consecutive sessions. All rats were acclimated to s.c. injections with vehicle prior to testing for the first time to reduce confounds of stress from injections. During abstinence, rats remained in their home cage and were handled and weighed daily and given i.v. administration of heparin/saline to maintain catheter patency

Experiment 4a and 4b: Effects of the 5-HT$_{1B}$R agonist, CP 94,253, on cocaine relapse.

Male rats (n = 35) underwent a minimum of 15 days of cocaine SA as described above and were placed on a 21-day period of abstinence after reaching stability criteria for infusion rates. After, rats were tested twice using a within-subjects design, in which they

received an injection of vehicle (1 ml/kg s.c.) prior to one test and CP 94,253 (5.6 mg/kg, s.c.) prior to the other test. On the test day, rats received their randomly assigned injection of either vehicle or CP 94,253 15 min prior to the test session. During the 2-h SA test, rats had access to the training dose of cocaine (0.75 mg/kg, i.v.) for the first hour and access to a low dose of cocaine (0.075 mg/kg, i.v.) for the second hour, as detailed above. On the second test day, rats received the treatment injection opposite of their first treatment; i.e. rats that received vehicle on test day 1 received CP 94,253 on test day 2, and vice versa. Rats remained in their home cages for three days in between test days. Three days allowed time for CP 94,253 to be eliminated and to reinstate an abstinence period. The doses of cocaine and CP 94,253 were chosen based on previous research conducted by Pentkowski et al. (2014) showing that the low and high cocaine doses are on the ascending and descending limbs of the dose-response function, respectively, and at the low dose there is an abstinence-dependent switch in the functional effects of CP 94,253 on cocaine SA as described above.

Following the second post-abstinence test, a subset of rats (n = 10) resumed cocaine SA on a FR5 reinforcement schedule for a minimum of 15 consecutive sessions. This number of SA sessions was chosen to approximate the number of SA sessions during the original training and constituted a *relapse* period. Upon meeting the stability criteria, rats received an injection of either vehicle or CP 94,253, as previously described. Rats were allowed to re-stabilize for a minimum of three days before receiving a second test with the opposite treatment.

Experiment 4c: Effects of CP 94,253 on cue reactivity of cocaine-conditioned stimuli.

After the last relapse test, a subset of rats (n = 6) were placed into abstinence for an additional 21 days. Rats were randomly assigned to receive an injection of either vehicle or CP 94,253 (5.6 mg/kg, s.c.) 15 min prior to the first test of cue reactivity. Reactivity sessions lasted for 1 h and all response-contingent cocaine-conditioned stimuli were available on an FR1 schedule but no cocaine was available. After the first test, rats continued abstinence for five days to allow time for CP 94,253 to be eliminated and to reinstate an abstinence period prior to the second test. On the second test, rats received a treatment injection opposite that of the first treatment; i.e., rats that received vehicle on test day 1 received CP 94,253 on test day 2, and vice versa.

Data Analysis

Statistical analyses were conducted with IBM SPSS Statistics® v. 25. Descriptive statistics are reported as the mean ± standard error of the mean. Self-administration data, including active and inactive lever responses, infusions, total intake (mg/kg, i.v.), and latency to first lever response were analyzed by either repeated measures ANOVA with drug pretreatment and cocaine dose as within-subjects factors or paired-samples t-test. Latencies were subjected to logarithm transformations to homogenize variance. All sources of significant effects were further analyzed by Tukey's post-hoc tests or t-tests. Final n/group are reported in the methods and figure captions and the timeline for each experiment is outlined along with its respective figure.

Results

Experiment 4a: Pretreatment with CP 94,253 following 21 days of forced abstinence decreased cocaine infusions, intake, and active lever responses as previously

reported (Fig. 16). Analysis of cocaine infusions, intake, and active lever responses all revealed main effects of cocaine dose [F (1, 19) = 35.31, 38.27, and 22.98, respectively, p's < 0.05]. Analyses of cocaine infusions revealed main effects of treatment dose [F(1,19) = 16.88, $p < 0.05$], and a treatment dose by cocaine dose interaction [F(1,19) = 4.70, $p < 0.05$]. For intake, there were main effects of treatment dose [F(1,19) = 32.79, $p < 0.05$], and a treatment dose by cocaine dose interaction [F(1,19) = 22.34, $p < 0.05$]. Analysis of active lever responses revealed main effects of treatment dose [F(1,18) = 16.43, $p < 0.05$], and a cocaine dose by treatment dose interaction [F(1,18) = 6.15, $p < 0.05$]. Rats received a higher number of cocaine infusions (Fig. 16A) and pressed the active lever more (Fig. 16C) while the low cocaine dose was available, but their consumption of cocaine was higher when the high cocaine dose was available (Fig. 16B; p's < 0.05) as expected. CP 94,253 pretreatment attenuated cocaine infusions, intake, and active lever responses across both doses of cocaine relative to vehicle pretreatment following 21 days of abstinence (Fig. 16; p's < 0.05). There were no effects of CP 94,253 on inactive lever responding (Fig. 16C) nor on latency to first lever response (Fig. 16D). Collectively, these results indicate that after 21-days of abstinence rats are similarly *motivated* to initiate cocaine-seeking but CP 94,253 treatment significantly decreases cocaine-taking and related behaviors.

Experiment 4b: All rats reliably resumed daily cocaine SA during the relapse phase (Fig. 17). Similar to the effects following abstinence, CP 94,253 given prior to test sessions during relapse decreased cocaine SA during this phase (Fig. 18). Analyses of cocaine infusions, total intake (mg/kg, i.v.), and active lever responses revealed main effects of cocaine dose [F(1,9) = 90.36, 86.63, 42.72, p's < 0.05], CP 94,253 dose [F(1,9) = 44.82, 132.90, and 16.11, p's < 0.05] and a cocaine dose by CP 94,253 dose interaction

[F(1,9) = 8.96, 31.08, 7.36, *p's* < 0.05] for each respective measure. Rats received a higher number of cocaine infusions (Fig. 18A) and pressed the active lever more (Fig. 18C) while the low cocaine dose was available, but their consumption of cocaine was higher when the high cocaine dose was available (Fig. 18B; *p's* < 0.05) as expected. Importantly, rats had fewer cocaine infusions, intake, and active lever responses when pretreated with CP 94,253, compared to vehicle, following stable performance on resumption of daily cocaine SA (Fig. 18A-C). There were no effects for inactive lever responses (Fig. 18C) and no effects on latency to first lever response (Fig. 18D). These results suggest that CP 94,253 pretreatment had the same effect during relapse to cocaine SA as it did after prolonged abstinence.

Experiment 4c: CP 94,253 pretreatment decreased drug-seeking during a cue reactivity test (Fig. 19). Analyses revealed a significant effect of CP 94,253 on active lever responses [Fig. 19A; t (5) = 2.96, $p < 0.05$] and total number of cue presentations [Fig. 19C; t (5) = 2.99, $p < 0.05$]. There were no effects on inactive lever responses (Fig. 19B) or on latency to first lever response (Fig. 19D). These results suggest that CP 94,253 attenuates the incentive motivation for cocaine elicited by presentation of previously cocaine-paired stimuli.

Discussion

The results from this study support my hypothesis that selective 5-HT_{1B}R agonists may be useful for treating cocaine use disorders. We successfully replicated previous results from our lab in demonstrating that CP 94,253 (5.6 mg/kg, s.c.) produced a downward shift in the dose-effect function for cocaine infusions, intake, and active lever responses following 21 days of forced abstinence (Fig. 16; Pentkowski, et al., 2014).

Furthermore, we made the exciting discovery that CP 94,253 continues to be efficacious in attenuating drug intake following cocaine relapse. Again, during this phase, CP 94,253 significantly decreased cocaine infusions, intake, and active lever responses after rats resumed daily cocaine SA sessions (Figs.18). CP 94,253 was not only effective at reducing cocaine-taking but also cocaine-seeking. We found that CP 94,253 was also effective at reducing the incentive motivation elicited by presentation of previously drug-paired stimuli. This was evident by a reduction of responses on the active lever and total cue presentations under a cue reactivity challenge following 21 days of forced abstinence (Fig. 19).

There are several possible reasons for the 5-HT_{1B}R agonist-induced decrease in cocaine SA, including an effect on motor capability, anxiety, or an effect on general motivation. It is unlikely that the decrease in cocaine intake is due to motor impairment. In fact, treatment with CP 94,253 and other 5-HT_{1B}R agonists increases cocaine-induced hyperlocomotion (Cheetham & Heal, 1993; Przegalinski et al., 2001; 2004). Furthermore, pharmacological activation of 5-HT_{1B}Rs either fail to alter or increase spontaneous locomotion in rats with a history of cocaine exposure (Koe et al., 1992; Pentkowski et al., 2009). Viral-mediated gene transfer for overexpression of 5-HT_{1B}Rs in the nucleus accumbens also increase cocaine-induced hyperlocomotion without affecting spontaneous locomotor activity (Neumaier et al., 2002; Pentkowski et al., 2012). In our experiments there were no changes in inactive lever responding or latencies to initiate active lever responding during testing, suggesting that motor capability was intact. It is also unlikely that the attenuating effects on the behaviors were a result of carry-over effects of CP

94,253. All treatments were counterbalanced and all rats demonstrated reliable SA on days without CP 94,253 treatment.

The ability of CP 94,253 to modulate anxiety is important to consider in interpreting the present findings. The role of 5-HT_{1B}Rs in modulating anxiety is complex, often yielding mixed results. CP 94,253 treatment increases anxiety-like behavior in both drug-naïve rats and in rats with a history of cocaine SA and abstinence in the elevated plus maze test (Lin and Parsons, 2002; Pentkowski et al, 2009). Furthermore, viral-mediated overexpression of 5-HT_{1B}Rs in the rostrocaudal portion of the dorsal raphe nucleus increases anxiety-like behaviors following a stress-induced procedure (Clark et al., 2002). However, other studies have reported anxiolytic-like effects following pharmacological activation of 5-HT_{1B}Rs or no effect following 5-HT_{1B}R overexpression. For instance, CP 94,253 treatment in rats produces a decrease in anxiety-like behaviors as assessed by the elevated plus maze (Tatarczynska et al., 2004) and overexpression of 5-HT_{1B}Rs in the nucleus accumbens-shell did not alter anxiety-like behavior in rats (Pentkowski et al., 2012). Mice lacking 5-HT_{1B}Rs show either no change or a decrease in anxiety-like behaviors compared to wildtype mice (Brunner et al., 1999; Malleret et al., 1999; Nautiyal et al., 2016). It is unlikely that CP 94,253 attenuated cocaine intake by producing stress-induced anxiety since stress generally increases drug intake, even after prolonged abstinence (Erb, Shaham, & Stewart, 1996; Goeders, 2002; Sarnyai, 2006). Finally, a CP 94,253-induced anxiogenic effect would manifest as a decrease in sucrose reinforcement as well but the effects of CP 94,253 are specific to attenuating cocaine-seeking and cocaine-taking behaviors (Pentkowski et al., 2009, Pentkowski et al., 2014).

The CP 94,253-induced attenuation on cocaine SA may be an effect on general motivation. Earlier reports indicated that CP 94,253 at 3.2 mg/kg decreased food intake (Koe et al., 1992). Similarly, CP 94,253 decreases intake of food pellets and 10% sucrose solution (Lee & Simansky, 1997). These results suggest that CP 94,253 promotes satiety and affects general motivation. However, the effects of CP 94,253 in these studies occurred at lower doses (5 - 40 μmol/kg or 3.2 mg/kg) than we used here and these effects on food intake disappeared with repeated CP 94,253 administration. Our lab found that CP 94,253 (0.3 – 10.0 mg/kg) has no effect on sucrose reinforcement in drug-naïve rats with *ad libitum* food access (Pentkowski et al., 2009). CP 94,253 enhances the rewarding effects of cocaine but is aversive when given alone in a place preference model (Cervo et al., 2002). Furthermore, CP 94,253 does not support SA when substituted for cocaine (Parsons, Koob, & Weiss, 1998) and therefore does not likely have abuse potential on its own.

5-HT_{1B}R agonists were considered contraindicated as treatments for cocaine abuse because agonists, including CP 94,253, enhance the rewarding and reinforcing effects of cocaine (Rocha et al., 1998 Castanon et al., 2000; Cervo et al., 2002; Parson, Koob, & Weiss, 1998; Pentkowski et al., 2014). However, our lab showed that these enhancing effects were dependent on when 5-HT_{1B}R agonists were administered during the drug-taking cycle. If cocaine-experienced rodents undergo a period without the drug, such as during extinction training or forced abstinence, 5-HT_{1B}R agonists attenuate the rewarding and reinforcing effects of cocaine (Pentkowski et al., 2009; 2014; Der-Ghazarian et al., 2017). These results suggest that 5-HT_{1B}R agonists may be useful as adjunctive treatments to prevent relapse to cocaine after a detoxification period. Indeed, CP 94,253 is effective at attenuating cocaine reinforcement during a single SA session following abstinence and

here, we show that the attenuating effects of CP 94,253 persist even after resumption of cocaine SA similar in length to initial training. Furthermore, CP 94,253 effectively attenuated cue-induced motivation to seek drug following resumption and abstinence from cocaine intake. Overall, our results provide support for the clinical investigation of CP 94,253 and other 5-HT_{1B}R agonists as a potential treatment for cocaine use disorders.

CHAPTER 5

CONCLUDING REMARKS

In this dissertation, I aimed to test the hypotheses that 5-HT_{1B}Rs modulate psychostimulant-related behaviors and that 5-HT_{1B}R agonists have therapeutic potential for treating psychostimulant use disorders. The main findings that support these hypotheses include: 1) the selective 5-HT_{1B}R agonist CP 94,253 attenuated the reinforcing and motivational effects of methamphetamine in a 5-HT_{1B}R-mediated manner, and unexpectedly this occurred regardless of prior abstinence, 2) the less selective but clinically-available 5-$HT_{1D/1B}$R agonist, zolmitriptan, attenuated the reinforcing effects of methamphetamine also regardless of prior abstinence, 3) zolmitriptan attenuated cocaine reinforcement in male and female rats and these effects were blocked by either a selective 5-HT_{1B}R or 5-HT_{1D}R antagonist, 4) the effects of CP 94,253 were long-lasting and persisted even after cocaine resumption, and 5) the 5-HT_{1B}R agonist-induced attenuation of psychostimulant reinforcement occurred at doses that did not alter sucrose reinforcement or spontaneous locomotion. Collectively, these exciting results support the hypothesis that 5-HT_{1B}Rs are suitable targets for novel treatment development to attenuate psychostimulant dependence.

Potential Mechanisms

The leading hypothesis involving 5-HT_{1B}R modulation of cocaine-related behaviors suggests that 5-HT_{1B}R agonists potentiate the effects of cocaine by elevating extracellular dopamine release in the NAc and reducing extracellular GABA release in the VTA (Guan & McBride, 1989; Cameron & Williams, 1994; Wise, 1996; Parsons, Koob, & Weiss, 1998; Parsons, Weiss, & Koob, 1999). Indeed, local infusion of 5-HT_{1B}R agonists

in the VTA increases extracellular dopamine release in the NAc and VTA while producing a decrease of GABA extracellular levels in the VTA (Yan & Yan, 2001; O'Dell & Parsons, 2004; Yan, Zheng, & Yan, 2004). These changes in neurotransmitter efflux are blocked by the selective 5-HT_{1B}R antagonist, SB 224,289, but not by WAY 100,635 or BRL 15,572, a 5-HT_{1A}R and 5-HT_{1D}R antagonist, respectively. Furthermore, overexpression of 5-HT_{1B}Rs in the NAc shell and VTA produce an increase in cocaine-induced hyperactivity and enhance the rewarding and reinforcing effects of cocaine (Neumaier et al., 2002: Pentkowski et al., 2012).

The above findings suggest that the VTA acts as the integrating unit where activation of 5-HT_{1B}Rs potentiates the reinforcing effects of cocaine. Indeed, intra-VTA administration of the less selective 5-HT_{1B}R agonist, CP 93,129 has been shown to increase both amphetamine and cocaine induced hyperlocomotion (Papla, Filip, & Przegalinksi, 2002; Przegalinski et al., 2004). Furthermore, in water-deprived rats, intra-VTA administration of CP 93,129 facilitates discrimination of cocaine stimulus effects (Filip et al., 2002). In a preliminary study, I found further support for the role of VTA 5-HT_{1B}Rs in modulating cocaine reinforcement in rats with bilateral cannula implanted into the VTA. After establishing stable cocaine SA, these rats were tested for cocaine (0.075 mg/kg, i.v.) SA after receiving bilateral infusions into the VTA of vehicle on one test day and CP 94,253 (1 µg/0.3 µl) on another test day, with order counterbalanced across subjects. We found that CP 94,253 infusion into the VTA produced an increase in cocaine intake (Fig. 20), similar to the increase in cocaine intake observed with systemic CP 94,253 administration in rats that have not undergone abstinence. Furthermore, the intra-VTA administration of CP 94,253 failed to alter spontaneous locomotion. This latter effect is

consistent with intra-VTA administration of less selective 5-HT_{1B}R agonists on locomotor activity (Papla, Filip, & Przegalinksi, 2000). Collectively, these studies provide support for the modulating role of VTA 5-HT_{1B}Rs on cocaine-related behaviors.

The 5-HT_{1B}R-induced potentiation of dopamine neurons does not explain the abstinence-dependent decrease in cocaine SA. Our lab first discovered that following extinction training, 5-HT_{1B}R stimulation attenuates cocaine-primed and cue-induced reinstatement of extinguished cocaine-seeking behavior in rats (Acosta et al., 2005). Additionally, overexpression or pharmacological stimulation of 5-HT_{1B}Rs increases cocaine intake during maintenance of drug taking but attenuates cocaine intake following 21 days of abstinence (Pentkowski et al., 2009, 2014). Activation of 5-HT_{1B}Rs following abstinence also attenuates incentive motivational effects of cocaine priming in mice (Der-Ghazarian et al., 2017). These results suggest 5-HT_{1B}Rs undergo a functional "switch" depending on a history of abstinence following cocaine exposure (Pentkowski et al., 2012; Neisewander, Cheung, & Pentkowski, 2014; Pentkowski et al, 2014).

The mechanism for the abstinence-induced functional switch in 5-HT_{1B}R agonist effects remains unclear. Chronic stimulation of 5-HT_{1B}Rs downregulates 5-HT_{1B}R binding sites in several brain structures (Pranzatelli & Razi, 1994). Supraphysiological stimulation of 5-HT_{1B}Rs following accumulation of 5-HT during cocaine SA would be expected to downregulate 5-HT_{1B}Rs. Conversely, a decrease in 5-HT extracellular levels upregulates 5-HT_{1B}Rs (Offord, Ordway, & Frazer, 1987) and 5-HT extracellular levels have been shown to decrease following withdrawal from cocaine SA (Parsons, Koob, & Weiss, 1995). Indeed, 5-HT_{1B}Rs are upregulated in numerous brain structures following a 5-day withdrawal period from chronic cocaine exposure (Przegalinski et al., 2003). Similar 5-

$HT_{1B}R$ upregulation and increased sensitivity has been found with cocaine and other psychostimulants following 14 days of abstinence (O'Dell & Parsons, 2006; Miszkiel et al., 2018). We recently found that 5-HT_{1B}Rs modulate VTA dopamine neuronal firing through interactions with dopamine D2 autoreceptors (D_2AR). Specifically, the selective 5-$HT_{1B}R$ agonist CP 94,253 alone mildly inhibits dopamine neuron firing; however, when D_2ARs are stimulated, CP 94,253 counteracts the D_2AR-induced inhibition of dopamine neuronal firing in the VTA. However, these effects may depend on D_2AR sensitivity because 5-$HT_{1B}R$ agonist effects are not observed in mice that have undergone chronic cocaine exposure, which desensitizes D_2ARs. In further support, 5-$HT_{1B}R$ antagonist administration, which has no effect on dopamine neuron firing under normal conditions, restores sensitivity to D_2ARs. CP 94,253 may have counteracting effects under normal D_2AR sensitivity but after chronic cocaine exposure its inhibitory effects may predominate under low D_2AR sensitivity (Gao et al., 2020). These dual effects may be responsible for the abstinence-induced 5-$HT_{1B}R$ effects observed with cocaine-related behaviors.

Limitations and future directions

Our findings are exciting as we demonstrate the therapeutic potential of 5-$HT_{1B}R$ agonists to reduce psychostimulant intake. However, several limitations of this dissertation need to be addressed in future studies to explore the generalizability of our effects across drug class, age, and other behavioral and psychiatric disorders. Polydrug use is common with individuals co-using cocaine and heroin, methamphetamine, or alcohol (Darke & Hall, 1995; Leri et al., 2004; Booth et al., 2006). Therefore, it is important to examine interactions of polydrug use and 5-$HT_{1B}Rs$ in future studies. Here we investigated the effects of 5-$HT_{1B}R$ agonists during maintenance of SA and following abstinence with

cocaine or methamphetamine but not during co-exposure with other drugs of abuse like alcohol. Psychostimulant co-abuse with alcohol is very common among substance-dependent individuals (Furr, Delva, & Anthony, 2000; Althobaiti & Sari, 2016). It seems likely that 5-HT_{1B}Rs will prove effective in treating this polydrug use because 5-HT_{1B}Rs have also been implicated in alcohol-related behaviors in rodents and humans. Mice lacking the 5-HT_{1B}R gene consume twice as much ethanol relative to wildtype mice (Crabbe et al., 1996) and upregulation of 5-HT_{1B}Rs are found in the cortex and hippocampus following chronic but not acute ethanol consumption in rats (Pandey et al., 1996). Furthermore, activation of 5-HT_{1B}Rs attenuate ethanol SA in rats (Maurel, De Vry, Schreiber, 1999; Tomkins & O'Neill, 2000) and attenuates alcohol-induced aggression in rodents and humans (Fish, Faccidomo, & Miczek, 1999; Miczek & De Almeida, 2001; Faccidomo, Bannai, & Miczek, 2008; Gowin et al., 2010). Therefore, future research will need to address the effects of 5-HT_{1B}R manipulations following polydrug use with psychostimulants and alcohol.

Susceptibility to psychostimulant use disorders varies with age. Adolescents are at an increased risk for developing behavioral and drug-related problems (Compas, Hinden, & Gerhardt, 1995; Breslau & Peterson, 1996; Winters et al., 2018). In animal models, adolescent rodents have demonstrated greater sensitivity to the rewarding effects of drugs as well as a faster progression from initial drug taking to dependence (Clark, Kirisci, & Tarter, 1998; Caster, Walker, & Kuhn, 2005; O'Dell, 2009). It is difficult to use rodent SA models to test therapeutic potential of treatments during adolescence because of the limited period to establish psychostimulant dependence and test within rodent adolescence;

however, it remains interesting to investigate if 5-HT_{1B}R activation affects initial psychostimulant consumption during this development stage.

5-HT_{1B}R alterations during early life may increase vulnerability for developing substance use disorders in adulthood. Vazquez and colleagues found a decrease in 5-HT_{1B} mRNA levels in the hippocampus of infant rats (post-natal day ~14) after the rats had undergone chronic intermittent stress with injections of vehicle or the antidepressant, desipramine (2002). A separate group of infant rats that underwent identical stress procedures consumed more ethanol when tested in adulthood (post-natal day 80, Vazquez et al., 2002). This research suggests that downregulation of 5-HT_{1B}Rs following early life stress may contribute to the susceptibility for developing substance use disorders in adulthood.

There is a high prevalence of psychiatric comorbidity, including affective disorders, among psychostimulant abusers (Ross, Glaser, & Germanson, 1988; Kilbery, Breslau, & Andreski, 1992; McHugh & Kneeland, 2019). Depressive disorders are the most diagnosed comorbid conditions among cocaine abusers entering treatment (Kleinman et al., 1990; Kilbey, Breslau, & Andreski, 1992). 5-HT_{1B}Rs modulate depression in humans and depressive-like symptoms in animals. Patients diagnosed with major depressive disorder (MDD) have reduced 5-HT_{1B}R expression in the ventral striatum, ventral pallidum (Murrough et al., 2010), and anterior cingulate cortex (Tiger et al., 2016). Similarly, reduced levels of the 5-HT_{1B}R trafficking protein, p11, are found in postmortem tissue of depressed patients (Svenningsson et al., 2006; Svenningsson & Greengard, 2007). In animal models of depression, 5-HT_{1B}R mRNA and 5-HT_{1B}R binding sites increase throughout parts of the brain following stress and learned helplessness (Edwards et al.,

1991; Neumaier et al., 1997). Furthermore, systemic pharmacological activation of 5-HT_{1B}Rs or overexpression of 5-HT_{1B}Rs in neurons originating in the dorsal raphe nucleus produce antidepressant-like effects in mice and rats (Redrobe, MacSweeney, & Bourin, 1996; O'Neill & Conway, 2001; McDevitt et al., 2011). Prolonged withdrawal from cocaine has been associated with depression in humans and depressive-like effects in rats as well as induced alterations in 5-HT receptor sensitivity (Baumann & Rothman, 1998; Markou, Kosten, & Koob, 1998; Lopez & Becona, 2007). Therefore, future work will need to investigate the role of 5-HT_{1B}R agonists on depressive-like symptoms following chronic cocaine exposure. This holistic approach can help elucidate the mechanisms and symptoms for characterization of a preclinical model that covers multiple facets of the substance use cycle.

My dissertation examined effects of acute 5-HT_{1B}R treatments given at specific times during the cocaine abuse cycle. An important future direction is to examine effects of chronic 5-HT_{1B}R treatment as such a regimen is likely needed to treating cocaine use disorders. Repetitive treatment with 5-HT_{1B}R agonists may produce tolerance that require incremental increases in dose to maintain efficacy at reducing psychostimulant intake. Zolmitriptan's efficacy at providing migraine relief following multiple treatments has been established (Tuchman et al., 2008). Here we demonstrated that zolmitriptan continues to be effective at reducing methamphetamine intake following intermittent treatment for three post-abstinence tests with two-three days of SA in between tests. Additionally, we found that zolmitriptan is effective at reducing cocaine intake following repeated testing regardless of abstinence. Therefore, it is likely that 5-HT_{1B}R agonists like zolmitriptan will remain efficacious following a chronic treatment regimen.

The reason for this discrepancy between the effects of CP 94,253 and zolmitriptan on cocaine reinforcement during the active drug-taking phase remains unclear. Differences in cocaine intake may arise from differences in receptor binding affinity of each compound and how each receptor modulates cocaine reinforcement. CP 94,253 has greater affinity for 5-HT_{1B}Rs than zolmitriptan (K_i = 2 nM vs. 5.01 nM, respectively) and zolmitriptan has greater affinity for 5-HT_{1D}Rs than CP 94,253 (K_i = 0.63 vs. 49 nM). However, the extent that binding affinity of CP 94,253 and zolmitriptan have on cocaine reinforcement remains equivocal for a few reasons. First, 5-HT_{1D}Rs are expressed at lower levels in the rodent brain compared to 5-HT_{1B}Rs (Hoyer et al., 1994; Hannon & Hoyer, 2002). Second, 5-HT_{1B} and 5-HT_{1D}Rs function as auto- and heteroreceptors negatively couple to adenylyl cyclase activity via G-proteins that function to inhibit neurotransmitter release (Hen 1992; Maura et al., 1993; Ghavami, Baruscotti, & Robinson, 1997; Pullar et al., 2004; Sari, 2004). Third, 5-HT_{1B} and 5-HT_{1D}Rs are expressed in many of the same brain regions (Palacios et al., 1990; Hamblin & Metcalf, 1991; Bonaventure, Langlois, & Leysen, 1998; Hannon & Hoyer, 2002). And fourth, the 5-HT_{1B} and 5-HT_{1D}R share more than 60% amino acid sequence homology (Hamblin et al., 1992) and 5-HT_{1B}Rs have been reported to form homodimers as well as heterodimers with 5-HT_{1D}Rs when co-expressed *in vitro* (Xie et al., 1999). It is unclear if these receptors form oligomers *in vivo* and the extent of 5-HT_1R ligand interaction with these molecular complexes has yet to be determined. To our knowledge, we are the first to investigate the effects of 5-HT_{1D}Rs on cocaine reinforcement and therefore more research is needed to determine the contribution of 5-HT_{1D}Rs to the effects observed with cocaine intake in the present studies.

Clinical relevance

Clinical evidence implicates 5-HT_{1B}Rs in substance use disorders. Single nucleotide polymorphisms in the 5-HT_{1B}R gene have been associated with cocaine, alcohol, and heroin abuse (Cao, LaRocque, & Li, 2012; Contini et al., 2012). In alcohol-dependent patients there is an increase in 5-HT_{1B}R binding density in the NAc and globus pallidus (Hu et al., 2009) and in cocaine-dependent patients there is a decrease of 5-HT_{1B}R availability in the hypothalamus, anterior cingulate, and frontal cortex (Matuskey et al., 2015). Discrepancies in 5-HT_{1B}R availability in these studies may arise from the length of withdrawal from alcohol and cocaine (4 weeks vs. 6 days, respectively), as well as their pharmacological mechanisms of action. Importantly, zolmitriptan has been shown to attenuate alcohol-induced aggression in humans (Gowin et al., 2010). Future studies will need to investigate if zolmitriptan has repurposing potential for treating cocaine or methamphetamine intake in clinical populations.

Conclusions

This dissertation supports the hypothesis that 5-HT_{1B}R agonists have therapeutic potential for attenuating psychostimulant abuse. The data from these experiments suggests that CP 94,253 and zolmitriptan are efficacious at reducing cocaine and/or methamphetamine intake at different stages of the addiction cycle. While the exact neural mechanisms involved are unclear; evidence suggests that 5-HT_{1B}Rs within the mesolimbic signaling pathway modulate psychostimulant-related behaviors. Future research will need to investigate which neuronal subtypes and whether 5-HT_{1B}-auto vs. -heteroreceptors are involved in psychostimulant reinforcement. Nevertheless, our promising preclinical

evidence warrants further investigation of the therapeutic efficacy of zolmitriptan to treat psychostimulant-dependent patients.

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APPENDIX A

TABLES

Compound Name	Receptor Subtype	Biological Activity	Affinity (K_i, nM)		Selectivity over 5-HT_{1D}R	References
			1B	**1D**		
CP 94,253	5-HT_{1B}R	agonist	2	49	25-fold	Koe et al., 1992
SB 224,289	5-HT_{1B}R	antagonist	1.8	108	60-fold	Sarhan et al., 1999
					Selectivity over 5-HT_{1B}R	
Zolmitriptan	5-$HT_{1D/1B}$R	agonist	5.01	0.63	8-fold	Martin, 1997
BRL 15,572	5-HT_{1D}R	antagonist	774	12.9	60-fold	Schlicker et al., 1997

Table 1. Summary of 5-HT_{1B}R and 5-HT_{1D}R ligands and their biological activity, and receptor affinity and selectivity.

Zolmitriptan	Cocaine infusion baseline (SEM)	
Dose (mg/kg)	**Males**	**Females**
0	23.87 (±1.28)	23.57 (±1.62)
3.0	23.48 (±0.95)	26.19 (±2.38)
5.6	22.76 (±0.99)	25.10 (±2.15)
10	23.02 (±1.52)	23.71 (±2.03)

Table 2. Summary of cocaine infusion baselines preceeding treatment with zolmitriptan in male and female rats. Rats (n = 14/sex) stabilized for cocaine infusion rates for three consecutive sessions prior to testing with zolmitriptan (0-10 mg/kg, s.c.) using a within-subjects design. No differences were found in baseline infusion rates.

Treatment	Cocaine infusion baseline (SEM)/sample size			
Dose (mg/kg)	**Males**	***n***	**Females**	***n***
SB 3.2	27.56 (±1.67)	13	30.33 (±3.18)	10
SB 5.6	28.57 (±2.40)	14	27.02 (±1.33)	10
SB 10	28.10 (±2.11)	13	29.70 (±3.28)	10
SB 3.2 + Zol 5.6	26.58 (±2.44)	13	29.85 (±1.51)	10
SB 5.6 + Zol 5.6	27.73 (±2.63)	13	28.05 (±1.86)	10
SB 10 + Zol 5.6	27.57 (±1.92)	14	27.82 (±2.12)	10
BRL 0.3	26.90 (±1.73)	10	22.50 (±1.58)	8
BRL 1	28.43 (±2.25)	14	27.42 (±1.54)	11
BRL 3	27.85 (±1.96)	14	27.47 (±2.95)	12
BRL 0.3 + Zol 5.6	26.72 (±1.64)	10	22.10 (±1.68)	8
BRL 1 + Zol 5.6	27.92 (±1.92)	12	27.49 (±2.96)	13
BRL 3 + Zol 5.6	28.07 (±2.33)	12	26.44 (±2.20)	13

Table 3. Summary of cocaine infusion baselines preceeding treatment with 5-HT_{1B}R and 5-HT_{1D}R ligands in male and female rats. Rats stabilized on cocaine infusion rates for three consecutive sessions prior to testing with either SB 224,289 (3.2 – 10 mg/kg, s.c.) or BRL 15,572 (0.3 – 3 mg/kg, i.p.) alone or in combination with zolmitriptan (5.6 mg/kg, s.c.) using a between-subjects design. No differences were found in baseline infusion rates. SB = SB 224,289; BRL = 15,572; Zol = Zolmitriptan.

APPENDIX B

FIGURES

Timeline:

FR1-FR5 Acquisition/SA	Test 1	SA	Test 2	Abstinence	Test 1	Abstinence	Test 2
20-48 days	1 day	3-5 days	1 day	21 days	1 day	3 days	1 day

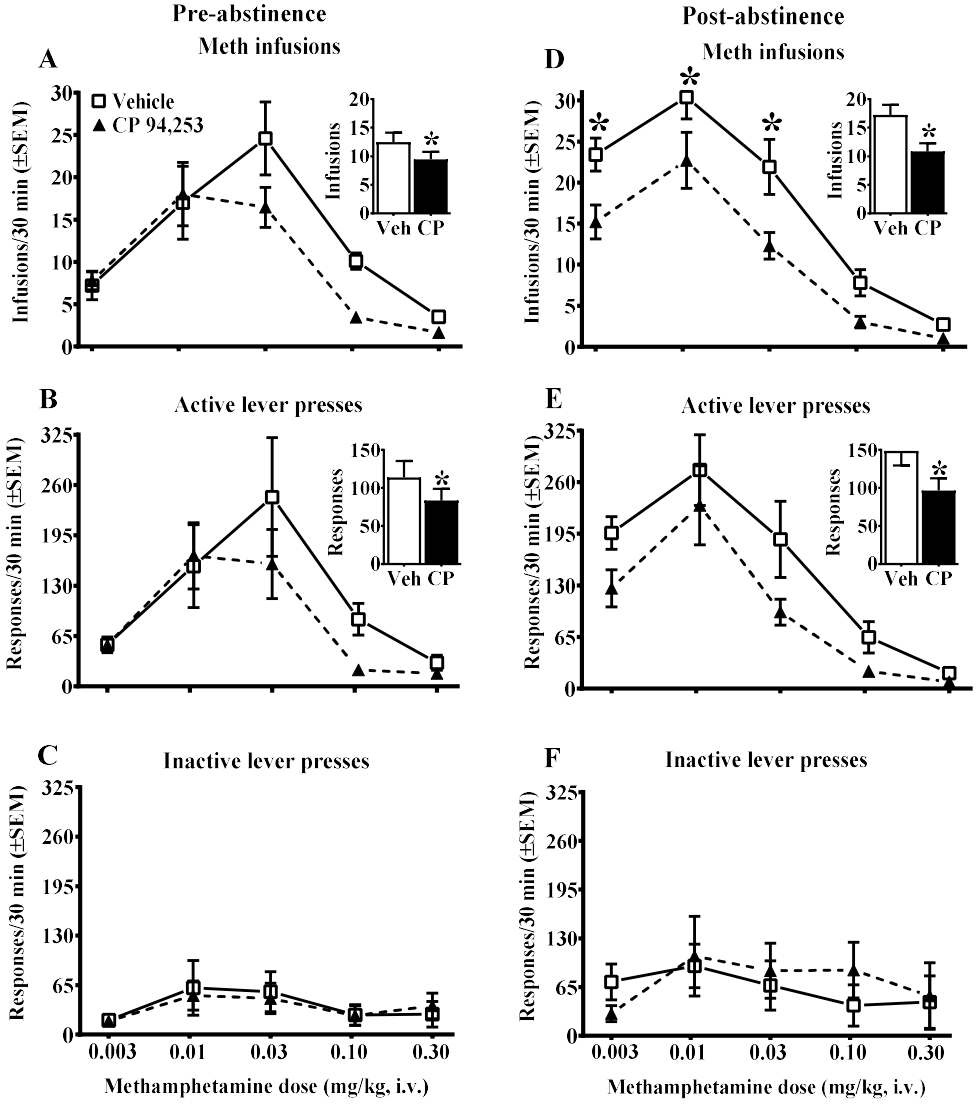

Figure 1. Effects of the 5-HT_{1B} receptor agonist, CP 94,253 on infusions (**A**, **D**), active lever (**B**, **E**) and inactive lever (**C**, **F**) responses on a FR5 schedule of methamphetamine (0.1 mg/kg, i.v.) reinforcement during pre- and post-abstinence tests (left and right panels, respectively). Data are expressed as the mean (± SEM) during the 30 min test period for each of the methamphetamine doses tested (0.003 – 0.30 mg/kg, i.v.). Rats (n = 10) were tested twice, receiving pretreatment with vehicle (1 ml/kg, s.c.; open squares) prior to one test and CP 94,253 (5.6 mg/kg, s.c.; filled triangles) prior to the other test, with order of pretreatment counterbalanced. Insets in **A-D** show a main effect of CP 94,253 averaged across methamphetamine doses. Asterisks (*) represent a difference from vehicle condition ($p < 0.05$).

Timeline:

FR1-VR5 Acquisition	PR SA	Test	Abstinence	Test
11-13 days	10-18 days	1 day	21 days	1 day

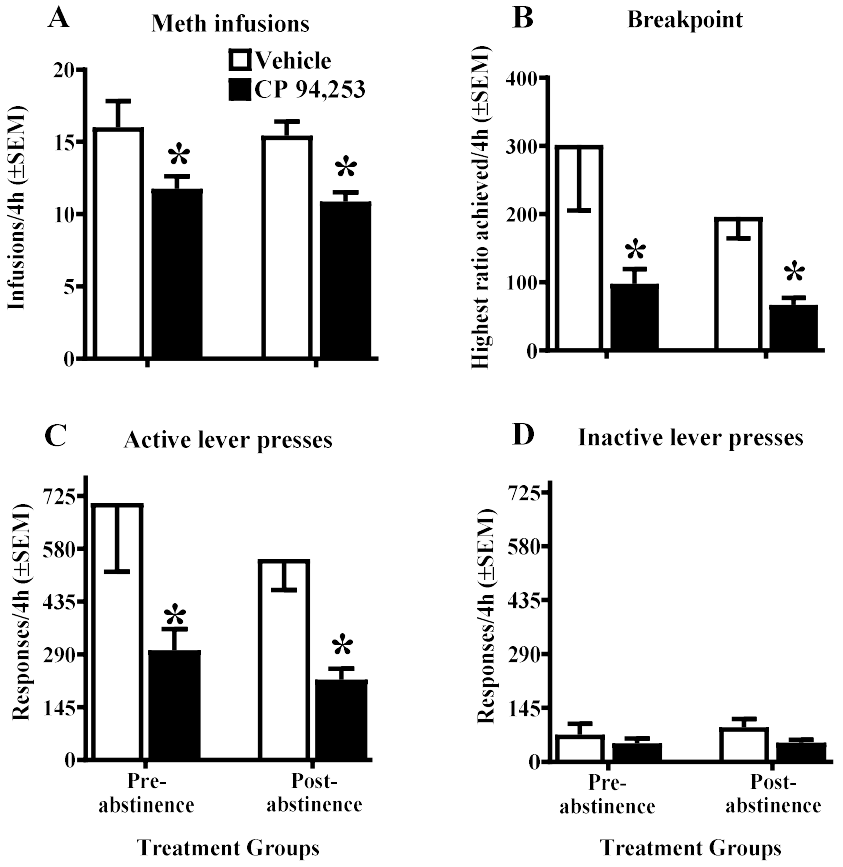

Figure 2. Effects of the 5-HT_{1B} receptor agonist, CP 94,253, on infusions **(A)**, breakpoints **(B)**, active lever **(C)**, and inactive lever **(D)** responses under a progressive ratio schedule of methamphetamine (0.05 mg/kg, i.v.) reinforcement during pre- and post-abstinence tests. Data are expressed as the mean (± SEM) during 4 h sessions. Rats were pretreated 15 min prior to the start of the test sessions with either vehicle (1 ml/kg, s.c.; white bars; n = 7) or CP 94,253 (10 mg/kg, s.c.; black bars; $n = 8$). Asterisks (*) represent a difference from vehicle at each time point ($p < 0.05$).

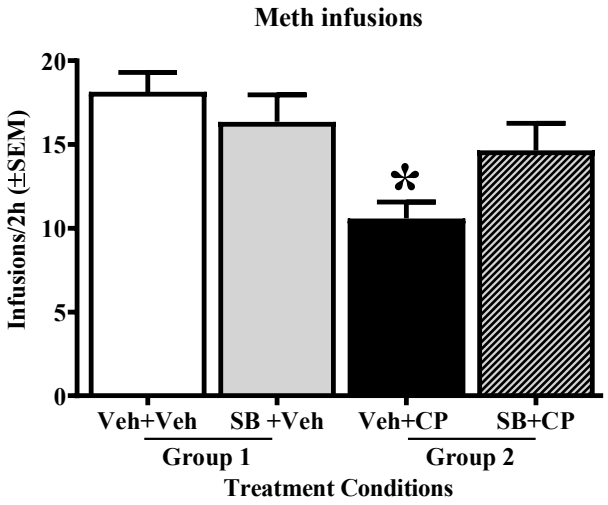

Figure 3. Reversing the attenuating effects of CP 94,253 on methamphetamine (0.1 mg/kg, i.v.) self-administration with SB 224,289 during tests that occurred pre-abstinence. On the first test day, rats (group 1: n = 14, group 2: n = 17) received a pretreatment of either vehicle (Veh, 1 ml/kg, white bar) or SB 224,289 (SB; 10 mg/kg, i.p., gray bar) 30 min before the 2 h session. They then received a treatment of either vehicle or CP 94,253 (CP; 5.6 mg/kg, s.c.; black bar) 15 min prior to the test session that commenced under a VR5 schedule of reinforcement. Conditions were identical on the second test day for all rats except that the pretreatment given 30 min before session start was reversed such that rats that had received vehicle previously were given SB 224,289 and rats that had received SB 224,289 previously were given vehicle. Data are expressed as the mean (+ SEM). Asterisk (*) represents a difference from all other groups ($p < 0.05$).

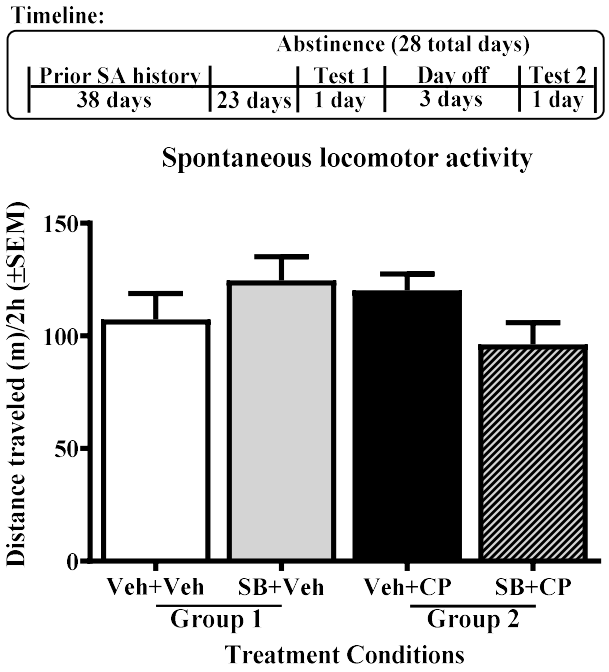

Figure 4. Effects of 5-HT_{1B} receptor ligands on spontaneous locomotion. Rats were placed on abstinence from methamphetamine for a total of 29 days following acquisition and stabilization on a progressive ratio schedule of reinforcement. On the first test day, rats (group 1: n = 7, group 2: n = 7) received a pretreatment of either vehicle (Veh, 1 ml/kg, white bar) or SB 224,289 (SB; 10 mg/kg, i.p., gray bar) 30 min before commencement of the session. They then received a treatment of either vehicle or CP 94,253 (CP; 10 mg/kg, s.c., black bar) 15 min prior to the same session. Conditions were identical on the second test day for all rats except that the pretreatment given 30 min before session start was reversed such that rats who had received vehicle previously were given SB 224,289 and vice versa. All rats underwent 3 days off between tests 1 and 2 while remaining in abstinence. Data are expressed as the mean (+ SEM) total distance traveled in meters across the 2 h session. Pretreatment with 5-HT_{1B} receptor drugs produced no significant differences in spontaneous locomotion.

Timeline:

Prior SA history	Test 1	Abstinence	Test 2	SA	Test 3	SA	Test 4
23-40 days	1 day	29-36 days	1 day	2 days	1 day	3 days	1 day

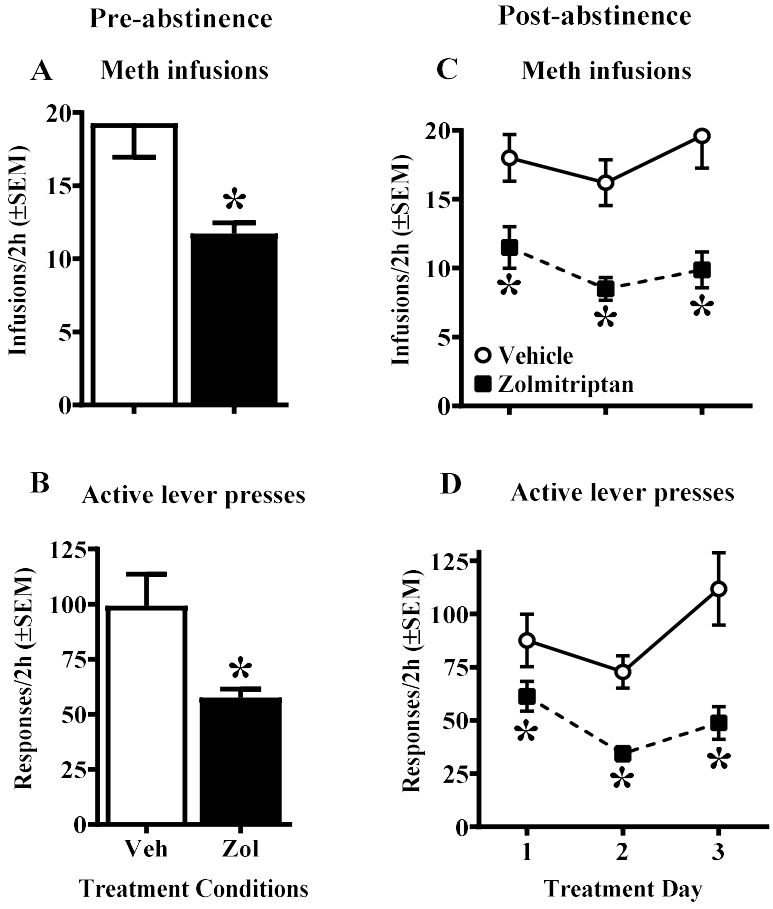

Figure 5. Effects of zolmitriptan, a 5-$HT_{1D/1B}$ receptor agonist, on infusions (**A**, **C**), and active lever (**B**, **C**) responses on a VR5 schedule of methamphetamine (0.1 mg/kg, i.v.) reinforcement during pre- and post-abstinence tests (left and right panels, respectively). Data are expressed as the mean (± SEM). Rats were pretreated 15 min prior to the start of the 2 h sessions with either vehicle (1 ml/kg, white bar/open circles; $n = 6$) or zolmitriptan (10 mg/kg, s.c.; black bar/filled squares; $n = 9$). Asterisks (*) represent a difference from vehicle condition ($p < 0.05$).

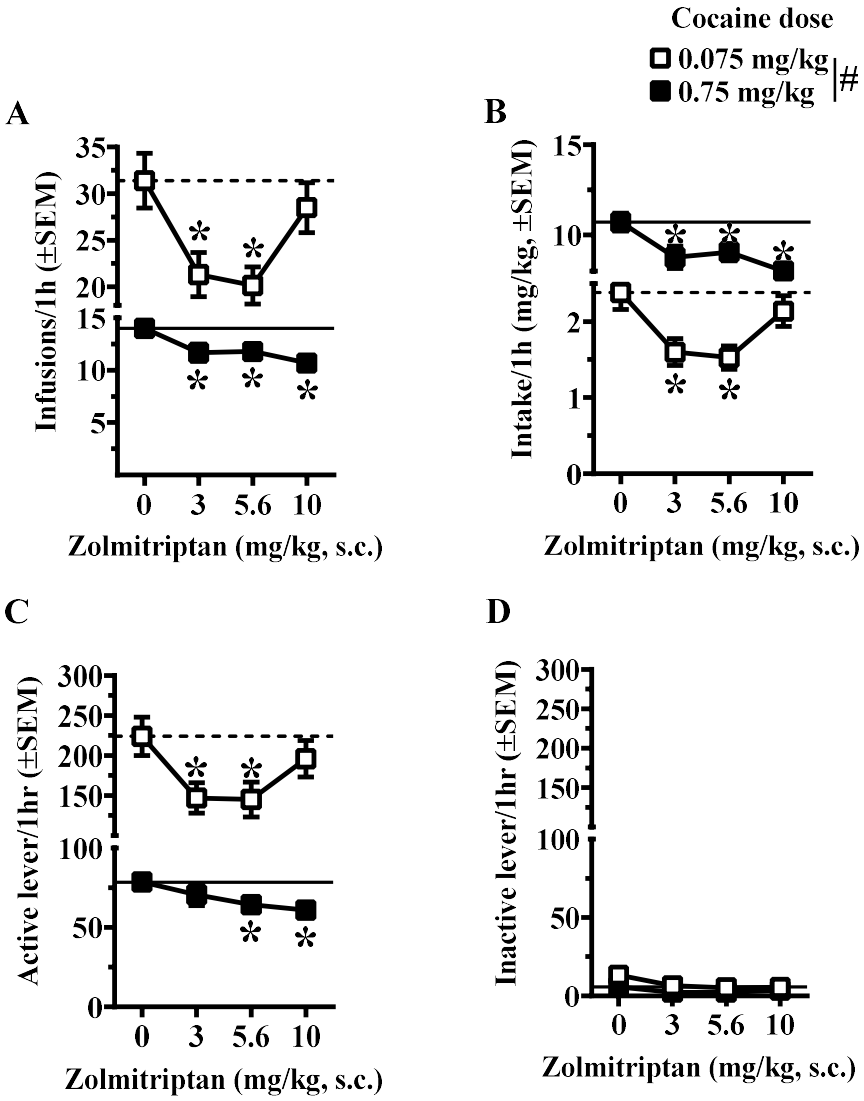
A
B
C
D
Cocaine dose
0.075 mg/kg
0.75 mg/kg
#
Infusions/1h (±SEM)
Intake/1h (mg/kg, ±SEM)
Active lever/1hr (±SEM)
Inactive lever/1hr (±SEM)
Zolmitriptan (mg/kg, s.c.)
0
3
5.6
10

Figure 6. Effects of the 5-$HT_{1D/1B}$ receptor agonist, zolmitriptan, on cocaine infusions **(A)**, total intake **(B)**, and active **(C)** and inactive **(D)** lever responding are expressed as the mean (± SEM) during test sessions in which 0.75 mg/kg, i.v. cocaine was available for 1 h, followed by 0.075 mg/kg, i.v. cocaine available for 1 h. Because there were no effects or interactions with sex the data are collapsed across sex. Horizontal lines correspond to the mean of vehicle pretreatment values under the low (dashed line) and high (solid line) cocaine doses. Rats were pretreated 15 min prior to the start of the test sessions with either vehicle or zolmitriptan (3.0, 5.6, or 10 mg/kg, s.c.) using a within-subjects design (n = 28/dose). Asterisks (*) represent a difference from vehicle and pound sign (#) represents a difference between low and high cocaine dose ($p < 0.05$).

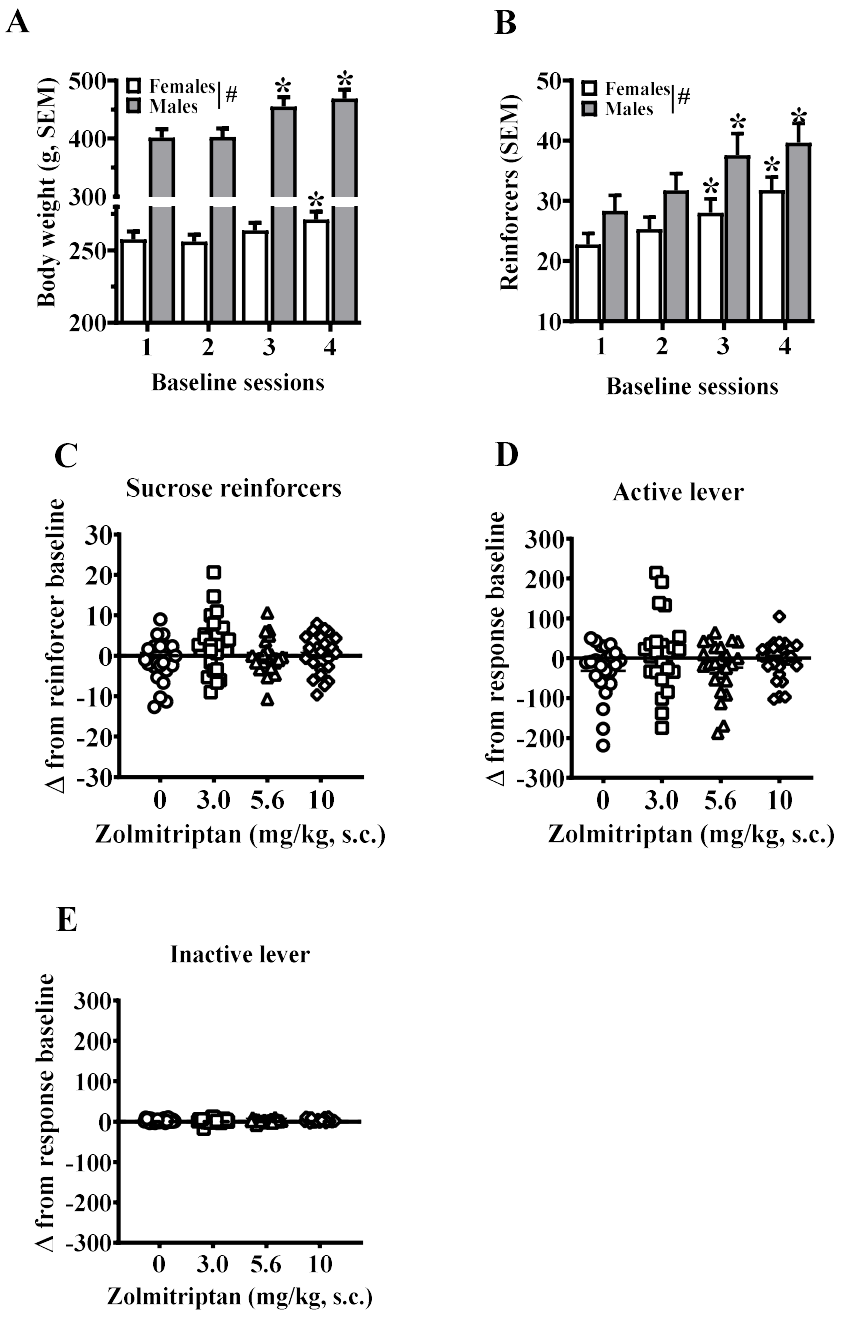
A
Females
Males
#
Body weight (g, SEM)
500
400
300
250
200
1
2
3
4
Baseline sessions
B
Females
Males
#
Reinforcers (SEM)
50
40
30
20
10
1
2
3
4
Baseline sessions
C
Sucrose reinforcers
Δ from reinforcer baseline
30
20
10
0
-10
-20
-30
0
3.0
5.6
10
Zolmitriptan (mg/kg, s.c.)
D
Active lever
Δ from response baseline
300
200
100
0
-100
-200
-300
0
3.0
5.6
10
Zolmitriptan (mg/kg, s.c.)
E
Inactive lever
Δ from response baseline
300
200
100
0
-100
-200
-300
0
3.0
5.6
10
Zolmitriptan (mg/kg, s.c.)

Figure 7. Effects of the 5-$HT_{1D/1B}$ receptor agonist, zolmitriptan, on sucrose reinforcement in male and female rats. Data are expressed as the mean (± SEM) during 30-min sessions. Across the baseline sessions we observed differences in body weight **(A)** and sucrose reinforcement rates **(B)** in male and female rats. Therefore, data are expressed as a difference between the test day minus the appropriate baseline across zolmitriptan doses. Analyses indicated no main effect of sex nor interactions, and data are collapsed across males and females for the effects of zolmitriptan. Rats (n = 24) were pretreated 15 min prior to the start of the test sessions with either vehicle or zolmitriptan (3.0, 5.6, and 10 mg/kg, s.c.; in descending order) using a within-subjects design to assess the effects of zolmitriptan on reinforcers obtained **(C)**, and active **(D)** and inactive **(E)** lever responding. Asterisks (*) represent a difference from 1st and 2nd baseline and pound sign (#) represent a difference between male and female rats ($p < 0.05$).

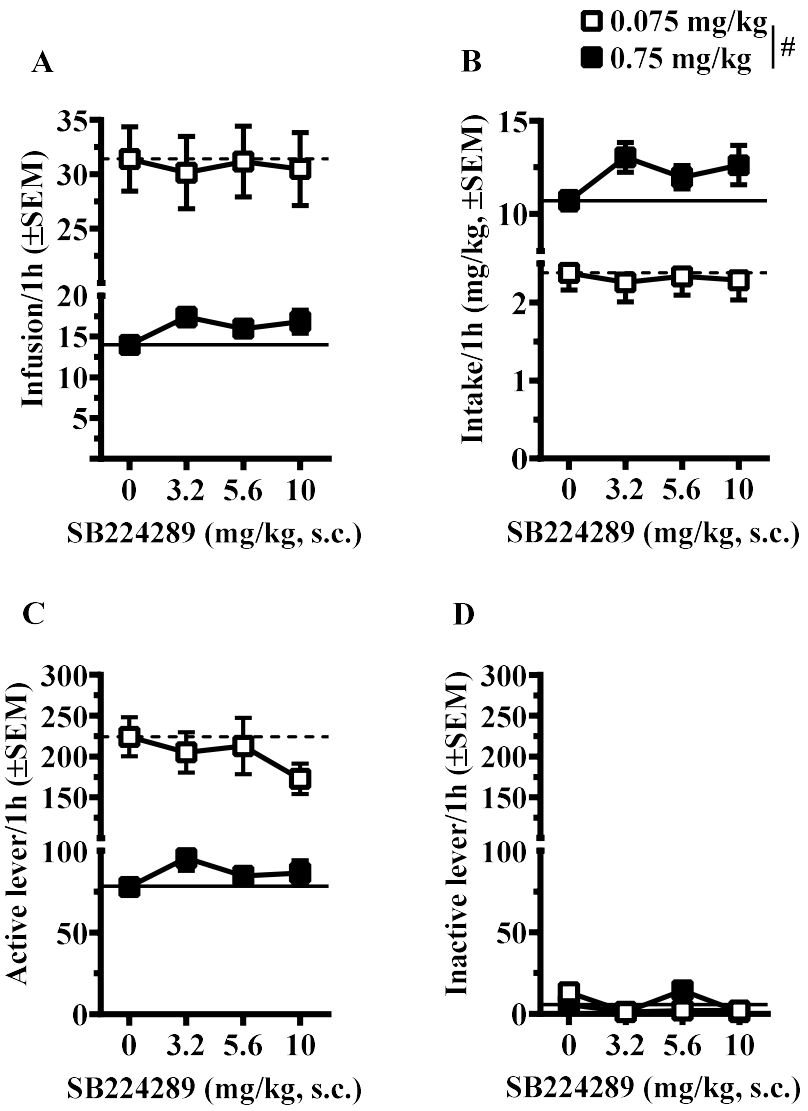
A
B
0.075 mg/kg
0.75 mg/kg
#
Infusion/1h (±SEM)
Intake/1h (mg/kg, ±SEM)
SB224289 (mg/kg, s.c.)
SB224289 (mg/kg, s.c.)
C
D
Active lever/1h (±SEM)
Inactive lever/1h (±SEM)
SB224289 (mg/kg, s.c.)
SB224289 (mg/kg, s.c.)

Figure 8. Effects of the 5-HT_{1B} receptor antagonist, SB 224,289, on cocaine infusions **(A)**, cocaine intake **(B)**, and active **(C)** and inactive **(D)** lever responding are expressed as the mean (± SEM) during test sessions in which 0.75 mg/kg, i.v. cocaine was available for 1 h, followed by 0.075 mg/kg, i.v. cocaine available for 1 h. Because there were no effects or interactions with sex the data are collapsed across sex. Horizontal lines correspond to the mean of vehicle pretreatment values under the low (dashed line) and high (solid line) cocaine doses. Rats were pretreated 30 min prior to the start of the test sessions with either vehicle or SB 224,289 (3.2, 5.6, or 10 mg/kg, s.c.) using a between-subjects design (n = 23-28/dose). Pound sign (#) represents a difference between low and high cocaine dose ($p < 0.05$) for all measures except inactive lever presses.

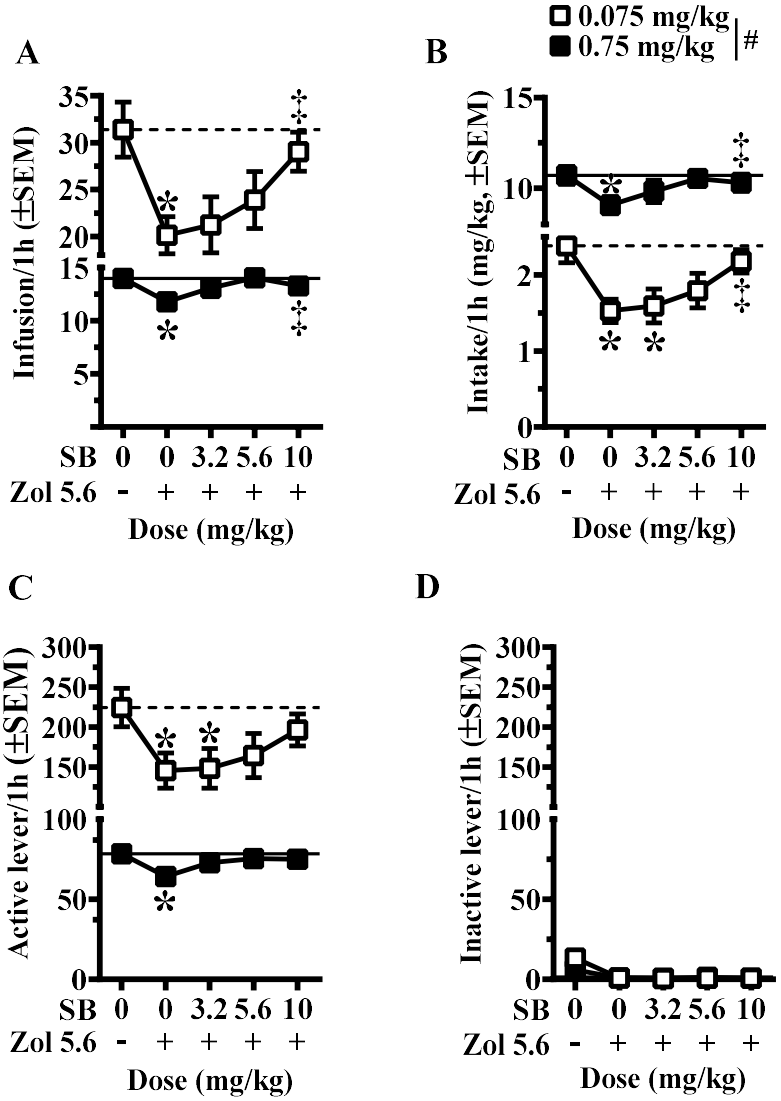
A
B
0.075 mg/kg
0.75 mg/kg
#
Infusion/1h (±SEM)
Intake/1h (mg/kg, ±SEM)
SB 0 0 3.2 5.6 10
Zol 5.6 - + + + +
Dose (mg/kg)
C
D
Active lever/1h (±SEM)
Inactive lever/1h (±SEM)

Figure 9. Effects of the 5-HT_{1B} receptor antagonist, SB 224,289, on the zolmitriptan-induced attenuation of cocaine infusions **(A)**, cocaine intake **(B)**, and active **(C)** and inactive **(D)** lever responding are expressed as the mean (± SEM) during test sessions in which 0.75 mg/kg, i.v. cocaine was available for 1 h, followed by 0.075 mg/kg, i.v. cocaine available for 1 h. Because there were no effects or interactions with sex the data are collapsed across sex. Horizontal lines correspond to the mean of vehicle pretreatment values under the low (dashed line) and high (solid line) cocaine doses. Rats were pretreated 30 min prior to the start of the test sessions with either vehicle or SB 224,289 (3.2, 5.6, or 10 mg/kg, s.c.) followed by pretreatment 15 min later with vehicle or zolmitriptan (5.6, mg/kg, s.c.) using a between-subjects design (n = 20-28/ dose). Asterisks (*) represent a difference from vehicle, pound sign (#) represents a difference between low and high cocaine dose, and double dagger (‡) represents difference from zolmitriptan alone ($p < 0.05$) for all measures except inactive lever presses.

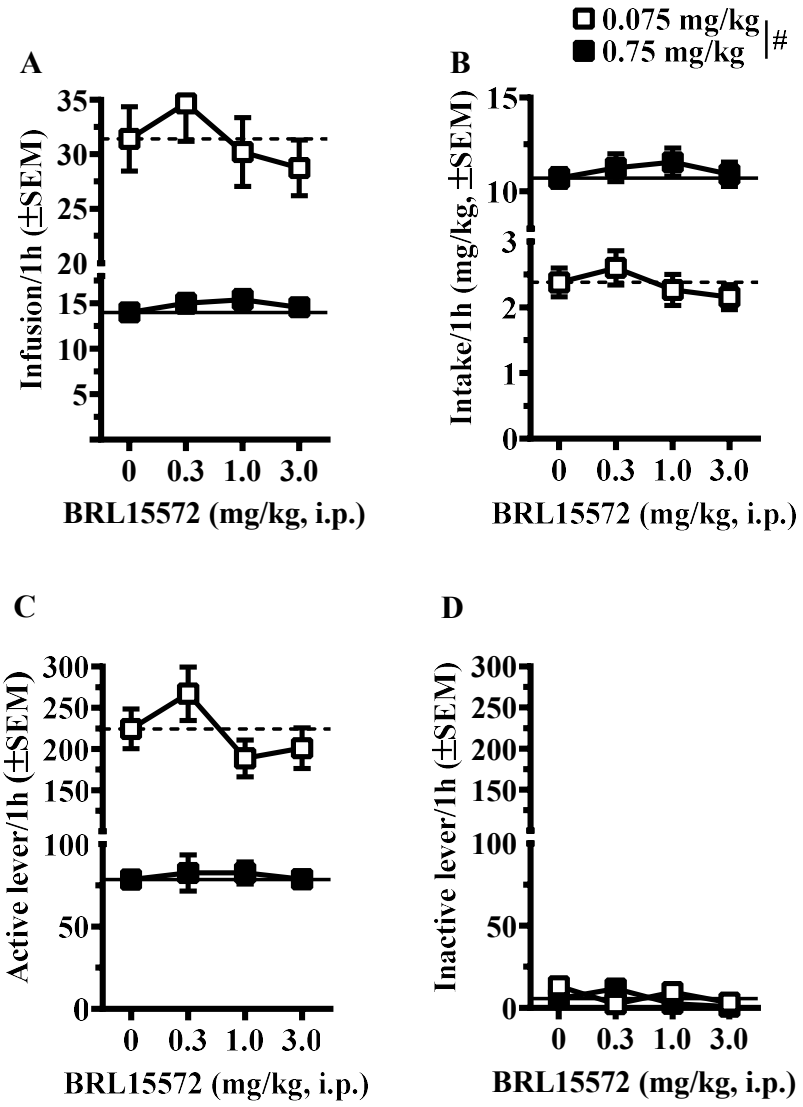
A
Infusion/1h (±SEM)
35
30
25
20
15
10
5
0 0.3 1.0 3.0
BRL15572 (mg/kg, i.p.)
B
0.075 mg/kg
0.75 mg/kg
#
Intake/1h (mg/kg, ±SEM)
15
10
3
2
1
0
0 0.3 1.0 3.0
BRL15572 (mg/kg, i.p.)
C
Active lever/1h (±SEM)
300
250
200
150
100
50
0
0 0.3 1.0 3.0
BRL15572 (mg/kg, i.p.)
D
Inactive lever/1h (±SEM)
300
250
200
150
100
50
0
0 0.3 1.0 3.0
BRL15572 (mg/kg, i.p.)

Figure 10. Effects of the 5-HT_{1D} receptor antagonist, BRL 15,572, on cocaine infusions **(A)**, cocaine intake **(B)**, and active **(C)** and inactive **(D)** lever responding are expressed as the mean (± SEM) during test sessions in which 0.75 mg/kg, i.v. cocaine was available for 1 h, followed by 0.075 mg/kg, i.v. cocaine available for 1 h. Because there were no effects or interactions with sex the data are collapsed across sex. Horizontal lines correspond to the mean of vehicle pretreatment values under the low (dashed line) and high (solid line) cocaine doses. Rats were pretreated 30 min prior to the start of the test sessions with either vehicle or BRL 15,572 (0.3, 1.0, or 3.0 mg/kg, i.p.) using a between-subjects design (n = 18-28). Pound sign (#) represents a difference between low and high cocaine dose ($p < 0.05$) for all measures except inactive lever presses.

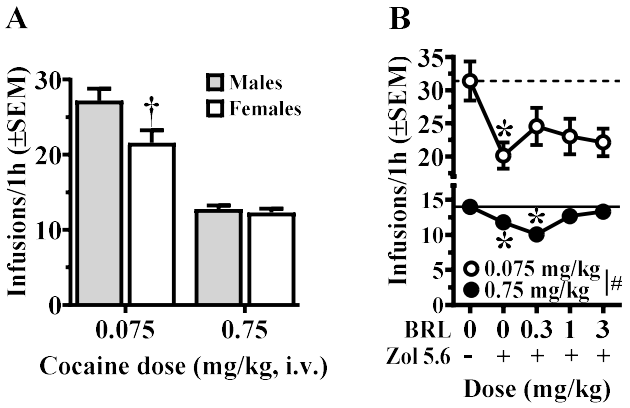

Figure 11. Effects of sex and the 5-HT_{1D} receptor antagonist, BRL 15,572, in reversing zolmitriptan effects on cocaine infusions. Analyses indicated sex by cocaine dose **(A)** and BRL 15,572 dose group by cocaine dose interactions **(B)**. Data are expressed as the mean (± SEM) during test sessions in which 0.75 mg/kg, i.v. cocaine was available for 1 h, followed by 0.075 mg/kg, i.v. cocaine available for 1 h. Horizontal lines correspond to the mean of vehicle pretreatment values under the low (dashed line) and high (solid line) cocaine doses. Rats were pretreated 30 min prior to the start of the test sessions with either vehicle or BRL 15,572 (0.3, 1.0, or 3.0 mg/kg, i.p.) using a between-subjects design (n = 18-28). Asterisks (*) represent a difference from vehicle, pound sign (#) represents a difference between low and high cocaine dose, and dagger (†) represents a difference from male rats ($p < 0.05$).

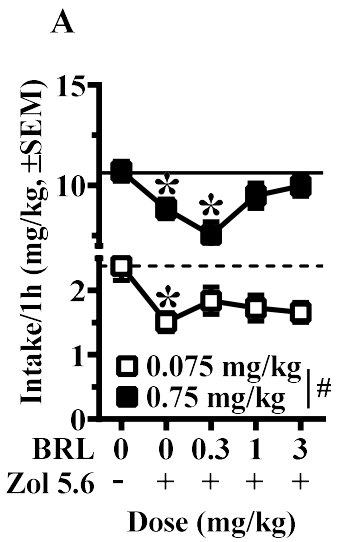

Figure 12. Effects of the 5-HT_{1D} receptor antagonist, BRL 15,572, in reversing zolmitriptan effects on cocaine intake. Data are expressed as the mean (± SEM) during test sessions in which 0.75 mg/kg, i.v. cocaine was available for 1 h, followed by 0.075 mg/kg, i.v. cocaine available for 1 h. Horizontal lines correspond to the mean of vehicle pretreatment values under the low (dashed line) and high (solid line) cocaine doses. Rats were pretreated 30 min prior to the start of the test sessions with either vehicle or BRL 15,572 (0.3, 1.0, or 3.0 mg/kg, i.p.) using a between-subjects design (n = 18-28). 15 min later, rats received vehicle of 5.6 mg/kg, s.c. zolmitriptan (Zol5.6). Asterisks (*) represent a difference from vehicle and a pound sign (#) represents a difference between low and high cocaine dose ($p < 0.05$).

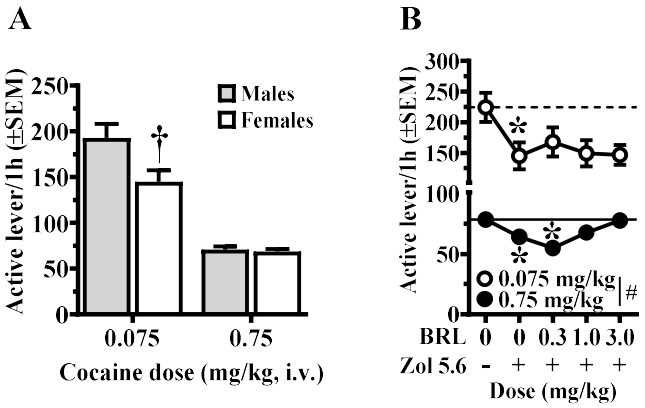

Figure 13. Effects of the 5-HT_{1D} receptor antagonist, BRL 15,572, in reversing zolmitriptan effects on active lever responses. Analyses indicated sex by cocaine dose **(A)** and BRL 15,572 dose group by cocaine dose interactions **(B)**. Data are expressed as the mean (± SEM) during test session in which 0.75 mg/kg, i.v. cocaine was available for 1 h, followed by 0.075 mg/kg, i.v. cocaine available for 1 h. Horizontal lines correspond to the mean of vehicle pretreatment values under the low (dashed line) and high (solid line) cocaine doses. Rats were pretreated 30 min prior to the start of the test sessions with either vehicle or BRL 15,572 (0.3, 1.0, or 3.0 mg/kg, i.p.) using a between-subjects design (n = 18-28). 15 min later, rats received vehicle of 5.6 mg/kg, s.c. zolmitriptan (Zol5.6). Asterisks (*) represent a difference from vehicle, pound sign (#) represents a difference between low and high cocaine dose, and dagger (†) represents a difference from male rats ($p < 0.05$).

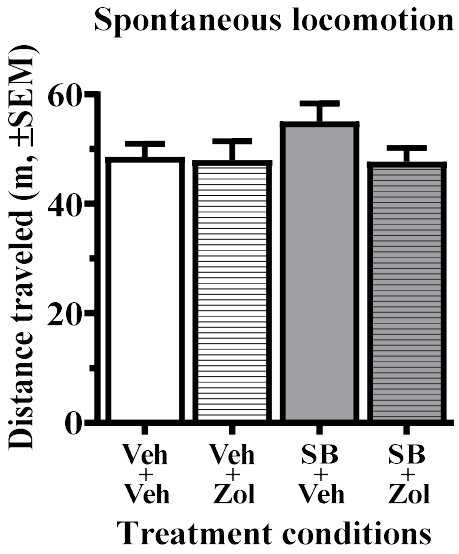

Figure 14. Effects of 5-HT_{1B} receptor drugs on spontaneous locomotion. Male and female rats were placed on abstinence from cocaine self-administration for a minimum of 10 days prior to conducting the test for effects of the 5-HT_{1B} receptor drugs on locomotion. Data are expressed as mean (± SEM) distance traveled in meters (m) across a 1-h session, collapsed across sex because no sex differences were observed. Drug combinations were tested using a within-subjects design. Rats (n = 13) were pretreated 30 min prior to the start of the session with either vehicle or SB 224,289 (10 mg/kg, s.c.; top line of treatment condition) followed 15 min later with pretreatment of either vehicle or zolmtriptan (5.6 mg/kg, s.c.; bottom line of treatment condition). Pretreatment with 5-HT_{1B} receptor drugs failed to alter spontaneous locomotion.

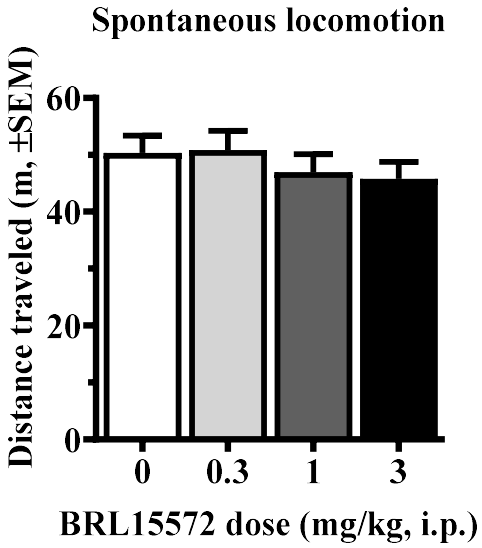

Figure 15. Effects of the 5-HT_{1D} receptor antagonist, BRL 15,572, on spontaneous locomotion. Male and female rats underwent a minimum of 5 rest days after the last test with the 5-HT_{1B} receptor drugs on locomotion. Data are expressed as mean (± SEM) distance traveled in meters (m) across a 1-h session, collapsed across sex because no sex differences were observed. Drug combinations were tested using a within-subjects design. Rats (n = 13) were pretreated 30 min prior to the start of the session with either vehicle or BRL 15,572 (0.3 – 3.0 mg/kg, i.p.). Pretreatment with the 5-HT_{1D} receptor antagonist failed to alter spontaneous locomotion.

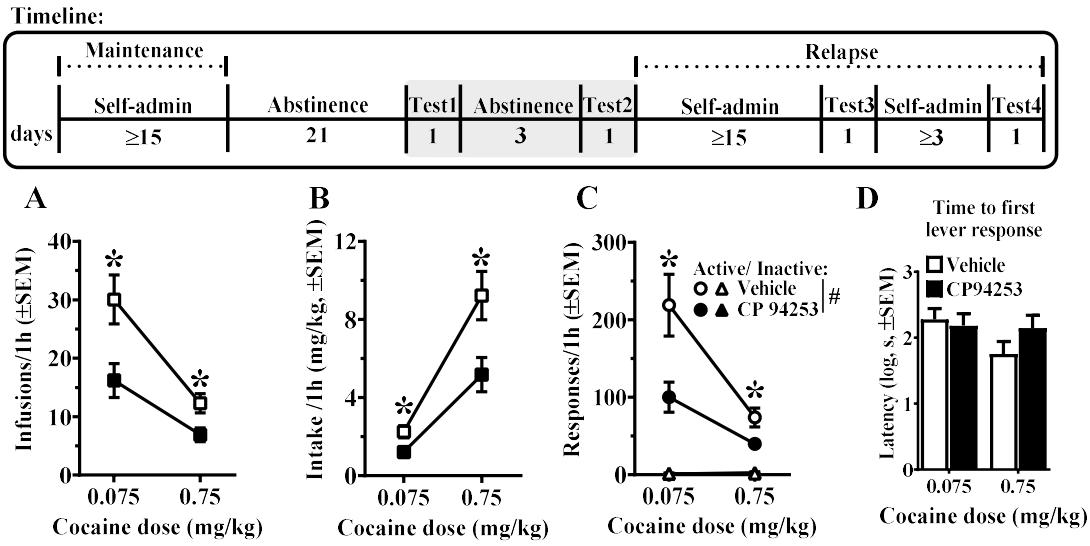

Figure 16. Effects of the selective 5-HT$_{1B}$ receptor agonist, CP 94,253 on cocaine infusions **(A)**, total intake **(B)**, active and inactive lever responding **(C)**, and latency to first **(D)** response on the active lever following a 21-day period of abstinence (shaded area on timeline). Data are expressed as the mean (± SEM) during test session in which 0.75 mg/kg, i.v. cocaine was available for 1 h, followed by 0.075 mg/kg, i.v. cocaine available for 1 h. Rats (n = 20) were pretreated 15 min prior to the start of the test sessions with either vehicle (open symbols or white bars) or CP 94,235 (5.6 mg/kg, s.c.; closed symbols or black bars) using a within-subjects design. Asterisks (*) represent a difference from vehicle and pound sign (#) represents difference between low and high cocaine ($p < 0.05$).

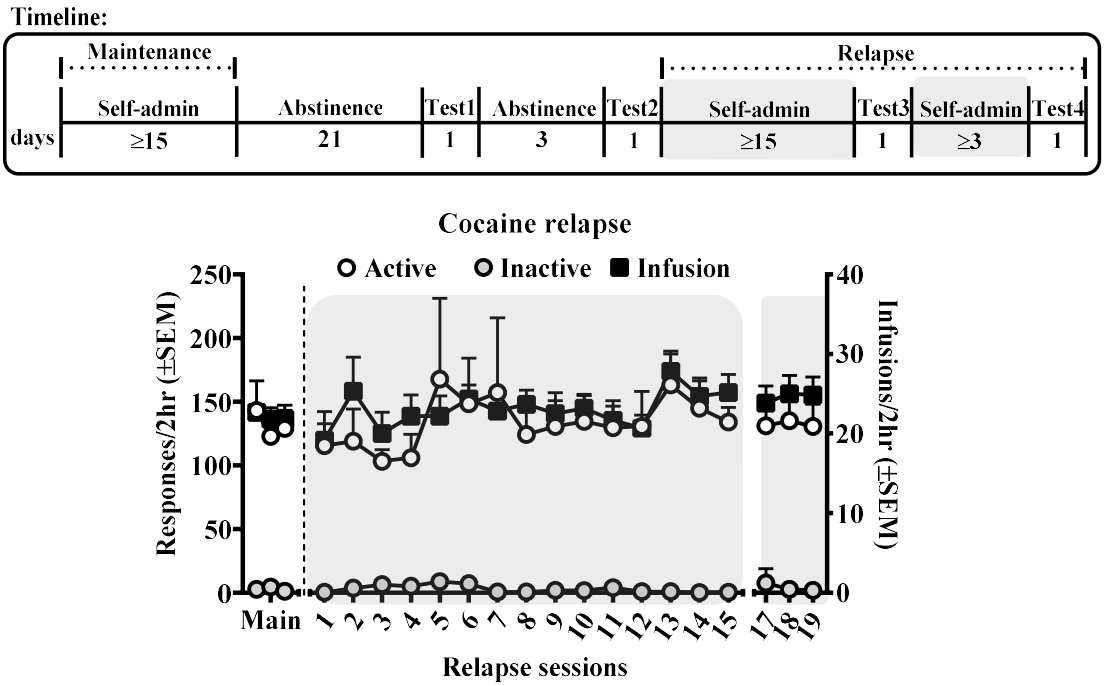

Figure 17. Cocaine SA in male rats during the last 3 sessions of maintenance (Main) and during the relapse sessions which followed a 21-day abstinence period. Data are expressed as the mean (± SEM) during 2-h sessions. Rats were trained to lever press for cocaine (0.75 mg/kg, i.v.) on a FR5 reinforcement schedule. After a minimum of 15 consecutive sessions, rats were placed in abstinence for 21 days before undergoing a period of relapse for 15 sessions, approximating the original acquisition/maintenance phase of cocaine SA. Rats had a minimum of three days between treatment sessions to re-stabilize cocaine infusions. Active (white circles) and inactive (grey circles) lever presses, and infusions (black squares) are shown for the last three sessions of cocaine SA maintenance, and following abstinence, which is represented by the vertical dashed line, and during resumption of daily cocaine SA sessions (grey shaded areas).

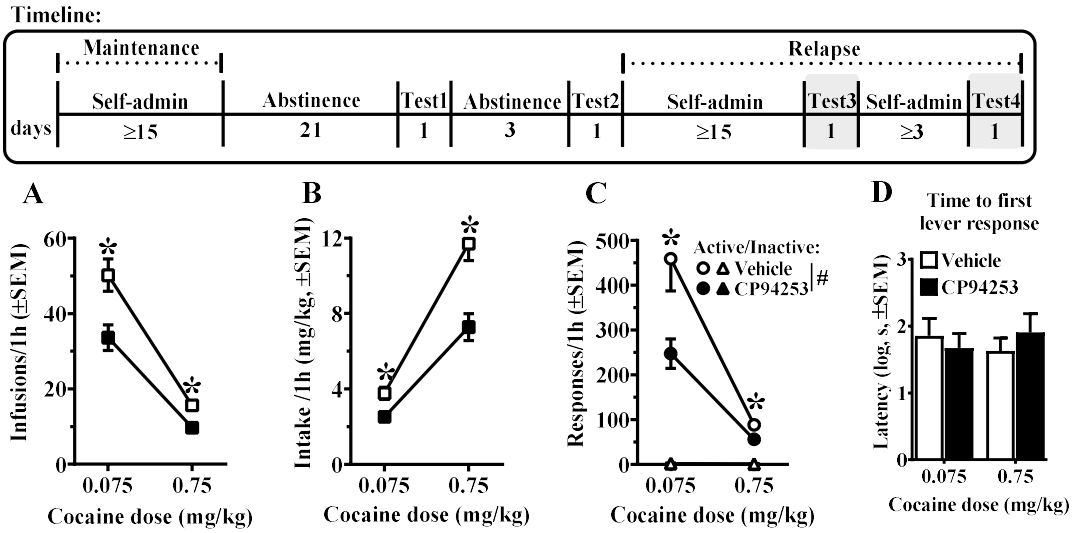

Figure 18. Effects of the selective 5-HT_{1B} receptor agonist, CP 94,253 on cocaine infusions **(A)**, total intake **(B)**, active and inactive lever responding **(C)**, and latency to first **(D)** response on the active lever during a period of relapse to cocaine (shaded area on timeline). Data are expressed as the mean (± SEM) during test session in which 0.75 mg/kg, i.v. cocaine was available for 1 h, followed by 0.075 mg/kg, i.v. cocaine available for 1 h. Rats (n = 10) were pretreated 15 min prior to the start of the test sessions with either vehicle (open symbols or white bars) or CP 94,235 (5.6 mg/kg, s.c.; closed symbols or black bars) using a within-subjects design. Asterisks (*) represent a difference from vehicle and pound sign (#) represents difference between low and high cocaine ($p < 0.05$).

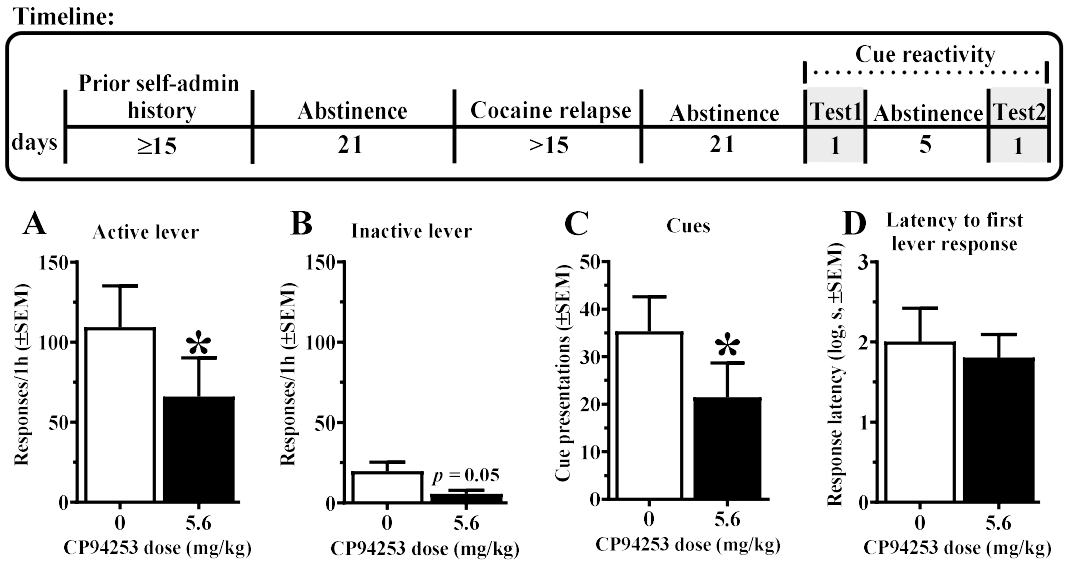

Figure 19. Effects of the selective 5-HT_{1B} receptor agonist, CP 94,253 were assessed on active **(A)** and inactive **(B)** lever responding, cue presentations **(C)**, and latency to first response on the active lever **(D)** during cue reactivity (shaded area on timeline). Data are expressed as the mean (± SEM) during 1 h sessions where cocaine-seeking behavior was measured on the active lever on a FR1 schedule. Rats (n = 6) were pretreated 15 min prior to the start of each test sessions with either vehicle (white bars) or CP 94,235 (5.6 mg/kg, s.c.; black bars) using a within-subjects design. Asterisks (*) represent a difference from vehicle ($p < 0.05$).

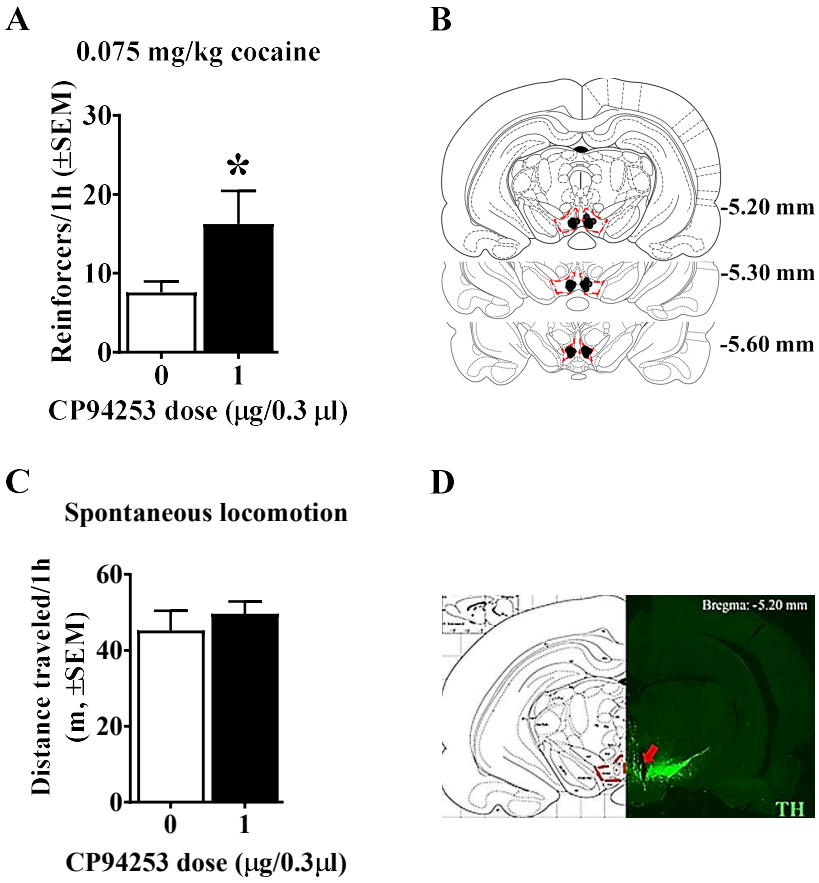
A
0.075 mg/kg cocaine
Reinforcers/1h (±SEM)
30
20
10
0
*
0
1
CP94253 dose (μg/0.3 μl)
B
-5.20 mm
-5.30 mm
-5.60 mm
C
Spontaneous locomotion
Distance traveled/1h
(m, ±SEM)
60
40
20
0
0
1
CP94253 dose (μg/0.3μl)
D
Bregma: -5.20 mm
TH

Figure 20. Effects of local administration of the selective 5-HT_{1B} receptor agonist, CP 94,253 on maintenance of cocaine (0.075 mg/kg, i.v.) intake. Data are expressed as the mean (± SEM). Administration of intra-tegmental effects of CP 94,253 were assessed on cocaine infusions **(A)**. Schematic representation of coronal sections show injection sites **(B)** in rat brains taken at -5.20, -5.30, & -5.60 mm from bregma (Paxinos & Franklin, 2013). Intra-tegmental infusion of CP 94,253 failed to alter spontaneous locomotion **(C)**. Representative immunoreactivity for tyrosine hydroxylase **(D)** is shown with a red arrow indicating cannula placement targeting the VTA. Rats (n = 12) were pretreated 15 min prior to the start of the test sessions with vehicle (white bars) or CP 94,253 (1 µg/0.3 µl; black bars) using a within-subjects designs. Asterisks (*) represent a difference from vehicle ($p < 0.05$).

APPENDIX C

LIST OF ABBREVIATIONS

5-HT: serotonin
5-HT_{1B}R: serotonin 1B receptor
5-HT_{1D}R: serotonin 1D receptor
BRL: BRL 15,572
CP: CP 94,253
D_2AR: dopamine D2 autoreceptor
DAT: dopamine transporter
DMSO: dimethyl sulfoxide
DRN: Dorsal raphe nucleus
FDA: Food and Drug Administration
FR: Fixed ratio
GIRK: G protein-coupled Inwardly Rectifying Potassium Channel
NAc: Nucleus Accumbens
NET: norepinephrine transporter
PR: Progressive ratio
SA: Self-administration
SB: SB 224,289
SERT: serotonin transporter
SSRI: selective serotonin reuptake inhibitor
SUD: Substance use disorder
TH: Tyrosine Hydroxylase
Veh: Vehicle
VMAT: vesicular monoamine transporter
VR: Variable ratio
VTA: ventral tegmental area
Zol: Zolmitriptan

APPENDIX D

ANIMAL SUBJECTS APPROVAL

All experiments proceeded in accordance with a protocol and standard operating procedures approved by the Arizona State University Institutional Animal Care and Use Committee.

Institutional Animal Care and Use Committee (IACUC)
Office of Research Integrity and Assurance
Arizona State University
660 South Mill Avenue, Suite 315
Tempe, Arizona 85287-6111
Phone: (480) 965-4387 *FAX:* (480) 965-7772

Animal Protocol Review

ASU Protocol Number:	**16-1482R**
Protocol Title:	**Neural Mechanisms of Drug Seeking Behavior**
Principal Investigator:	**Janet Neisewander**
Date of Action:	**1/22/2016**

The animal protocol review was considered by the Committee and the following decisions were made:

The protocol was approved as modified.

If you have not already done so, documentation of Level III Training (i.e., procedure-specific training) will need to be provided to the IACUC office before participants can perform procedures independently. For more information on Level III requirements see https://researchintegrity.asu.edu/training/animals/levelthree.

Total # of Animals:	**310**	
Species:	**Rats**	**Pain Level: D**

Protocol Approval Period: **1/22/2016 – 1/21/2019**

Sponsor:	**National Institute of Drug Abuse**
ASU Proposal/Award #:	**027587**
Title:	**Neural mechanisms of drug seeking**

Signature: Augustowski for C Johnson
IACUC Chair or Designee

Date: 1/25/2015

Cc: IACUC Office
IACUC Chair

Institutional Animal Care and Use Committee (IACUC)
Office of Research Integrity and Assurance
Arizona State University
660 South Mill Avenue, Suite 312
Tempe, Arizona 85287-6111
Phone: (480) 965-6788 *FAX:* (480) 965-7772

Animal Protocol Review

ASU Protocol Number:	**19-1679R**
Protocol Title:	**Serotonin involvement in drug addiction-related behavior**
Principal Investigator:	**Janet Neisewander**
Date of Action:	**11/1/2018**

The animal protocol review was considered by the Committee and the following decisions were made:

The protocol was approved.

If you have not already done so, documentation of Level III Training (i.e., procedure-specific training) will need to be provided to the IACUC office before participants can perform procedures independently. For more information on Level III requirements see https://researchintegrity.asu.edu/animals/training.

Total # of Animals:	**3130**	
Species:	**Rats**	**Unalleviated Pain/Distress: No-1560**
Species:	**Mice**	**Unalleviated Pain/Distress: No-1570**

Protocol Approval Period:	**11/1/2018 – 10/31/2021**
Sponsor:	**National Institute of Drug Abuse**
ASU Proposal/Award #:	**AWD00027587**
Title:	**Neural Mechanisms of Drug Seeking**

Signature: _____ Date: 11/2/2018
IACUC Chair or ***Designee***

Cc: IACUC Office
IACUC Chair

APPENDIX E

CURRICULUM VITAE

Raul Garcia

Contact Information

Arizona State University
427 E Tyler Mall
Mail Code: 4601
Tempe, AZ 85287
Email: rgarci27@asu.edu

Education & Professional Training

08/2014 – 05/2020	PhD Candidate/ Graduate Research Assistant Interdisciplinary Graduate Program in Neuroscience School of Life Sciences Mentor: Janet Neisewander, PhD Arizona State University, Tempe, AZ
08/2013 – 08/2014	Post-baccalaureate Research Education Program (PREP) Department of Psychology Behavioral Neuroscience Program Mentor: Federico Sanabria, PhD Arizona State University, Tempe, AZ
08/2009 - 05/2013	Bachelor of Science in Psychological Science Graduated Summa Cum Laude Arizona State University, Tempe, AZ

Research Interests

Neurobiology of reward, learning and memory, decision-making and motivation.

Research Experience

08/2014 – Present	Neisewander Drug Addiction Lab P.I.: Janet Neisewander, PhD Interdisciplinary Graduate Program in Neuroscience Arizona State University
04/2015 – 08/2015	Lab Rotation in Newbern Developmental Neuroscience Lab P.I.: Jason Newbern, PhD Interdisciplinary Graduate Program in Neuroscience Arizona State University
05/2012 – 08/2014	Basic Behavioral Processes Lab P.I.: Federico Sanabria, PhD Behavioral Neuroscience Program Arizona State University

08/2012 - 05/2013	Memory and Language Lab P.I.: Stephen Goldinger, PhD Cognitive Science Program Arizona State University

Funding

01/2020 – 05/2020	Graduate College Completion Fellowship
01/2019 – 05/2019	Graduate College Special Tuition Fellowship, ASU Graduate College
05/2018 – 08/2018	Doctoral Fellowship for First-Generation College Graduates, ASU College of Liberal Arts and Science
08/2016 – 05/2018	Recipient of the Initiative for Maximizing Student Development (IMSD), School of Life Sciences, Arizona State University (GM099650)
08/2014 – 08/2016	Recipient of a Diversity Supplement Training Grant, Graduate Research Assistant, Neural Mechanisms of Drug Seeking, National Institute on Drug Abuse (DA0110615)
08/2013 – 08/2014	Recipient for the Post-baccalaureate Research Education Program (PREP) in Biomedical Research, National Institute of General Medical Sciences (GM071798)

Teaching Experience

01/2019 – 05/2019	Biology 467: Neurobiology
08/2018 – 12/2018	Biology 340: General Genetics
01/2018 – 05/2018	Biology 182: General Biology

Peer-reviewed Publications

In prep	Garcia, R., Scott, S., Blattner, K., Blass, B., & Neisewander, J.L. 5-HT1BR attenuation of cocaine self-administration persists after a period of relapse.
In prep	Garcia, R., Cotter, A. R., Charmchi, D., & Neisewander, J.L. Zolmitriptan, the FDA-approved 5-HT1B/1DR agonist, decreases cocaine but not sucrose self-administration.
In print	Garcia, R., Cotter, A. R., Leslie, K., Olive, M.F., & Neisewander, J.L. (2017). Preclinical evidence that 5-HT1B receptor agonists show promise as medications for psychostimulant use disorders. International Journal of Neuropsychopharmacology, 20 (8), 644-653. doi.org/10.1093/ijnp/pyx025

In print Daniels, C. W., Watterson, E., Garcia, R., Mazur, G. J., Brackney, R. J., & Sanabria, F. (2015). Revisiting the effect of nicotine on interval timing. Behavioural Brain Research, 283, 238-250. doi:10.1016/j.bbr.2015.01.027

Seminar Presentations

11/2019 R. Garcia. 5-HT_{1B}R agonists show therapeutic potential for treating psychostimulant abuse. Interdisciplinary Graduate Program in Neuroscience Seminar, 2019, Tempe, AZ.

07/2018 R. Garcia, and J.L. Neisewander. The role of 5-HT1BR agonists in cocaine reinforcement and their potential as treatments for psychostimulant use disorders. International Society for Serotonin Research 2018, Cork, Ireland.

01/2018 R. Garcia and J.L. Neisewander. Evaluating the efficacy of zolmitriptan as an anti-relapse treatment for cocaine self-administration. Behavioral Neuroscience Seminar 2018, Tempe, AZ.

10/2017 R. Garcia and J.L. Neisewander. Using 5-HT1BR agonists as treatments for psychostimulant use disorders. National Hispanic Science Network 2017, Phoenix, AZ.

06/2015 R. Garcia, N. Pentkowski, J. Venault, K. Leslie, M.F. Olive, J. Neisewander. "The effects of the 5-HT1B receptor agonist, CP 94,253, on methamphetamine self-administration." College on Problems of Drug Dependence 2015, Phoenix, AZ.

Abstracts & Poster Presentations

10/2019 R. Garcia, S. Scott, T. Le, S. Doyle, J. Valenzuela, K.M. Blattner, B.E. Blass, & J. L. Neisewander. 5-HT1B receptor agonist attenuation of cocaine self-administration persists after a period of relapse. Society for Neuroscience 2019, Chicago, IL.

11/2018 R. Garcia, D. Charmchi, A. Cotter, J.P. Bonadonna, & J. L. Neisewander. FDA-approved 5-HT1B/1D receptor agonist, zolmitriptan, decreases cocaine self-administration in male and female rats. Society for Neuroscience 2018, San Diego, CA.

07/2018 R. Garcia, A. Cotter, D. Charmchi, J. L. Neisewander. Evaluating the role of 5-HT1BR agonists on cocaine reinforcement and their potential as an anti-relapse treatment. International Society for Serotonin Research 2018, Cork, Ireland.

03/2018 R. Garcia, D. Charmchi, G. Powell, J. Hesterman, J.L. Neisewander. Intracranial administration of the serotonin1B receptor agonist, CP 94,253, in the ventral tegmental area modulates cocaine self-administration. Behavior, Biology, & Chemistry 2018, San Antonio, Texas.

11/2017 C.D. Gipson, G.L. Powell, J.G. Goenaga, A.P. Del Franco, M.C. Holter, R. Garcia, A. Vannan, J. L. Neisewander. Developmental nicotine exposure induces persistent alterations in accumbens glutamatergic circuitry. Society for Neuroscience 2017, Washington DC.

06/2017 S. N. Scott, R. Garcia, A. Bralich, J. Hesterman, A. Stone, J. L. Neisewander. Ovarian hormonal status influences 5-HT1B receptor agonist effects on cocaine self-administration in rats. College on Problems of Drug Dependence 2017, Montreal, CA

03/2017 G.L. Powell, J. Goenaga, A. Del Franco, M. Holter, R. Garcia, A. Vannan, J.L. Neisewander, C.D. Gipson. Developmental nicotine exposure induces persistent alterations in accumbens glutamatergic circuitry. Research on Nicotine and Tobacco 2017, Florence, Italy.

11/2016 R. Garcia, A.R. Cotter, K. Leslie, K. Ennis, T. Benson, M.F. Olive, J.L. Neisewander. The 5-HT1B receptor agonists, CP 94,253 and zolmitriptan, attenuate the reinforcing and motivational effects of methamphetamine. Society for Neuroscience Conference 2016, San Diego, CA.

07/2016 R. Garcia, A. Cotter, K. Ennis, M.F. Olive, J.L. Neisewander. The 5-HT1B receptor agonist, CP 94253, attenuates the reinforcing and motivational effects of methamphetamine. International Society for Serotonin Research Conference 2016, Seattle, WA.

10/2015 R. Garcia, N. Pentkowski, J. Venault, K. Leslie, J.P. Bonadonna, A. Cotter, A. Campagna, M.F. Olive, J.L. Neisewander. The 5-HT1B receptor agonist, CP 94253, modulates methamphetamine self-administration. Society for Neuroscience Conference 2015, Chicago, IL.

08/2015 R. Garcia, N. Pentkowski, J. Venault, K. Leslie, J.P. Bonadonna, A. Cotter, A. Campagna, T. Benson, K. Ennis, M.F. Olive, J. Neisewander. Different modulatory effects of the 5-HT1B receptor agonist, CP 94253, on methamphetamine self-administration compared to cocaine self-administration. American Psychological Association Convention 2015, Toronto, CA.

04/2015 R. Garcia, N. Pentkowski, J. Venault, K. Leslie, M.F. Olive, J.L. Neisewander. Different modulatory effects of the 5-HT1B receptor agonist CP 94,253 on methamphetamine self-administration compared to cocaine self-administration. National Institute on Drug Abuse Diversity Supplement Workshop 2015, Bethesda, MA.

03/2015 R. Garcia, N. Pentkowski, J. Venault, K. Leslie, J.P. Bonadonna, A. Cotter, M.F. Olive, J.L. Neisewander. Effects of the 5-HT1B receptor agonist CP 94,253 on methamphetamine self-administration. Behavior Biology and Chemistry Conference 2015, San Antonio, TX.

11/2014 T. Der-Ghazarian, S. Brunwasser, K. Dai, R. Garcia, M. Gao, N. Pentkowski, J. Wu, J.L. Neisewander. Effects of 5-HT1BR Agonist CP 94,253 on Cocaine-Induced Locomotion Before and After Abstinence from Repeated Cocaine Administration. Society for Neuroscience Conference 2014, Washington D.C.

03/2013 R. Garcia, R.J. Brackney, A. Spitzer, F. Sanabria. Extinction learning deficit in an animal model of ADHD. Annual MGE@MSA/WAESO Student Research Conference 2013, Tempe, AZ.

Undergraduate Student Thesis Committee

2020 Tien Le, Barrett's the Honor's College, Bachelor of Science
2019 Margaret Zheng, Barrett's the Honor's College, Bachelor of Science
2018 Austin Cotter, Barrett's the Honor's College, Bachelor of Science

Journal Reviewer

2018 Pharmacology, Biochemistry, and Behavior
2017 International Journal of Neuropsychopharmacology

Awards & Honors

09/2019 Travel Award – ASU Graduate College
09/2019 Travel Award - ASU SOLs Graduate Student Programs
07/2019 NIDA-IRP Scientific Director's Fellowship for Diversity in Research
04/2019 College Graduate Excellence Award
11/2018 Travel Award - ASU SOLs Graduate Student Programs
11/2018 Travel Award - 2018 NIDA-NIAAA Diversity Scholar
07/2018 Travel Award - 2018 International Society for Serotonin Research
06/2018 Travel Award – ASU SOLs Graduate Student Programs
04/2018 Graduate Excellence Award – ASU College of Liberal Arts & Sciences
04/2018 Outstanding Research Award – Graduate & Professional Student Association
03/2018 Travel Award - 2018 Behavior, Biology, & Chemistry Conference
10/2017 Travel Award - Initiative for Maximizing Student Development
04/2017 Graduate Excellence Award – ASU College of Liberal Arts & Sciences

10/2016	Travel Award - Initiative for Maximizing Student Development
07/2016	Travel Award - 2016 APA Convention
04/2015	Travel Award - 2015 APA Convention
2012, 2013	Western Alliance to Expand Student Opportunities (WAESO) Award
2012, 2013	Dean's List, ASU – College of Liberal Arts & Sciences
08/2007	The Susan Thompson Buffett Foundation Scholarship

Professional Memberships & Affiliations

2016 - Present	International Society for Serotonin Research (ISSR)
2016 - 2018	ASU's Graduate Association of Interdisciplinary Neuroscience Students (GAINS) – Primary Role: President
2016 - 2018	ASU's Latin@ Graduate Student Alliance (LGSA) – Primary Role: Vice-President
2016 - 2017	International Behavioral Neuroscience Society (IBNS)
2015 - 2016	ASU's Graduate Association of Interdisciplinary Neuroscience Students (GAINS) – Primary Role: Student Program Representative
2015 - Present	ASU's Latino Graduate Student Association (LGSA)
2015 - Present	ASU's Graduate Women Association (GWA)
2015 - Present	Society for Advancement of Chicanos/Latinos and Native Americans in Science (SACNAS)-ASU chapter
2014 - Present	Society for Neuroscience
2014 - 2015	American Association for the Advancement of Science
2014 - Present	Graduate Association of Interdisciplinary Neuroscience Students (GAINS)
2013 - 2014	Cognitive Neuroscience Society
2012 - 2012	Arizona's Foundation for Suicide Prevention – Out of the Darkness Campus Walk
2012	ASU's Well Devil Council
2011 – Present	Living United for Change in Arizona (LUCHA)
2011 - 2012	To Write Love on Her Arms – ASU Chapter
2011 - 2013	Association for Psychological Sciences
2009 - 2014	American Psychological Association
2007 - 2008	Mexican American Student Association (MASA) @ UNL

Skills

- Bilingual in English & Spanish
- Extensive experience running human subjects & handling animals (rats, mice, pigeons, pigs)
- Experience with subcutaneous, intraperitoneal, and intracranial injections in rodents
- Stereotaxic/Femoral & Vein Catheter Implantation Surgeries
- Brain Extractions (rats & mice)
- Paraformaldehyde Perfusions
- Cryostat/Vibratome Experience

- Immunohistochemistry/Cresyl Violet Staining
- Fluorescence/Confocal Microscopy
- Antero/Retrograde Tracing
- Experience with Chemogenetic Techniques (DREADDs & Vivo-Morpholinos)

Outreach, Volunteer & Community Service

2018 Graduate career panelist - ASU's Society for Advancement of Chicanos/Latinos and Native Americans in Science (SACNAS)
2018 Archway Arete Classical Academy Science Outreach
2018 Brain & You 2018, Graduate Association of Interdisciplinary Neuroscience Students
2018 Kyrene Elementary DISCOVERoom Science Outreach
2018 Gavilan Peak Elementary Science Exposition
2018 Madison Elementary School Neuroscience Outreach
2017 South Mountain High School Outreach. Topic: Methods of Scientific Inquiry
2017 Brain & You 2017, Graduate Association of Interdisciplinary Neuroscience Students
2017 Madison Elementary School Neuroscience Outreach
2017 Kyrene Elementary DISCOVERoom Science Outreach
2017 Gavilan Peak Elementary Science Exposition
2016 Hispanic Heritage Month Celebration Committee
2016 Brain & You 2016, Graduate Association of Interdisciplinary Neuroscience Students
2016 Invited STEM Professional Panel at South Mountain Community College
2016 Gavilan Peak Elementary Science Exposition
2014 ASU-West campus STEM for minority and underrepresented students

Mentorship & Supervisory Roles

Juliette Venault[e]	Katelin Ennis	Pacifique Rukundo[a]
Kenneth Leslie[c]	Delaram Charmchi[c]	Oscar Villarreal[ab]
Austin Cotter[bcd]	Tien Le[bcd]	Sophia Doyle[f]
Allegra Campagn	Margaret Zheng[bd]	Gokul Karthik[f]
Thomas Benson	Aracely Esquer[ab]	Jamie Sprout

Note: [a]Under-represented student in science. [b]SOLUR (School of Life Sciences Undergraduate Research) student. [c]Student co-author. [d]Assisted with student's thesis. [e]International Exchange Student. [f]High School Student