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Noradrenergic regulation of cocaine-seeking behaviors

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Noradrenergic regulation of cocaine-seeking behaviors

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An abstract of

A dissertation submitted to the Faculty of the

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Abstract

Noradrenergic regulation of cocaine-seeking behaviors

By Karl T. Schmidt

Norepinephrine (NE) has been shown in human and laboratory studies to modulate relapse-like behaviors of drugs of abuse. The neuroanatomy and receptor subtypes mediating these effects are not as well characterized. Here, we discuss the contributions of noradrenergic signaling to psychostimulant-induced behaviors in animal models. The experiments in this dissertation focus on noradrenergic projections from the locus coeruleus to mesocorticolimbic brain regions and how these circuits influence drug-seeking and other operant behaviors. Alpha 1 adrenergic receptors in the medial prefrontal cortex, but not ventral tegmental area or nucleus accumbens, are necessary for drug-primed reinstatement, but not sufficient to reinstate in the absence of cocaine. Furthermore, blockade of all noradrenergic signaling prevents cocaine induced glutamate release in the prefrontal cortex. Stimulating NE neurons in the locus coeruleus with Designer Receptors Exclusively Activated by Designer Drugs (DREADDs) drives drug-seeking behaviors, but only when tested under conditions commonly used to test stress-induced reinstatement. Finally, selective activation of the locus coeruleus with optogenetics reinforces operant behavior at levels matching identical stimulation of dopamine neurons in the ventral tegmental area. Overall, these studies identify regions of noradrenergic influence important in relapse-like behavior and other motivated behaviors.

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CHAPTER I:

MODULATION OF PSYCHOSTIMULANT ADDICTION-RELATED BEHAVIORS BY NOREPINEPHRINE

Adapted from:

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1.1 Abstract

Psychostimulants, such as cocaine and amphetamines, act primarily through the monoamine neurotransmitters dopamine (DA), norepinephrine (NE), and serotonin (5-HT). While stimulant addiction research has largely focused on DA, medication development efforts targeting the dopaminergic system have thus far been unsuccessful, leading to alternative strategies aimed at abating stimulant abuse. Noradrenergic compounds have shown promise in altering the behavioral effects of stimulants in rodents, non-human primates, and humans. In this chapter, we discuss the contribution of each adrenergic receptor (AR) subtype (α1, α2, and β) to 5 stimulant-induced behaviors relevant to addiction: locomotor activity, conditioned place preference, anxiety, discrimination, and self-administration. AR manipulation has diverse effects on these behaviors; each subtype profoundly influences outcomes in some paradigms, but is inconsequential in others. Furthermore, optogenetic and chemogenetic techniques are used to control noradrenergic activity and provide new approaches to investigate interactions between NE and stimulant-induced behaviors.

1.2 Cocaine prevalence and abuse in society

Despite thousands of years of usage for its stimulant properties, cocaine has become a highly abused drug in modern society. Cocaine today is purified from the leaves of the coca plant into a fine powder that can be injected, snorted, or transformed into a freebase form and smoked (crack). In 2014, an estimated 1.5 million Americans were active cocaine users with over 900,000 of those meeting criteria for a cocaine use disorder (NSDUH, SAMSHA, 2015). Of emergency room visits related to drug use, 40% included cocaine (DAWN, 2011). The increased medical care, criminal activity, loss of productivity, and other issues that arise from substance use is estimated by the Office of National Drug Control Policy to total $193 billion with approximately $28 billion coming from direct purchase of cocaine (whitehouse.gov, 2014). Unfortunately, we currently have no FDA approved pharmacotherapies for cocaine-dependence.

Although we have been unable to identify effective treatments, decades of research have identified the mechanism of action of cocaine. Cocaine has two primary effects: 1) it acts as a local anesthetic which leads to its Schedule II classification as it can be prescribed for certain surgeries and 2) it activates the brain's reward pathway. Cocaine blocks the reuptake of dopamine (DA), norepinephrine (NE), and serotonin (5-HT) thereby increasing the availability of these neurotransmitters in the synapse (Figure 1.1). The "high" and primary reinforcing effects of cocaine result from cocaine's action at the dopamine transporter and increase of DA in the mesocorticolimbic reward pathway (Roberts et al. 1977; Di Chiara and Imperato, 1988; Thomsen et al., 2009). However, as I will discuss in this chapter and those that follow, the neurochemical mechanisms mediating other aspects of cocaine use and abuse, in particular those related to relapse,

are sensitive to alterations in signaling of noradrenergic receptors. Because relapse prevention would have the greatest impact from a treatment perspective, I will focus my experiments on animal models of relapse, but will discuss other effects of cocaine and related stimulants.

1.3 Psychostimulants and catecholamines

For many years, medication development efforts for psychostimulant abuse therapies revolved around understanding and modifying DA transmission. Because DA mediates the primary rewarding/reinforcing effects of psychostimulants, the focus on DA was understandable. However, after decades of research, dopaminergic compounds have failed to gain FDA approval or general acceptance as treatments for stimulant dependence. Several reasons likely contribute to this lack of efficacy. For example, dopaminergic drugs showed abuse liability themselves (Mariani and Levin, 2012). In addition, experienced drug abusers often report that, although the drug may no longer produce a subjective euphoric effect, they continue to use the drug for other reasons, rendering medications that alter the positive subjective effects of psychostimulants impotent.

Although it is generally accepted that the abuse-related effects of psychostimulants occur primarily through dopaminergic activity, this class of drugs alters several of neurotransmitter systems. In particular, both cocaine and amphetamine-like compounds also increase extracellular levels of NE and 5-HT by preventing reuptake by their respective plasma membrane transporters (i.e. DAT, NET, and SERT) and/or inducing release. Based on recent studies implicating NE in relapse-like behavior, interest

in the contribution of this neurotransmitter to addictive processes has reemerged (Weinshenker and Schroeder, 2007; Gaval-Cruz and Weinshenker, 2009). Some noradrenergic compounds have already shown promise in human laboratory studies and initial clinical trials (Gaval-Cruz and Weinshenker, 2009; Fox et al., 2012; Haile et al., 2012; Newton et al., 2012; Shorter et al., 2013; K. Cunningham, personal communication). Yet, our knowledge of how the NE system reacts to, and interacts with, psychostimulants is remarkably incomplete, and the neurobiological mechanisms underlying the effects of these compounds are not well understood.

This chapter will focus on how adrenergic receptors (ARs) influence responses to psychostimulants, how these systems could be targeted for novel addiction therapies, and novel techniques for modulating adrenergic activity. First, I will begin with an overview of adrenergic receptor subtypes, followed by pharmacological compounds used to manipulate noradrenergic signaling.

1.4 Adrenergic receptor subtypes

ARs are G protein-coupled receptors (GPCRs) that bind, and are activated by, NE and its derivative transmitter, epinephrine (EPI). Because EPI levels in the brain are very low (Mefford, 1988), it is likely that NE mediates most of the effects discussed in this review, although some evidence for EPI regulation of motor activity exists (Stone et al., 2003). Included in this family of receptors are 9 subtypes encoded by separate genes: three α1ARs (α1a, α1b, and α1d), three α2ARs (α2a, α2b, and α2c), and three βARs (β1, β2, and β3). α1ARs are $G\alpha_q$-coupled and their activation stimulates phospholipase C activity to cleave phosphatidylinositol 4,5-biphosphate (PIP2) and increase inositol triphosphate

(IP3) and diacylglycerol (DAG), causing an increase of intracellular calcium and activation of protein kinase C. βARs are typically $G\alpha_s$-coupled and activate protein kinase A (PKA) via stimulation of adenylate cyclase activity and cyclic adenosine monophosphate (cAMP) production. α2ARs are $G\alpha_i$-coupled and function as inhibitory autoreceptors on noradrenergic neurons, although both presynaptic and postsynaptic α2AR heteroreceptors on NE target neurons are also abundant in the brain. Activation of these receptors decreases PKA activation by suppressing cAMP production by adenylate cyclase. In addition, the Gβγ protein complex associated with ARs is capable of modulating intracellular signaling molecules and ion channels, including phospholipase C, G-protein receptor kinase (GRK), inwardly rectifying potassium channels (GIRK), and calcium channels, among others (Lin and Smrcka, 2011). However, the functional consequences of βγ signaling in the context of stimulant-induced behaviors have not been well investigated.

Although ARs typically signal via these molecules, several non-canonical AR signaling pathways that are independent of G-proteins and cAMP exist. For example, β2AR activation stimulates a GSK 3β/Akt pathway via β-arrestins, which previously were thought to only be important for GPCR internalization and sensitization but are now known to be scaffolds for multi-protein complexes and signaling (Yamamoto et al., 2007; Beaulieu et al., 2009). Additionally, β1ARs can signal through $G\alpha_{olf}$ to activate a receptor tyrosine kinase, which in turn stimulates the Ras/Raf/MEK/MAPK/MSK pathway to produce cyclic AMP response element-binding protein (CREB) phosphorylation and gene transcription (Meitzen et al., 2011). β2ARs can switch coupling from $G\alpha_s$ to $G\alpha_i$ via PKA-mediated phosphorylation of the receptor leading to

MAPK pathway activation (Daaka et al., 1997). β2AR activation can cause GRK phosphorylation, which activates a β-arrestin and phosphodiesterase-4 (PDE4) feedback circuit to decrease cAMP activity and PKA phosphorylation of the receptor (Baillie et al., 2003). Without this β-arrestin/PDE4 feedback, an enhanced switch from $G\alpha_s$ to $G\alpha_i$ coupling is observed. This β2AR- $G\alpha_i$ signaling also occurs in the brain and is reported to mediate learning and memory, potentially via PLC (Schutsky et al., 2011a; Schutsky et al., 2011b; Ouyang et al., 2012). The mechanisms underlying ligand-induced activation of these pathways are understudied and could provide critical insights into AR-mediated effects.

1.5 Compounds targeting adrenergic receptors

Because many compounds targeting these ARs with varying degrees of selectivity for one subtype versus another are used to determine the roles of each receptor in stimulant-induced behaviors, we provide a brief description of these compounds.

The prototypical α1AR antagonist is prazosin, but it is limited by its equal affinity for each of the α1AR subtypes (Zhong and Minneman, 1999). Terazosin is similar to prazosin but is favored in intracranial infusion studies because it is more soluble in artificial cerebrospinal fluid (aCSF) that is often used as a vehicle for these experiments (Stone et al., 1999). WB 4101 was the first subtype-selective α1AR antagonist, with an affinity for the α1aAR approximately 20-fold greater than the α1bAR; however its binding affinity does not differentiate the α1dAR (Morrow and Creese, 1986). Two compounds that are selective for α1a over α1b and α1d are 5-methylurapidil and (+)-niguldipine (Hanft and Gross, 1989; Boer et al., 1989). BMY 7378 has a 100-fold greater

affinity for α1d than α1a or α1b, but also acts as a partial 5HT1a receptor agonist (Goetz et al., 1995; Zhong and Minneman, 1999). Epinephrine activates α1ARs with the highest affinity, followed by NE and phenylephrine, respectively (Morrow and Creese, 1986). As measured by radioligand binding, epinephrine, NE, and phenylephrine show the highest affinities for α1d (Minneman et al., 1994), and only phenylephrine binds with a greater affinity for α1a than α1b (Morrow and Creese, 1986). However, when measuring intracellular responses in recombinant HEK 293 cells expressing only one subtype, similar potencies are found for epinephrine, NE, and phenylephrine regardless of the receptor subtype expressed (Minneman et al., 1994). Methoxamine, ST 587, and SDZ NVI 085 also activate α1ARs (Spealman, 1995; Munzar and Goldberg, 1999).

Agonists at α2ARs include clonidine, UK 14304, lofexidine, guanabenz, and dexmedetomidine (Aghajanian and VanderMaelen, 1982; Carter, 1997; Kleven and Koek, 1997; Sallinen et al., 1997; Erb et al., 2000). Guanabenz/guanfacine is preferential for α2a, but clonidine and dexmedetomidine has equal affinity at α2 subtypes (Gobert et al., 1998). α2AR antagonists include yohimbine, efaroxan, BRL-44408, dexefaroxan, idazoxan, and atipamezole (Dickinson et al., 1988; Villegier et al., 2003; Juhila et al., 2005; Jimenez-Rivera et al., 2006; Doucet et al., 2013;). Yohimbine and atipamezole show equal affinities at all three receptor subtypes, but atipamezole has a 200-fold greater selectivity than yohimbine for the α2AR over the α1AR (Schwartz and Clark, 1998). Because yohimbine interacts with a number of non-noradrenergic systems in addition to acting at the α2AR, its effects should be interpreted with caution (Feuerstein et al., 1985; Millan et al., 2000; Conrad et al., 2012).

The prototypical βAR antagonist is propranolol, and like prazosin, it is not selective for any of the β subtypes. Timolol and nadolol act equally at β1 and β2 ARs, but nadolol cannot cross the blood brain barrier (Colussi-Mas et al., 2005). Selective β1AR antagonists include atenolol, which also only acts peripherally, and betaxolol (Harris et al., 1996; Bernardi et al., 2009). ICI-118,551 has high affinity for the β2AR (O'Donnell and Wanstall, 1980; Bilski et al., 1983). SR58611A and SR59230A are selective agonists and antagonists, respectively, for the β3AR (Consoli et al., 2007).

Although not an exhaustive list of AR activators and inhibitors, these are some of the most commonly used compounds in psychostimulant studies that we will refer to later in this review.

1.6 Animal models of psychomotor stimulant effects

1.6.1 Locomotor activity

A characteristic trait of stimulant drugs, such as cocaine and D-amphetamine, is the ability to increase locomotor activity in rodents. This hyperactivity is robust and provides a reliable metric for assessing the contribution of different systems to simple drug effects. In these studies, subjects are placed in an open field-like chamber, and activity is measured via a grid of infrared photobeams across the chamber. The animal's position is monitored by beam breaks or by visual tracking software that uses contrast between the animal and the floor to identify location and movement. When initially placed in the chamber, animals will typically show an increased level of locomotion induced by the novelty of the chamber that is subject to habituation. Drug administration can occur either prior to this exploratory period or following habituation. Additionally,

repeated administration of stimulants leads to behavioral sensitization in which the same dose leads to greater levels of activity. Although the face validity of this behavioral measure for drug addiction per se is poor, locomotor activity can be a predictor of abuse liability (Marinelli and White, 2000; Simmons et al., 2013), and it has been suggested that the sensitization paradigm reflects the incentive salience value of drugs and models drug craving (Robinson and Berridge, 2001).

1.6.2 Place preference and aversion

The conditioned place preference (CPP) procedure is a popular paradigm used to measure the rewarding effects of addictive drugs (Tzschentke, 2007; Aguilar et al., 2009). This procedure uses a two- or three-compartment apparatus in which daily conditioning sessions pair the effects of a drug to one compartment and vehicle to the other. In the three-chamber version of the CPP paradigm, the third compartment is a neutral middle partition that is not paired with any stimuli and can be used as a "start box". To determine whether the animal has an initial bias toward one side, a preconditioning test occurs during which the subject can freely explore all compartments. Ideally, the paradigm follows a balanced design in which no initial side preference is observed. If the subjects show a preconditioning preference, the experimental design can be described as "biased" or "unbiased". In a biased design, the drug pairing is made with regards to the animals' initial preference. For example, to increase the probability of observing a place preference on the final test, drug is paired with the compartment each animal prefers less during preconditioning. In the preferred unbiased design, the drug-compartment pairing is random; some animals receive the drug on the "preferred" side, others on the "non-

preferred" side. Alternatively, one compartment can be designated as the drug-paired chamber for all animals regardless of initial preference. Following repeated pairings, a test session is used to assess the rewarding or aversive properties of the drug in question by allowing the animal to freely move between the compartments in a drug-free state. An increased amount of time spent in the drug-paired chamber is thought to reflect drug reward, while decreased time indicates a drug aversion. Because the paradigm requires learning, the conditioned effect can be extinguished, is subject to retrieval and reconsolidation processes, and can be reinstated after a drug-prime or stress exposure, which are believed to model aspects of relapse in human drug abusers.

1.6.3 Anxiety

In addition to the rewarding effects of stimulants, drugs like cocaine also have anxiogenic properties. The elevated plus maze can be used as a behavioral readout of anxiety-like behavior. In this task, an animal is placed in a plus-shaped apparatus raised a few feet from the floor. Two of the four arms are enclosed with walls, and the other two arms are open. Time spent in the closed arms is thought to represent anxiety-like behavior because (1) restriction to the open arms causes greater behavioral and physiological responses consistent with anxiety than closed arm restriction, (2) compounds that cause anxiety in humans increase time spent in the closed arms, and (3) clinically effective anxiolytic drugs selectively increase time spent in the open arms (Pellow et al., 1985).

To determine the expression of psychostimulant-induced anxiety/aversion, a runway model of self-administration has been used (Wenzel, et al 2014). In the runway

model, rats must walk down an alley to a goal box to receive an infusion of cocaine. In this paradigm, the approach-avoidance conflict of drug delivery can be assessed by analyzing the time to reach the goal box (approach), the frequency of retreating (avoidance), and the time to initiate approach behaviors. The runway model is an elegant way to separate the euphoric and anxiogenic properties of stimulants.

1.6.4 Drug discrimination

Drug discrimination is a measure of the interoceptive effects of a drug. In this procedure, animals are trained to respond on one operandum (drug-appropriate) following an experimenter-administered injection of a training drug and another operandum (vehicle-appropriate) following vehicle administration. Responses on the appropriate lever are reinforced by food, water, shock-termination, or other stimuli that maintain high, stable rates of behavior, but typically appetitive reinforcement is used. Once animals meet training criteria in which responses are made on the appropriate operandum with high selectivity (typically greater than 80-90%), a session occurs to test whether various doses of the training drug, other drugs, or pretreatments plus the training drug can alter the discriminative stimulus properties. When the test treatment produces interoceptive effects similar to the training drug, the subject responds predominantly on the drug-appropriate lever. When the test treatment produces interoceptive effects that are distinct from the training drug, the subject responds predominantly on the vehicle-appropriate lever. In this way, one can interrogate whether a test treatment produces a state that “feels” like that produced by the training drug. For example, when trained with cocaine, the psychostimulants amphetamine and methylphenidate engendered responding

on the cocaine-appropriate lever, while non-stimulant drugs such as fenfluramine and mescaline elicited responding on the vehicle lever (McKenna and Ho, 1980).

1.6.5 Self-administration

The gold standard for assessing the reinforcing properties of a drug is the operant self-administration paradigm, in which an animal performs a behavior reinforced by the delivery (intravenous, oral, etc.) of a drug that is usually paired with a sensory cue (e.g. light or tone). Psychostimulants and other drugs that are abused by humans are readily self-administered by animals and produce behavioral patterns that are reminiscent of aspects of human addiction. First, in the "acquisition" phase, the animal learns the operant task (e.g. lever press, nose poke, etc.) that results in reinforcer presentation, and the "maintenance" phase commences once the behavioral rates and drug intake stabilize. Maintenance responding is thought to model ongoing drug taking in humans, and alterations in this phase were used to determine the neurobiological basis of addiction and to test potential interventions. A variety of schedules can be employed during the maintenance phase to address specific aspects of reinforcer efficacy. For example, a fixed ratio (FR) schedule, in which the completion of a set number of responses (e.g. every response in an FR1, every 5^{th} response in an FR5, etc.) delivers a reinforcer, is a simple schedule frequently used to determine whether an animal will self-administer a compound. By comparison, a progressive ratio (PR) schedule, in which the response requirement increases exponentially during the course of the session until a "breakpoint" is reached when the subject stops responding to earn reinforcers, determines the relative reinforcing efficacy of the drug (Richardson and Roberts, 1996). More recently, interest

has piqued in two subsequent phases, “extinction” and “reinstatement”. During extinction, the drug is replaced with a non-reinforcing vehicle (e.g. saline, water). The animal learns that the operant task no longer precipitates reward presentation, and the conditioned behavior declines to low levels. Once the behavior is extinguished, administration of a drug prime, restoration of cues previously associated with the drug, or stress (e.g. mild electric footshock or pharmacological stressor like yohimbine) can “reinstate” the operant behavior to rates comparable to maintenance levels even though the operant behavior is not reinforced by drug presentation. Thus, reinstatement represents drug seeking and is thought to model relapse behaviors of human addicts. Because many addicts try repeatedly to quit but have difficulty staying drug-free, this phase has become a prime target for recent medication development efforts. The studies presented in this dissertation have used the reinstatement model to determine the role of NE in altering drug seeking and the potential as a therapeutic target.

1.7 Norepinephrine alteration of psychostimulant-induced behaviors

1.7.1 Locomotor Activity

Manipulations of NE receptor subtypes indicate opposing roles of α1AR and α2AR, with blockade of α1AR decreasing, and antagonism of α2AR increasing, the acute locomotor response to stimulants. Numerous studies have shown that α1ARs antagonists such as prazosin, terazosin, and WB-4101 decrease drug-induced motor activity and behavioral sensitization (Snoddy and Tessel, 1985; Dickinson et al., 1988; Blanc et al., 1994; Darracq et al., 1998; Drouin et al., 2002; Weinshenker et al., 2002; Wellman et al., 2002; Vanderschuren et al., 2003; Auclair et al., 2004; Salomon et al., 2006; Alsene et

al., 2010). Importantly, the effects seen with α1AR antagonism appear to be specific to drugs with abuse liability because prazosin did not impair basal locomotion or hyperactivity induced by the muscarinic antagonist scopolamine (Blanc et al., 1994; Wellman et al., 2002; Alsene et al., 2010). Compared to the wild type, mice genetically lacking the α1bAR subtype (α1b KO) had a decrease in acute and sensitized responses to amphetamine and cocaine despite normal basal dopaminergic function and DA receptor populations (Auclair et al., 2002; Drouin et al., 2002; Villegier et al., 2003; Auclair et al., 2004). Furthermore, the effects of prazosin on drug-induced hyperactivity were abolished in the α1b KO mice, indicating that the α1b subtype is the most important mediator of this psychostimulant response (Drouin et al., 2002). α1d KO mice showed a decreased locomotor response to amphetamine, suggesting a contribution of this subtype. However, spontaneous wheel running and novelty-induced rearing were also reduced in these animals, indicating a non-specific effect on motor activity (Sadalge et al., 2003). Intracerebroventricular (i.c.v) administration of the α1a receptor antagonist, 5-methylurapidil, failed to suppress cocaine hyperlocomotion (Clifford et al., 2007). The location of the α1ARs regulating stimulant-induced activity appears to be the medial prefrontal cortex (mPFC; Blanc et al., 1994; Darracq et al., 1998) and nucleus accumbens shell (Mitrano et al., 2012) because local infusions of α1AR antagonists into these regions reduced cocaine and/or amphetamine-induced locomotion.

Antagonism of α2AR, on the other hand, which facilitates NE transmission by blocking autoreceptor function, increased both acute stimulant-induced locomotion (Dickinson et al., 1988; Villegier et al., 2003; Jimenez-Rivera et al., 2006) and sensitized responses (Doucet et al., 2013) in mice and rats. Conversely, the α2AR agonist clonidine,

which suppresses NE release via autoreceptor stimulation, produced a decreased acute response to cocaine (Vanderschuren et al., 2003; Jimenez-Rivera et al., 2006), and prevented amphetamine sensitization (Doucet et al., 2013). None of these studies provided evidence for the neuroanatomical substrates mediating these α2AR responses.

Fewer studies have examined the role of βARs on locomotor responses to stimulants. Propranolol, a non-selective βAR blocker, increased the acute effects of cocaine in rats (Harris et al., 1996), but not mice (Al Hasani et al., 2013). Mixed results have been reported with amphetamine; low doses of propranolol (1.0-3.0 mg/kg) increased activity induced by amphetamine (1.0 mg/kg) in rats (Vanderschuren et al., 2003), but much higher doses of both drugs (30 mg/kg propranolol and 3.2 mg/kg amphetamine) revealed decreased locomotion when compared to mice treated with amphetamine alone (Snoddy and Tessel, 1985). Administration of a centrally acting βAR antagonist blocked the development of sensitization to amphetamine or cocaine, whereas peripherally acting antagonists did not (Colussi-Mas et al., 2005; Bernardi and Lattal, 2012a). These studies implicated the bed nucleus of the stria terminalis (BNST), which displayed induction of the immediate early gene c-fos following amphetamine administration, and intra-BNST infusion of the βAR antagonist timolol prevented sensitization (Colussi-Mas et al., 2005).

In summary, it appears that NE transmission has an overall facilitatory effect on stimulant-induced locomotion, and blocking postsynaptic ARs attenuates this behavior.

1.7.2 Place Preference and Aversion

Place Preference Induction

In general, drugs that modulate NE activity specifically are ineffective at creating conditioned preferences or aversions on their own. Neither the α1AR agonist phenylephrine nor the α1AR antagonist prazosin supported the formation of a place preference (Zarrindast et al., 2002; Sahraei et al., 2004). Similarly, α2AR antagonists have also showed either no effect (Morales et al., 2001; Sahraei et al., 2004; Tahsili-Fahadan et al., 2006) or a conditioned aversion (File, 1986). In contrast, the α2AR agonist clonidine elicited a CPP (Asin and Wirtshafter, 1985; Cervo et al., 1993), but this effect was only observed at specific doses and when paired with the less preferred compartment, and other α2AR agonists have not mimicked this effect (Sahraei et al., 2004; Tahsili-Fahadan et al., 2006). The βAR antagonist timolol also did not produce a preference on its own (Robledo et al., 2004). These results indicate that neither AR agonists nor antagonists, per se, have rewarding properties or abuse liability.

Despite a thorough search of the literature, surprisingly few studies were found that examined the effects of AR antagonists on the development of a psychostimulant CPP. One study reported that propranolol (10 mg/kg) failed to alter cocaine CPP (Al Hasani et al., 2013), and 2 others showed that mice lacking the α2aAR or both the β1AR and β2AR had normal amphetamine and cocaine CPP (Juhila et al., 2005; Vranjkovic et al., 2012). Surprisingly, no published studies have examined the influence of α1ARs on stimulant CPP. Considering the substantial evidence indicating a role for α1AR signaling on stimulant-induced locomotor activity, discussed above, and certain aspects of stimulant self-administration, discussed below, future efforts to determine the influence of α1ARs on the rewarding effects of cocaine and the neuroanatomy underlying any such findings are warranted.

Retrieval, Reconsolidation, and Extinction

While the effects of NE on the extinction of fear conditioning have been thoroughly investigated (Mueller and Cahill, 2010), only a few studies have examined the role of AR signaling in the extinction of conditioned drug effects. Because the neurobiological events mediating memory retrieval, reconsolidation, and extinction impact the ability to express or extinguish a CPP, care must be taken in the design and interpretation of studies to address the distinction between these processes. For the purposes of this review, we will be using the terms retrieval, reconsolidation, and extinction as the authors employed them to describe their work.

The retrieval of a cocaine CPP memory was blocked by pre-session administration of β-receptor antagonists (Otis and Mueller, 2011). These effects were localized to βARs in the PFC or dorsal hippocampus, but not basolateral amygdala (BLA; Otis et al. 2013; Otis et al., 2014). When administered prior to a retrieval trial, β1AR antagonists, but not β2AR antagonists inhibit subsequent expression of the memory (Fitzgerald et al., 2016). By contrast β2ARs mediate the reconsolidation of cocaine CPP as administration of propranolol and ICI-118,551 (β2 antagonist), but not betaxolol (β1 antagonist) immediately following retrieval impaired the expression of CPP in subsequent sessions (Bernardi et al., 2008; Fricks-Gleason and Marshall, 2008; Bernardi et al., 2009). Similar effects were observed with a high dose of prazosin, but not a lower dose (Bernardi et al., 2009), yet how and where these βAR-mediated effects occur was not determined.

In contrast to retrieval, the effects of βAR activation on reconsolidation were localized to the BLA; c-fos immunoreactivity was increased in the BLA following reconsolidation and local infusions of β antagonists blocked the effect (Bernardi et al., 2009; Otis et al., 2013).

Regarding extinction of CPP, a possible role of αARs has been identified, but the data are not clear-cut. Mice treated with prazosin immediately after daily drug-free test sessions extinguished at normal rates, yet reacquired cocaine CPP with a single re-exposure session that was ineffective in vehicle-treated animals (Bernardi and Lattal, 2010). However, when the data were reanalyzed to control for initial preference, a high dose of prazosin accelerated extinction in animals with a high initial preference score (Bernardi and Lattal, 2012b). Perhaps these findings could be explained by regional expression patterns of α1ARs, as NE depletion in the prelimbic PFC sped up extinction of amphetamine CPP, whereas NE depletion in the infralimbic PFC slowed extinction (Latagliata et al., 2016). Yohimbine impaired the extinction of cocaine CPP, although this effect was not replicated with a selective α2AR antagonist and may be mediated by orexin rather than NE (Davis et al., 2008; Conrad et al., 2012).

Reinstatement

After extinction of a drug-environment association, place preference can be reinstated with a non-contingent drug prime. Cocaine-primed reinstatement of CPP was unchanged by administration of propranolol, prazosin, or clonidine just prior to the cocaine prime (Al Hasani et al., 2013; Mantsch et al., 2010), yet βAR antagonism during retrieval and extinction sessions prevented subsequent reinstatement (Fricks-Gleason and

Marshall, 2008; Otis and Mueller, 2011; Otis et al., 2014). The mechanisms by which these compounds alter memory to prevent future cocaine-primed reinstatement but fail to impact the acute priming effects of cocaine should be further investigated.

Stress exposure can reinstate stimulant CPP in an AR-dependent manner. Stress-induced reinstatement of CPP depended on β2AR activity, but was preserved following α1AR blockade (Mantsch et al., 2010; McReynolds et al., 2014). The effect of β2AR blockade was localized to interactions with CRF neurons in the BNST (McReynolds et al., 2014). Stress-induced reinstatement could also be blocked by a cannabinoid receptor (CB1) antagonist, and a subthreshold dose of the α2AR antagonist BRL-44408 reinstated cocaine CPP when combined with a CB1 agonist (Vaughn et al., 2012), indicating an interaction of the NE and cannabinoid systems in this paradigm. Moreover, β2AR agonists and α2AR antagonists produced reinstatement on their own (Mantsch et al., 2010; Vranjkovic et al., 2012). κ-opioid receptors were necessary for stress-induced reinstatement, and κ-agonist-induced reinstatement of cocaine CPP required κ expression in the noradrenergic locus coeruleus (LC) and was enhanced by clonidine, propranolol, or betaxolol, but not ICI-118,551, at doses that did not reinstate on their own (Al Hasani et al., 2013). These results indicate that diverse stress-inducing compounds act via noradrenergic mechanisms, specifically β2AR activation, to facilitate reinstatement of a CPP.

1.7.3 Anxiety

Using the elevated plus maze, mice injected with cocaine decreased the amount of time spent in the open arms. This anxiety-like behavior was blocked by propranolol, but

not prazosin or yohimbine, implicating βARs (Schank et al., 2008). Thus, the noradrenergic system may act as a brake to limit the intake of cocaine by inducing negative side effects and suggests a therapeutic avenue to decrease drug use.

Additionally, withdrawal from chronic cocaine induced anxiety-like behavior in the elevated plus maze and the defensive burying task, another preclinical measure of anxiety, that was attenuated by the β1AR antagonist betaxolol and the α2AR agonist guanfacine, respectively (Rudoy and Van Bockstaele, 2007; Buffalari et al., 2012). β1AR protein levels and corticotropin-releasing factor (CRF) transcription in the amygdala were increased during cocaine withdrawal and reduced after betaxolol administration, indicating that a β1AR-mediated change of CRF is important for this behavior (Rudoy and Van Bockstaele, 2007; Rudoy et al., 2009). Furthermore, betaxolol returned intracellular PKA catalytic subunit abundance following cocaine withdrawal to levels of drug-naïve animals and prevented cocaine-induced CREB phosphorylation (Rudoy et al., 2009). Similarly, propranolol blocked cocaine withdrawal-induced anxiety in rats and in the clinical setting with patients dealing with severe withdrawal symptoms (Harris and Aston-Jones, 1993; Kampman et al., 2001). Because drug-dependent individuals continue taking drugs to decrease the aversive effects of withdrawal, targeting the receptors responsible for this anxiety could have substantial therapeutic efficacy.

Additional support for a role of βARs in the negative effects of stimulants was observed using the runway model of self-administration. Combined administration of betaxolol and ICI-118,551 infused in the central amygdala or BNST decreased retreat behaviors (Wenzel et al., 2014). Further work to understand the aversive underpinnings

of psychostimulants could prove beneficial to overcome the reinforcing properties to decrease drug taking.

1.7.4 Drug Discrimination

The discriminative stimulus effects of psychostimulants are largely dependent on DA activity and only partially mediated by αARs, yet βARs have greater influence. Cocaine and amphetamine cross-generalized, and their effects could be abolished after administration of the DA D2 antagonist haloperidol (Schechter and Cook, 1975; McKenna and Ho, 1980). Regarding αARs and discrimination, a mixed bag of results has been reported with some studies indicating effects and others failing to find significance depending on the training drug and species used. The αAR antagonist phenoxybenzamine failed to alter the discriminative effects of cocaine or amphetamine (Schecter and Cook, 1975; McKenna and Ho, 1980). Similarly, it was recently reported that the nonselective αAR antagonist phentolamine had no effect on the ability of amphetamine or ephedrine to substitute for amphetamine in pigeons (Ercil and France, 2003). The α1AR antagonist dibenamine also did not change cocaine's discriminative stimulus effects in rats (Colpaert et al., 1976). Prazosin has been the most thoroughly examined αAR antagonist, yet lacks a clear, consistent role in the interoceptive effects of stimulants. It failed to profoundly shift the dose-response curves of cocaine (Kleven and Koek, 1997), methamphetamine (Munzar and Goldberg, 1999), or amphetamine (West et al., 1995) in rats, yet produced rightward shifts in the curves of cocaine in pigeons (Johanson and Barrett, 1993) and squirrel monkeys (Spealman, 1995; Rowlett et al., 2004), methamphetamine in pigeons (Sasaki et al., 1995), and amphetamine in mice (Snoddy and Tessel, 1985). No effect on

cocaine or methamphetamine discrimination was observed following α1AR agonist administration in rats or monkeys (Spealman, 1995; Kleven and Koek, 1997; Munzar and Goldberg, 1999). Because no consistent, cross-species effects of αAR signaling has been observed, it appears that activity at these receptors is largely unnecessary for the interoceptive effects of stimulants.

A similar hodgepodge of results has been reported with compounds targeting α2AR. Whereas the α2AR agonist UK 14304 failed to alter the discriminative stimulus effects of cocaine (Spealman, 1995; Kleven and Koek, 1997), clonidine partially substituted for methamphetamine, amphetamine, and cocaine in rats (D'Mello, 1982; Wood et al., 1985; Munzar and Goldberg, 1999) and cocaine in pigeons (Johanson and Barrett, 1993), but not cocaine in squirrel monkeys (Spealman, 1995). Oddly, when tested in combination with methamphetamine doses higher than the training dose, clonidine decreased drug-appropriate responding (Munzar and Goldberg, 1999). Cocaine discrimination was unaffected by yohimbine or efaroxan, another α2AR antagonist (Wood et al., 1985; Spealman, 1995; Kleven and Koek, 1997). Thus, the data on α2AR compounds are inconsistent and confusing. These differential effects could be explained, at least in part, by the specificity of these drugs at different doses. At low doses, α2AR agonists and antagonists preferentially interact with the α2AR inhibitory autoreceptor, while at higher doses, these drugs can also engage α2AR heteroceptors on target neurons, as well as α1ARs (Gobert et al., 1998).

Experiments using βAR drugs have revealed some interesting results. Some studies found no effect of propranolol on the discriminative stimulus effects of amphetamine (Schecter and Cook, 1975; Snoddy and Tessel, 1985; Ercil and France,

2003), methamphetamine (Munzar and Goldberg, 1999), or cocaine (Spealman, 1995). However, propranolol and cocaine partially substituted for each other (Colpaert et al., 1979; Young and Glennon, 2009). Furthermore, in a discrimination test between 2.5 mg/kg cocaine and 10 mg/kg cocaine, propranolol, tertatolol and the β2AR antagonist ICI-118,551, but not the peripherally limited βAR antagonist nadolol nor the β1AR antagonist betaxolol, enhanced the ability of the low dose of cocaine to engender responding on the 10 mg/kg cocaine-associated lever (Kleven and Koek, 1997). When pretreated with prazosin, the enhancing effect of propranolol was blocked (Kleven and Koek, 1998; Young and Glennon, 2009). These results suggest a role for central β2ARs in cocaine's discriminative stimulus effects, particularly when low doses of cocaine are used, that is modulated by α1AR activity.

In summary, the interoceptive effects of stimulants are, at best, modestly susceptible to alteration by AR agonists or antagonists. Specifically, the clearest evidence supports the ability of βAR antagonists to influence the discriminative stimulus effects of psychostimulants in an α1AR-dependent fashion.

1.7.5 Self-Administration

Maintenance

Most noradrenergic compounds have not shown reinforcing properties on their own (e.g. Risner and Jones, 1976), although some, such as βAR agonists/antagonists, have not been tested. The one exception is the α2AR agonist, clonidine, which was self-administered by rats (Davis and Smith, 1977; Shearman et al., 1981), macaques (Woolverton et al., 1982), and baboons (Weerts and Griffiths, 1999). Interestingly, rats

self-administered clonidine even at doses that resulted in toxicity and occasionally death (Davis and Smith, 1977). However, methadone-dependent patients did not self-administer clonidine (Preston et al., 1985). Therefore, it appears that, at least in this clinical setting, the abuse liability of noradrenergic compounds is inconsequential.

The primary reinforcing effects of stimulants appear largely independent of noradrenergic activity, especially as measured with intravenous (IV) self-administration, yet exceptions exist under a few notable conditions. Interestingly, NE depletion with the synthesis inhibitor nepicastat decreases the reinforcing efficacy of cocaine as measured by reduced breakpoint on a progressive ratio, but 1) nepicastat has no effect on fixed ratio self-administration and 2) the AR mediating this effect is not known (Schroeder et al., 2013). Across various schedules of reinforcement, the αAR antagonists phentolamine, phenoxybenzamine, and prazosin failed to alter cocaine self-administration in non-human primates (Wilson and Schuster, 1974; Woolverton, 1987; Howell and Byrd, 1991). A similar lack of effect was observed in dogs self-administering amphetamine (Risner and Jones, 1976) or cocaine (Risner and Jones, 1980). Infusion of prazosin directly into the PFC or ventral tegmental area (VTA) likewise did not alter cocaine intake in rats (Ecke et al., 2012). Given the technical difficulties of IV self-administration in mice, the importance of ARs has not been tested in this species, although genetic ablation of the α1bAR reduced cocaine consumption in an oral self-administration paradigm (Drouin et al., 2002). α2AR agonists had no effect on amphetamine (Yokel and Wise, 1978), heroin/cocaine “speedball” (Highfield et al., 2001), or cocaine (Wee et al., 2008) IV self-administration. In rhesus macaques, acute administration of the α2AR agonist lofexidine decreased responding maintained by cocaine and food, while chronic treatment enhanced

cocaine self-administration (Kohut, et al., 2013). βAR antagonists also failed to impact cocaine responding when assessed under long-access (6 h/session) parameters (Wee et al., 2008), but have been reported to decrease cocaine self-administration in 3-h sessions with rats (Harris et al., 1996) and in 100 min sessions in squirrel monkeys (Goldberg and Gonzalez, 1976). However, food-maintained responding also decreased following propranolol administration in rats, suggesting a non-specific suppression of operant behavior (Harris et al., 1996). A similar result was observed in a choice study in which rats were allowed to respond for either cocaine or food pellets. When pretreated with propranolol, food intake decreased, as did cocaine intake, and by contrast, terazosin pretreatment increased food pellet choice without altering cocaine self-administration (Perry, et al. 2015). Combined, these results indicate that stimulants maintain their reinforcing effects through mechanisms other than ARs. With the abundance of research identifying DA as the neurotransmitter mediating the primary reinforcing effects of stimulants, these data are not surprising.

Oral self-administration of amphetamine is vastly different than IV self-administration because rats develop an aversion to oral amphetamine and consume mostly water in a two bottle choice procedure. Propranolol, but not haloperidol, increased the intake of amphetamine, indicating that the aversive effects of oral amphetamine are mediated by βAR signaling, not DA (Kongyingyoes et al., 1988). Perhaps, this NE-dependent increase in the aversive effects of stimulants can be advantageous if used as a strategy to develop noradrenergic therapies that would counteract the euphoria experienced by drug abusers.

An important contribution of α1AR signaling emerged during various paradigms of escalated stimulant self-administration. For example, rats that underwent a cocaine pre-exposure regimen showed escalated cocaine self-administration that was abolished when prazosin was co-administered with cocaine during the sensitization phase (Zhang and Kosten, 2007). Furthermore, prazosin decreased breakpoint on a PR schedule of cocaine self-administration in rats under 6-h long-access conditions that typically showed escalated drug intake (Wee et al., 2008). α1AR abundance was decreased in the bed nucleus of the stria terminalis (BNST) by high levels of cocaine exposure (Wee et al., 2008), which the authors speculated occurred as a compensatory response to inflated NE overflow during prolonged self-administration and produced antagonist sensitivity. However, the behavioral consequences of intra-BNST prazosin infusions remain to be tested in this paradigm.

Thus, it appears that chronic drug exposure recruits α1AR-dependent pathways that are necessary for escalated drug taking in experimental animals. Because these escalation procedures are believed to more closely resemble addiction and binge drug taking in humans compared with short-access maintenance schedules, these effects of α1AR signaling may have clinical relevance. Only 2 human studies have examined the clinical utility of α1ARs in stimulant dependence, and the findings parallel the escalated intake effects in the rodent literature. For example, the α1AR antagonist doxazosin decreased self-report of “high”, “stimulating”, and “like cocaine” following cocaine (20 mg, IV; Newton et al., 2012) and increased cocaine-negative urines in treatment-seeking cocaine dependent people compared to placebo under some dosing regimens (Shorter et

al., 2013). These results indicate a need for further investigation of the promising therapeutic capabilities of α1AR antagonists.

Extinction

Only a few studies have examined the role of NE in the extinction of stimulant self-administration. Stemming from evidence that extinction of drug self-administration and fear conditioning requires activity in the infralimbic cortex, one study dissected the contribution of NE to the extinction of cocaine self-administration via microinfusions of GABAergic, glutamatergic, and noradrenergic compounds (LaLumiere et al., 2010). Silencing activity in the region with GABA agonists impaired extinction learning. Intra-infralimbic administration of the β2AR agonist clenbuterol immediately after extinction sessions enhanced the retention of extinction learning, while ICI-188,551 infused immediately prior to extinction sessions impaired extinction. In another study, repeated exposure to yohimbine during the first few extinction sessions slowed the rate of extinction (Kupferschmidt et al., 2009). This effect may be a result of increased LC activity during extinction tests as elevated c-fos expression has been seen following the beginning of extinction (Cason et al., 2016). Pretreatment with CRF1 receptor antagonism decreases drug seeking responding on the first day of extinction and LC c-fos levels indicating a relationship between stress, LC activity, and extinction responding. Clearly, further studies examining other AR subtypes are needed.

Reinstatement

The most profound effects of AR signaling manipulations occur during the reinstatement phase of stimulant self-administration, which is thought to reflect relapse-like drug-seeking behavior in humans. Stress, cues previously associated with the drug, or the non-contingent administration of the drug itself can trigger drug-seeking behaviors. Interestingly, NE alone has been shown to reinstate cocaine-seeking when administered i.c.v. (Brown et al., 2009). The authors attributed this phenomenon to a stress effect because it was associated with activation of neurons in the BNST and central amygdala that are part of the brain's stress pathway (Brown et al., 2011).

Yohimbine, an α2AR antagonist that increases NE release by blocking the primary noradrenergic inhibitory autoreceptor, is an anxiogenic drug that reinstates psychostimulant-seeking behavior in rats and monkeys (Lee et al., 2004; Shepard et al., 2004; Schroeder et al., 2013). However, it is unclear whether the effects of yohimbine occur through the noradrenergic system at all. In monkeys, the reinstating effects of yohimbine were blocked by clonidine and replicated with a selective α2AR antagonist (Lee et al., 2004), implicating the α2AR. Conversely, clonidine had no effect on yohimbine-primed reinstatement in rats (Brown et al., 2009), suggesting a contribution of non-adrenergic receptors, although the preferential α2aAR-selective agonist guanfacine did reduce yohimbine-primed reinstatement in this species (Buffalari et al., 2012).

NE signaling has a critical role in stress-induced, cue-induced, and drug-primed reinstatement in rats, but through distinct combinations of receptor subtypes. Either systemic administration of α2AR agonist (clonidine, lofexidine, or guanabenz) or β2AR antagonists infused directly into the ventral BNST or central amygdala prevented footshock stress induced reinstatement of cocaine seeking (Erb et al., 2000; Leri et al.,

2002, Vranjkovic et al., 2014), clonidine prevented reinstatement induced by κ-opioid pharmacological stressors (Valdez et al., 2007), prazosin attenuated cocaine-primed reinstatement (Zhang and Kosten, 2005), and a combination of prazosin and propranolol modestly reduced cue-induced reinstatement, but not either when administered alone (Smith and Aston-Jones, 2011; Dunbar and Taylor 2016). Clonidine also blocked stress-induced, but not cue-induced reinstatement of cocaine + heroin "speedball" in rats (Highfield et al., 2001). Thus, βARs mediate the effects of NE on stress-induced reinstatement, α1ARs are required for cocaine-primed reinstatement, and α1ARs and βARs contribute to cue induced-reinstatement but via redundant pathways. α2AR agonists appear to diminish multiple forms of reinstatement by decreasing overall NE release via inhibitory autoreceptor activation.

A somewhat different picture emerges for primates. For example, adrenergic compounds had no effect on cocaine-primed reinstatement in squirrel monkeys (Platt et al., 2007). However, reinstatement paradigms differ between rats and non-human primates in important ways that may affect interpretation of these results. For instance, non-human primate self-administration typically involves a second-order schedule of reinforcement in which cues associated with cocaine serve as conditioned reinforcers. During "cocaine-primed reinstatement", a combination of drug-prime and drug-associated cues drives cocaine-seeking behavior. Because prazosin, on its own, had no effect on cue-induced reinstatement in rodents, the lack of effect in the monkey cocaine + cue reinstatement paradigm is not entirely surprising. The only human studies germane to this subject found that clonidine and guanfacine decreased stress- or cue-induced craving

for cocaine (Jobes et al., 2011; Fox et al., 2012; 2014), however another lab could not replicate these results (Moran-Santa Maria et al., 2015).

1.8 Optogenetic/chemogenetic control of noradrenergic neurons

In recent years, manipulations to alter neuronal activity have become more targeted and sophisticated with the development of optogenetics and chemogenetics. Optogenetics allows the control of cellular activity via expression of light-sensitive ion channels (e.g. channelrhodopsin or halorhodopsin) and exposure to light pulses. Chemogenetics can be used to control neural firing through the expression of mutated G protein-coupled receptors that respond only to exogenously administered compounds that are otherwise physiologically inert.

The strength of optogenetics lies in its ability to temporally control activity at a millisecond timescale and the cell type specificity that can be achieved using selective promoters and targeted infusion of viral vectors bearing the light-sensitive proteins, in addition to local delivery of the light source. One approach to obtaining noradrenergic-specific expression uses a tyrosine hydroxylase promoter to drive Cre recombinase in catecholaminergic neurons and stereotaxic injections of Cre-dependent channelrhodopsin viruses. Following viral injection, fiber optic ferrules are surgically implanted just dorsal to the target cells and connected to either LEDs or lasers for light delivery to activate the channels. The selectivity of neuronal targeting and light delivery allows for anatomical dissection of circuits never before possible. With sufficient time, viral injection into the LC would express channels to all terminal regions the LC targets. By targeting the fiber optic ferrule to the mPFC, for example, only the projections in the cortex that originate in

the LC would be activated. Similar single tract activation can be achieved with injection of a retrograde virus into a target terminal region and fiber optic placement targeting the cell bodies. Optogenetic activation of the LC in mice influences sleep/wake cycles, causes behavioral arrest, produces stress responses, and increases anxiety-like behaviors (Carter et al., 2010; McCall et al., 2015). Optogenetic inhibition of the LC impaired cortex-dependent cognitive function in an attentional set-shifting task (Janitzky et al., 2015). Expression of channelrhodopsin in the LC of rats has also been achieved using this approach, but behavioral consequences were not tested (Witten et al., 2011). Finally, a new branch of optogenetics is emerging that uses chimeric rhodopsin/GPCR proteins to enhance control of neuronal excitability in ways that better mimic physiological signaling. For example, a light-sensitive βAR has been developed and used to induce anxiety-like behaviors when expressed in the BLA (Suida et al., 2015).

Chemogenetics provides selective control of neuronal activity, similar to optogenetics, but trades the millisecond temporal control for ease of administration. Expression of so-called designer receptors exclusively activated by designer drugs (DREADDs) has been achieved with a similar transgenic targeting approach (McCall et al., 2015) as well as a more targeted viral expression technique. Taking advantage of the specificity of the phox2b transcription factor in regulating noradrenergic neuron gene expression a promoter with eight copies of the phox regulatory sequence (PRSx8) packaged in a viral vector is used to drive selective noradrenergic expression of DREADDs (Hwang et al., 2001). To activate these receptors, injections of the synthetic ligand clozapine-n-oxide (CNO) can be systemically administered via intraperitoneal or subcutaneous routes or locally microinfused through cannulae. Activation of the LC with

DREADDs reduces time to emerge from anesthesia in rats and enhances memory in mice (Vazey et al., 2014; Fortress et al., 2015). Inhibitory Gi-coupled DREADDs have been used to silence the LC during restraint stress and block the subsequent stress-induced anxiety (McCall et al., 2015).

Optogenetics and chemogenetics provide the ability to control the activity of select populations of neurons, but each technique comes with its own limitations that need to be considered when designing experiments. Optogenetics requires a substantial amount of hardware (lasers, fiber optics, cannulae, etc.) which could impact behavioral results in a number of ways. Delivery of light to activate the channels can be technically challenging, especially in a small and deep nucleus like the LC. Also, depending on the behavior, fiber optic tethering to the light source could be restrictive (e.g. simultaneous optogenetics and IV self-administration). However, emerging technologies are beginning to address some of these technical challenges as wireless, implantable light sources are under development (McCall et al., 2013). As activation of DREADDs requires a mere systemic injection of CNO, chemogenetics avoids many of the hardware challenges of optogenetics. However, DREADDs lose the ability to precisely control the firing frequency of the neurons they target. This lack of temporal resolution requires careful consideration in regions like the LC where tonic and phasic firing rates encode different behavioral states.

With regards to stimulant behaviors, optogenetics and chemogenetics provide the opportunity to further elucidate the circuitry and activity driving drug-induced behaviors. For example, Mahler and Aston-Jones have used Gq DREADDs to determine that activation of DA neurons in the VTA induces reinstatement and cue-induced and

cocaine-primed reinstatement (Mahler, S., personal communication). The circuit was further probed with administration into projection regions of the mPFC, NAc, or BLA to reveal that activation of all three projections was able to enhance cued reinstatement, but only activation of DA terminals in the NAc was able to drive drug-seeking under extinction conditions. This approach could be used to determine the critical elements of adrenergic circuitry to drive reinstatement. Once the circuitry is established, optogenetics could be used to elucidate the firing frequencies of these neurons that lead to alterations in drug-related behaviors. More applications of optogenetics and chemogenetics to noradrenergic interactions with stimulants will be discussed in Chapters 3 and 4.

1.9 Summary

In summary, while all ARs appear to impact stimulant responses, the relative contributions of α1, α2, and βAR subtypes differ depending on the behavioral paradigm employed (Figure 1.2). With the addition of optogenetic and chemogenetic techniques, we will know the timing and anatomical networking of these neurons in addition to the receptors mediating influences of NE on stimulant-induced behaviors. The focus of this dissertation is to expand on the understanding of the interactions between NE and cocaine-induced behaviors, with a focus on cocaine-primed reinstatement. NE signaling is modulated via adrenergic receptor blockade, inhibition of NE synthesis with nepicastat and disulfiram, optogenetic stimulation of the LC with channelrhodopsin, and chemogenetic stimulation of the LC with DREADDs.

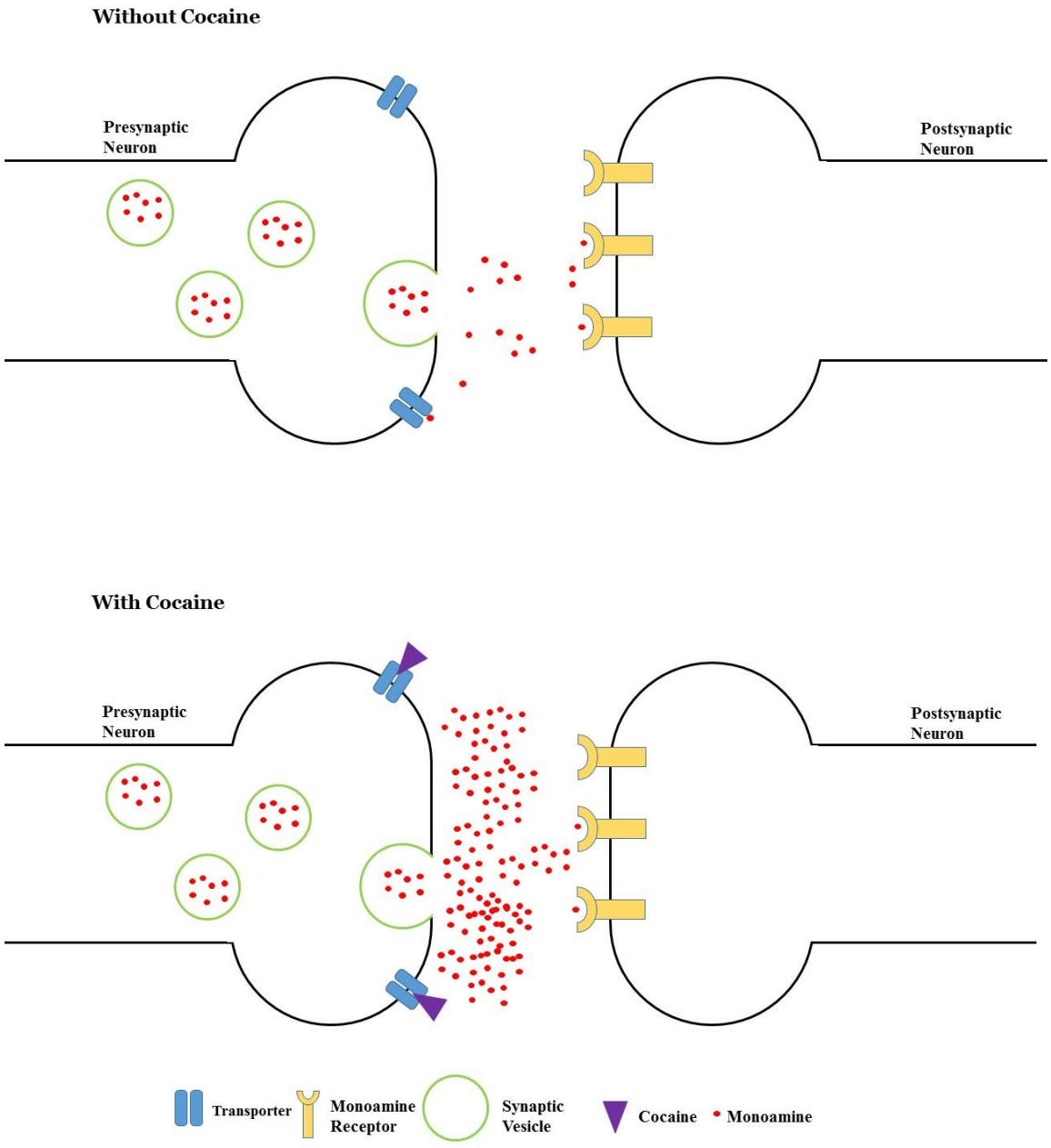

Figure 1.1. Cocaine effects at a monoamine synapse. Cocaine binds to monoamine transporters which prevents reuptake and thereby increases the amount of neurotransmitter in the synapse.

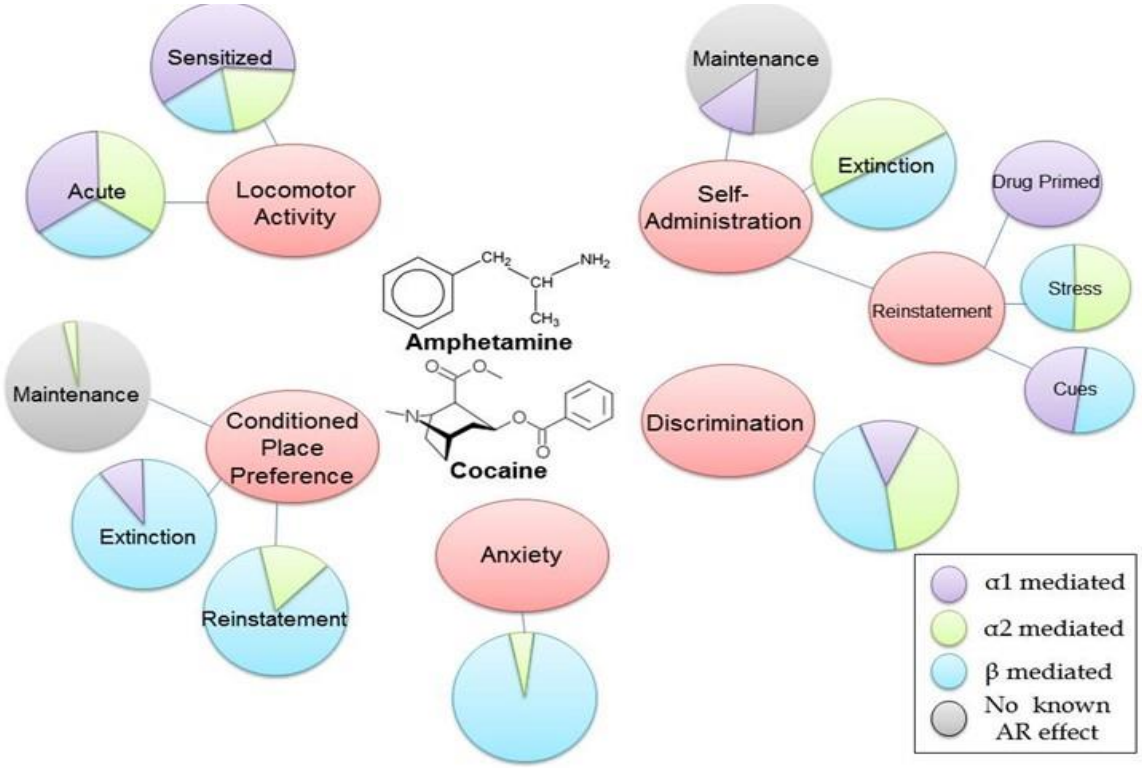

Figure 1.2. Relative contribution of adrenergic receptor subtypes to stimulant-induced behaviors. Stimulants, such as amphetamines and cocaine, produce behaviors including locomotor activity, conditioned place preference, drug-induced anxiety, drug discrimination, and self-administration. Various aspects of these behaviors are subject to control by adrenergic receptor (AR) signaling. Based on the available literature, it appears that each AR subtype exerts different relative effects on various stimulant-induced behaviors. Shown is a qualitative representation of the relative influences of α1 (lilac), α2 (lime), and β (cyan) AR subtypes to each stimulant-induced behavior, with greater influence represented by colored shading of a larger fraction of the pie graph. Gray shading indicates no known effect, which includes negative results as well as instances where the contribution of the receptor has never been tested.

CHAPTER II:

NOREPINEPHRINE REGULATES COCAINE-PRIMED REINSTATEMENT VIA α1-ADRENERGIC RECEPTORS IN THE MEDIAL PREFRONTAL CORTEX

2.1 Abstract

Drug-primed reinstatement of cocaine seeking in rats is thought to reflect relapse-like behavior and is mediated by the integration of signals from mesocorticolimbic dopaminergic projections and corticostriatal glutamatergic innervation. Cocaine-primed reinstatement can also be attenuated by systemic administration of dopamine β-hydroxylase (DBH) inhibitors, which prevent norepinephrine (NE) synthesis, or by α1-adrenergic receptor (α1AR) antagonists, indicating functional modulation by the noradrenergic system. In the present study, we sought to further discern the role of NE in cocaine-seeking behavior by: (1) determining whether α1AR activation can induce reinstatement on its own or is sufficient to permit cocaine-primed reinstatement in the absence of all other AR signaling, (2) identifying the neuroanatomical substrate within the mesocorticolimbic reward system harboring the critical α1ARs, and (3) assessing the neurochemical consequences of NE deficiency after cocaine administration. We found that, while intracerebroventricular infusion of the α1AR agonist phenylephrine did not induce reinstatement on its own, it did overcome the blockade of cocaine-primed reinstatement by the DBH inhibitor nepicastat. Furthermore, administration of the α1AR antagonist terazosin in the medial prefrontal cortex (mPFC), but not the ventral tegmental area (VTA) or nucleus accumbens (NAc) shell, attenuated cocaine-primed reinstatement. Finally, microdialysis experiments revealed that blockade of NE synthesis abolishes cocaine-induced increases in extracellular glutamate in the mPFC. Combined, these data indicate that α1AR activation in the mPFC is required for cocaine-primed reinstatement,

potentially via modulation of local glutamate release, and suggest that α1AR antagonists merit further investigation as pharmacotherapies for cocaine dependence.

2.2 Introduction

Cocaine abuse has persisted as a major public health concern within the United States for several decades. A prominent and problematic feature of addiction is the repeated occurrence of relapse episodes, even after prolonged periods of abstinence (O'Brien and Gardner, 2005), and there are no generally accepted or FDA-approved pharmacotherapies for cocaine dependence. The reinstatement model of relapse posits that non-contingent administration of the drug, cues previously associated with drug delivery, or stressful stimuli can induce the recurrence of extinguished drug-seeking behavior. This model has been useful for identifying neurotransmitters and neuroanatomical substrates involved in drug-seeking behavior that may represent targets for new addiction therapies.

Cocaine is a monoamine reuptake inhibitor that increases extracellular levels of dopamine (DA), serotonin, and NE. While DA is responsible for the primary reinforcing properties of cocaine, all efforts to develop DA-based medications for cocaine addiction have failed due to lack of efficacy, side effects, and/or abuse liability (Amato et al., 2011; Haile et al., 2012; Pierce et al., 2012; Shorter et al., 2011), suggesting that other systems might make a better therapeutic target. Compounds that block NE signaling, such as the NE synthesis inhibitors disulfiram and nepicastat, attenuate all three modalities of reinstatement in rats, although different adrenergic receptors (ARs) are involved in each type. Cocaine-primed reinstatement requires α1ARs, stress-induced reinstatement requires βARs, and both α1- and βARs play a role in cue-induced reinstatement (Devoto et al., 2016; Gaval-Cruz and Weinshenker, 2009; Leri et al. 2002; Schmidt and

Weinshenker, 2014; Schroeder et al., 2010, 2013; Smith and Aston-Jones, 2011; Weinshenker and Schroeder, 2007; Zhang and Kosten, 2005).

While the noradrenergic neural circuitry underlying stress-induced reinstatement has been identified (Leri et al., 2002; Vranjkovic et al., 2014), very little is known about how NE promotes drug-primed or cue-induced reinstatement. Previous work has implicated multiple nodes of the mesocorticolimbic reward circuit in cocaine-primed reinstatement; the NAc integrates coordinated DA signaling from the VTA and glutamate transmission from the mPFC to produce drug-seeking behavior (Anderson et al., 2008; McFarland and Kalivas, 2001). Each of these regions also receives noradrenergic innervation, contains α1ARs, and is influenced by α1AR signaling, making them ideal candidates for mediating the effects of NE transmission on cocaine-primed reinstatement.

Noradrenergic neurons in the locus coeruleus (LC), A1, and A2 brainstem nuclei innervate the VTA (Jones at al 1977; Mejias-Aponte et al., 2009; Simon et al 1979). We have shown that α1ARs are enriched on presynaptic GABAergic and glutamatergic elements in this brain region (Mitrano et al 2012; Rommelfanger et al 2009), which is consistent with electrophysiological studies showing that α1AR activation modulates GABA and glutamate release onto VTA DA neurons (Velasquez-Martinez et al 2012; 2015). α1AR agonists can also directly depolarize DA neurons and facilitate burst firing (Grenhoff and Svensson, 1989, 1993; Grenhoff et al 1993, 1995; Paladini et al 2001), and intra-VTA administration of α1AR antagonists decreases DA release in the NAc following cocaine administration (Goertz et al 2015). Therefore, it appears that α1ARs in the VTA fine-tune DA neuron activity via both direct and indirect mechanisms. The behavioral consequences of α1AR activation in the VTA are uncertain; we failed to

detect an effect of local α1AR antagonist administration on cocaine-induced locomotion (Mitrano et al., 2012).

The NAc is primarily innervated by the A2 noradrenergic cell group (Delfs et al., 1998), and α1ARs are enriched on presynaptic glutamatergic elements in this brain region, although they can also be found on GABAergic and dopaminergic terminals (Mitrano et al., 2012). Local blockade of α1ARs in the NAc attenuates cocaine-induced DA overflow and locomotor activity (Mitrano et al 2012; Sommermeyer et al 1995).

NE transmission in the mPFC originates from the LC (Florin-Lechner et al 1996; Morrison et al 1981; Swanson and Hartman 1975), and α1ARs are found mainly on glutamatergic elements. Selective lesions of NE terminals in the mPFC or local α1AR blockade prevent psychostimulant-induced locomotor activity, reward, and DA overflow (Auclair et al., 2002, 2004; Blanc et al 1994; Darracq et al 1998; Pan et al 2004; Ventura et al 2003; 2005; 2007). Intra-mPFC infusion of the α1AR antagonist terazosin has no effect on the maintenance phase of cocaine self-administration (Ecke et al., 2012), but the influence of this manipulation on reinstatement has not been tested.

To further define the role of α1ARs in cocaine-primed reinstatement, we first determined whether activation of α1ARs alone can trigger drug-seeking behavior, and whether α1AR signaling is permissive for cocaine-primed reinstatement in the absence of all other NE transmission. Next, we tested whether infusion of terazosin into the VTA, NAc shell, or mPFC could prevent cocaine-primed reinstatement. Finally, we examined the neurochemical consequences of cocaine administration following pharmacologic NE depletion.

2.3 Materials and Methods

Subjects

Adult male Sprague Dawley rats (Charles River; Wilmington MA) weighing 250-300 g prior to surgery were used. Rats were housed individually in a temperature-controlled environment on a reverse light/dark cycle with lights off at 8 am and lights on at 8 pm. Unless otherwise noted, all rats had *ad libitum* access to food and water. Rats were acclimated for 1 wk prior to experimentation. All procedures were conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals and approved by the Emory IACUC.

Operant Training

To facilitate operant behavior, animals used in self-administration studies were trained to lever press prior to surgery, as described previously (Schroeder et al 2010; 2013; Fuchs et al 2006). Briefly, rats were placed in rat operant conditioning chambers (Med Associates, St Albans, VT). Each chamber was housed within a sound-attenuating box and had a house light, two levers that extended during operant testing, and a light above each lever. Presses on the lever designated as active (right/left counterbalanced) were reinforced by a 45-mg food pellet (BioServ) on a FR1 schedule until 100 pellets were earned or 6 h elapsed. The criterion was 70% selectivity for active lever over inactive lever. Most rats met the criterion in 1 day, and all met the criterion within 3 days.

Jugular Catheter and Intracranial Cannula Surgery

Rats underwent surgery to implant jugular catheters and intracranial cannulae, as described previously (Schroeder et al 2010; 2013). Following isoflurane anesthesia and meloxicam (1 mg/kg, s.c.) analgesia, a catheter was inserted into the right jugular vein and threaded subcutaneously over the shoulder and exited via a mount placed between the shoulder blades. To maintain patency and prevent infections, catheters were flushed daily with 0.1 ml heparin solution (30 U/ml in saline) and 0.05 ml gentamicin (4 mg/ml). Immediately after catheter placement, rats were placed in a stereotaxic apparatus, and cannulae targeting the lateral ventricle, VTA, NAc shell, or mPFC were implanted. Coordinates in mm from bregma (Paxinos and Watson, 1998) were: lateral ventricle (unilateral) A/P -1.0; M/L -1.4; D/V -2.6; VTA (bilateral), A/P -5.8; M/L ± 0.7; D/V: -7.0; NAc shell (bilateral), A/P + 1.3; M/L ± 2.5; D/V -7.1, 10° angle; and dorsal mPFC (bilateral) A/P + 4.0; M/L ± 0.7 D/V -4.0. Animals were allowed 5-7 days to recover before behavioral testing.

Cocaine Self-Administration

Rats were allowed to self-administer cocaine on an FR1 schedule during 2-h sessions, as described previously (Schroeder et al, 2010; 2013). Each press on the active lever was reinforced by a cocaine infusion (0.5 mg/kg, i.v.), accompanied by illumination of a light above the active lever. For 20 s beginning with the infusion, the house light was extinguished to indicate a time-out period during which active lever presses were counted but did not lead to drug infusion. The maximum number of infusions per session was set at 40. Responses on the inactive lever were counted, but had no programmed consequences.

Rats were maintained on cocaine for at least 5 days with at least 20 infusions per session. Three consecutive days with less than 20% variability and at least 80% of response allocation on the active lever were required to progress to extinction. The average of these final three sessions was used to indicate the level of maintenance responding for each rat.

Extinction

During extinction, animals were run daily during 2-h sessions where responses on either lever had no programmed consequences. To meet extinction criteria, animals were required to respond at less than 30% of maintenance responding levels for 3 consecutive days.

Cocaine-Primed Reinstatement

Once extinction criteria were met, rats underwent cocaine-primed reinstatement sessions. For the nepicastat and phenylephrine experiments, rats were injected with nepicastat (50 mg/kg, i.p.) or vehicle 2 h prior to the test session, and then immediately prior to testing they were infused with phenylephrine (10 µg/0.5 µl, i.c.v.) or artificial cerebrospinal fluid (aCSF) and injected with cocaine (10 mg/kg, i.p.). Drug doses were chosen as follows. Cocaine (10 mg/kg) is a standard priming dose in the field that reliably reinstates cocaine seeking and is blocked by nepicastat (50 mg/kg), a dose that does not impair food seeking (Schroeder et al., 2010). Phenylephrine (10 µg) is in the range of behaviorally active doses when administered i.c.v. (Alsene et al., 2006; Stone et al., 2003) and does not have adverse interactions with cocaine (our unpublished data).

Treatments were administered in a counterbalanced order, and reinstatement sessions were separated by extinction sessions to ensure extinction criteria were reached prior to each test. Due to the occasional clogged cannula, lost head cap, etc., not all rats received all treatments, and data were analyzed as between-subjects instead of within-subjects because of these missing cells. For the α1AR antagonist experiments, rats were infused bilaterally with terazosin (3 µg/0.5 µl/side in the mPFC, VTA, or NAc shell) or aCSF and injected with cocaine (15 mg/kg, i.p.). Terazosin dose was chosen based on our previous studies showing that it can attenuate cocaine-induced locomotor activity when injected into the NAc shell (Mitrano et al., 2012). We selected a slightly higher dose of cocaine than in the phenylephrine experiment above because we have noted that cocaine-primed reinstatement is less effective when rats have implanted cannula and receive infusions in some brain regions, most notably the NAc (Fig. 2.2; our unpublished data). Each rat was subjected to a single treatment and reinstatement session. Following drug treatments, rats were placed in the testing chambers under extinction conditions. Active lever responses represented drug-seeking behavior, and data were analyzed in a between-subjects manner.

Food Self-Administration

Food self-administration occurred in the same operant conditioning chambers used for cocaine self-administration sessions. Responses on the active lever were reinforced on an FR1 schedule by 45-mg grain pellets. Responses on the inactive lever were counted, but had no programmed consequences. Sessions terminated with a

maximum number of 60 reinforcers or after 1 h. Rats undergoing food self-administration were restricted to 19 g of chow per day.

Food-Primed Extinction and Reinstatement

Criteria to progress through food maintenance and extinction were identical to those described for cocaine above. After meeting extinction criteria, rats were infused with terazosin 3 µg/0.5 µl/side in the mPFC) or aCSF, as described above. For food-primed reinstatement, sessions began with a non-contingent delivery of 3 grain pellets and continued with 1 pellet delivered non-contingently every min thereafter, as described (Schroeder et al., 2010). Responses on either lever had no programed consequences. Active lever responses represented food-seeking behavior, and data were analyzed in a between-subjects manner.

In Vivo Microdialysis

In vivo microdialysis was performed as described previously (Mitrano et al 2012; Vezina et al 2002; Kim et al 2005). Male Sprague Dawley rats (250-275 g on arrival) were housed individually in a 12 h light/dark reverse cycle room with food and water freely available. Following 5 days of acclimatization, rats were anesthetized with ketamine/xylazine (100 mg/kg, i.p. and 6 mg, i.p., respectively) and surgically prepared with a unilateral guide cannula aimed at the mPFC (A/P +4.0; M/L ±0.5; D/V -3.0 to -5.0 from skull). The D/V coordinates indicate the extent of the active portion of the microdialysis probe (Paxinos and Watson, 2005). Guide cannulae were angled at 8º to the vertical, and the interaural bar was set at +5.0.

Following a 7-10-day recovery period, rats were briefly anesthetized with isoflurane and a microdialysis probe (CMA Microdialysis) was lowered into the PFC. Rats were then placed in a microdialysis test box overnight with dialysate flow set at 0.3 µl/min. The following day (18-20 h later), the flow rate was increased to 1.5 µl/min, and the test session was initiated 1 h later, at which point samples were collected every 20 min. After 3 baseline samples were collected, rats were injected with saline or nepicastat (50 mg/kg, i.p.). After 6 more samples were collected, rats were injected with cocaine (10 mg/kg, i.p.), and then 6 final samples were collected. Neurotransmitter concentrations were corrected for the recovery capacity of the probes, which was determined for each probe at the end of the experiment. Because samples were collected for each rat at each time point, data were analyzed in a within-subjects manner. Rats were euthanized by perfusion with 1XPBS followed by 1.5% paraformaldehyde, brains were harvested, 40-µm coronal sections containing the PFC obtained, and these stained with cresyl violet for assessment of probe placement.

High-Performance Liquid Chromatography

HPLC was performed as described (Kim et al., 2005; Mitrano et al., 2012). Extracellular concentrations, corrected for individual probe recoveries, were estimated from peak areas using EZChrom Elite (Agilent Technologies, Santa Clara, CA) and Shimadzu CLASS-VP (Shimadzu Scientific Instruments, Columbia, MD) software.

Drugs

Phenylephrine and terazosin were purchased from Sigma-Aldrich (St. Louis, MO) and dissolved in aCSF. Nepicastat (SYN-117) was generously provided by Synosia Therapeutics (South San Francisco, CA) and injected as a suspension in sterile 0.9% saline containing 1.5% dimethyl sulfoxide (Sigma-Aldrich) and 1.5% Cremophor EL (Sigma-Aldrich). Cocaine HCl was generously provided by the National Institute on Drug Abuse Drug Supply Program (Bethesda, MD) and dissolved in sterile 0.9% saline.

Data Analysis

Data were analyzed by ANOVA, followed by Dunnett's or Sidak's post hoc tests, where appropriate, using Prism 6.0 for Macintosh.

2.4 Results

Central α1AR Stimulation Alone Does Not Induce Reinstatement, but Restores Cocaine-Primed Reinstatement to NE-Depleted Animals.

Blockade of α1ARs attenuates cocaine-primed reinstatement (Zhang and Kosten, 2005), but it is not known whether α1AR stimulation alone is sufficient to elicit cocaine-seeking behavior. Following cocaine self-administration and extinction, rats received phenylephrine (10 μg/0.5 μl, i.c.v.) just prior to a saline-primed reinstatement session. Active lever responding did not rise above extinction levels (Fig. 2.1). DBH inhibition, which reduces NE production, blocks signaling from all AR subtypes and prevents cocaine-primed reinstatement (Devoto et al., 2016; Schroeder et al., 2010, 2013). We next tested whether α1AR activation is sufficient to restore cocaine-primed reinstatement in the absence of all other AR signaling. Rats received vehicle or the DBH inhibitor

nepicastat (50 mg/kg, i.p.), followed 2 h later by aCSF or phenylephrine (10 μg/0.5 μl, i.c.v.). Rats were then given a priming injection of cocaine (10 mg/kg, i.p.) and placed in the operant conditioning chambers. As reported previously, cocaine elicited significant cocaine-seeking behavior in rats pretreated with vehicle, but not nepicastat. However, phenylephrine restored cocaine-primed reinstatement to nepicastat-treated animals (Fig. 2.1). A one-way ANOVA revealed a significant effect of treatment ($F_{5, 47}$=13.29, $p<0.0001$). Post hoc tests comparing each treatment combination to extinction levels showed significant reinstatement of active lever pressing for the vehicle + aCSF + cocaine ($q=5.79$, $p<0.0001$) and the nepicastat + phenylephrine + cocaine ($q=3.14$, $p<0.05$) groups, but not the nepicastat + aCSF + cocaine group ($q=0.07$, $p>0.05$). No significant differences in inactive lever responses were observed (data not shown). Combined, these data indicate that α1AR activation cannot elicit cocaine-seeking behavior on its own, but permits cocaine-primed reinstatement when endogenous NE transmission is quiescent.

α1AR Blockade in mPFC, but Not the VTA or NAc Shell, Attenuates Cocaine-Primed Reinstatement.

α1AR signaling is required for cocaine-primed reinstatement. To identify the underlying neuroanatomical substrates, aCSF or the α1AR antagonist was infused into the NAc shell, VTA, or mPFC just prior to a cocaine-primed (15 mg/kg, i.p.) reinstatement session following cocaine self-administration and extinction. We found that α1AR blockade in the mPFC (Fig. 2.2a), but not the VTA (Fig. 2.2b) or NAc shell (Fig. 2.2c), attenuated cocaine-primed reinstatement. Two-way repeated-measures ANOVAs

were run for each brain region tested. For the VTA, there was a main effect of phase ($F_{2,20}$=17.89, p<0.001) but not treatment ($F_{1,10}$=0.017, p>0.05) or phase x treatment interaction ($F_{2,20}$=0.006, p>0.05). For the NAc shell, there was a main effect of phase ($F_{2,20}$=8.648, p<0.02), but no treatment ($F_{1,10}$=0.5524, p>0.05) or phase x treatment interaction ($F_{2,20}$=0.3172, p>0.05). For the mPFC, there was a main effect of phase ($F_{2,18}$=12.13, p=0.0002), treatment ($F_{1,9}$=8.294, p=0.0182), and phase x treatment interaction ($F_{2,18}$=7.506, p=0.0043). Post hoc tests revealed a significant difference between the aCSF and terazosin groups on active lever presses during cocaine-primed reinstatement (t=4.82, p<0.001). No significant differences in inactive lever responses were observed (data not shown). These data indicate that α1ARs in the mPFC, but not the VTA or NAc shell, are required for cocaine-primed reinstatement.

α1AR Blockade in the mPFC Has No Effect on Food-Primed Reinstatement of Food Seeking.

To determine whether α1ARs in the mPFC modulate general reward seeking, rats were trained to self-administer food pellets. Following extinction, aCSF or terazosin was infused into the mPFC just prior to a food-primed reinstatement test. α1AR blockade had no effect on food-seeking behavior (Fig. 2.3). A two-way repeated measures ANOVA revealed a main effect of phase ($F_{2,14}$=20.37, p<0.001), but not treatment ($F_{1,7}$=4.366, p>0.05) or a phase x treatment interaction ($F_{2,14}$=0.6993, p>0.05). These results suggest that mPFC α1ARs are important for relapse-like behavior associated with drugs, but not natural rewards.

DBH Inhibition Prevents Cocaine-Induced Glutamate Overflow in the mPFC.

We and others have shown that DBH inhibition attenuates cocaine-primed reinstatement (Devoto et al., 2016; Schroeder et al, 2010; current study). Since DBH converts DA to NE, this treatment both decreases NE and increases DA in the mPFC, and some studies suggest that it is the supranormal DA transmission that prevents drug-seeking behavior (Devoto et al., 2012, 2014; 2016; Schroeder et al., 2010). However, our new results also implicate a reduction in NE-α1AR transmission. α1ARs are primarily localized on glutamatergic neuronal elements in the mPFC (Mitrano et al., 2012), and a combination of presynaptic and postsynaptic α1AR stimulation facilitates local glutamatergic transmission and depolarizes mPFC pyramidal neurons (Luo et al., 2014, 2015; Marek and Aghajanian, 1999). Since the activation of glutamatergic projections from the mPFC to the NAc is required for cocaine-primed reinstatement (Knackstedt and Kalivas, 2009; McFarland et al., 2003), we used *in vivo* microdialysis to test the hypothesis that DBH inhibition attenuates cocaine-induced glutamate overflow in the mPFC. Three 20-min baseline (B1-B3) microdialysis samples were collected prior to pretreatment with vehicle or the DBH inhibitor nepicastat (50 mg/kg, i.p.), followed by 6 more samples (N1-N6) and then a cocaine (10 mg/kg, i.p.) injection. Finally, 6 post-cocaine samples (C1-C6) were collected. We found that cocaine increased extracellular glutamate levels, and this effect was abolished by nepicastat (Fig. 2.4). A two-way repeated measures ANOVA showed main effects of time ($F_{14,140}$=2.94, $p<0.01$) and a time x treatment interaction ($F_{14,140}$=2.78, $p<0.01$). Post hoc tests revealed a significant increase in glutamate overflow from baseline (B3) following cocaine at several time

points in vehicle-treated rats (C1: $t=3.12$, $p<0.05$; C5: $t=3.78$, $p<0.01$; C6: $t=3.58$, $p<0.01$), but not nepicastat-treated animals.

2.5 Discussion

α1ARs, but not βARs, are known to be required for cocaine-primed reinstatement (Leri et al., 2002; Zhang and Kosten, 2005); what was not clear was whether stimulation of α1ARs would be sufficient for reinstatement. Furthermore, the neuroanatomical and neurochemical substrates governing α1AR control of cocaine-primed reinstatement had yet to be identified. The results of our study indicate that stimulation of α1ARs alone is not sufficient to induce reinstatement, but these receptors are permissive for cocaine-primed reinstatement in the absence of all other noradrenergic signaling. Moreover, α1AR activation is necessary in the mPFC, but not the VTA or NAc shell, for cocaine-primed reinstatement, and NE depletion prevents cocaine-induced glutamate overflow in the mPFC. In the following paragraphs, we will discuss each of these findings in greater detail.

Intracerebroventricular infusion of NE can trigger drug-seeking behavior in rats with a history of cocaine self-administration (Brown et al., 2009), although the receptor subtype mediating this effect was unknown. We found that i.c.v. infusion of the α1AR agonist phenylephrine, alone, failed to elicit cocaine seeking, suggesting that a different receptor was responsible for triggering NE-induced reinstatement. As mentioned in the Introduction, the ventral noradrenergic bundle, originating from the A1/A2 brainstem nuclei and projecting to the central nucleus of the amygdala and bed nucleus of the stria terminalis (BNST), is required for stress-induced reinstatement of cocaine seeking, (Leri

et al., 2002; Vranjkovic et al., 2014). This pathway mediates these effects via βARs; administration of βAR antagonists into the amygdala or BNST block footshock-induced reinstatement, while intra-BNST infusion of βAR agonists can provoke drug-seeking behavior in cocaine-experienced rats (Leri et al., 2002; Vranjkovic et al., 2014). Furthermore, i.c.v. administration of NE induces c-Fos expression in the amygdala and BNST (Brown et al., 2011). Thus, it seems likely that the ability of i.c.v. NE to promote reinstatement depends on βARs, not α1ARs, and engages stress circuitry rather than primary nodes of the mesocorticolimbic reward system directly.

For over a decade, we have known that activation of α1ARs is necessary for the full expression of cocaine-primed reinstatement (Zhang and Kosten, 2005). Although insufficient to induce reinstatement on its own, we found that α1AR stimulation restored cocaine-primed reinstatement to NE-depleted rats, further implicating this AR subtype. Thus, we next aimed to identify the brain region harboring the critical α1ARs. There were good reasons to suspect the 3 main nodes of the mesocorticolimbic reward system (VTA, NAc, and mPFC): all express α1ARs, and blockade of α1AR transmission in each of these regions attenuates several behavioral and/or neurochemical effects of cocaine (see Introduction). Our results clearly show that α1ARs in the mPFC, but not the VTA or NAc shell, are required for cocaine-primed reinstatement. Both positive and negative controls for the terazosin experiments support this conclusion. The same dose of terazosin in the NAc shell can block cocaine-induced locomotor activity (Mitrano et al., 2012), and local blockade of α1ARs in the mPFC had no effect on food-primed reinstatement of food seeking (Fig. 2.3), indicating specificity of mPFC α1ARs for drug-induced relapse-like behavior. We note that overall magnitude of responding during

reinstatement varied between experiments; cocaine-primed reinstatement was the lowest when aCSF was infused into the NAc, and highest when infusions were aimed at the mPFC. The reasons for these differences are not clear, but we have observed it before in other experiments (our unpublished data), and it is possible that NAc function is particularly sensitive to mechanical damage from the cannula combined with an endogenous neurotransmitter diluting effect of aCSF infusion.

Cocaine-primed reinstatement requires coordinated DA transmission from the VTA and glutamatergic transmission from mPFC projection neurons in the NAc (Knackstedt and Kalivas, 2009; McFarland et al., 2003). Because cocaine does not act on glutamate directly, a monoamine must mediate cocaine-induced activation of mPFC pyramidal neurons, and our results implicate NE-α1AR transmission. We have shown that α1ARs are expressed on both presynaptic and postsynaptic glutamatergic elements in the mPFC (Mitrano et al., 2012), and previous studies indicate that activation of these receptors increases local glutamate transmission and excitation of pyramidal neurons (Luo et al., 2014, 2015; Marek and Aghajanian, 1999). Our new microdialysis results demonstrate that cocaine-induced glutamate release in the mPFC is abolished by NE depletion. Thus, we propose that a cocaine prime blocks NE reuptake in the mPFC, which acts on presynaptic α1ARs to facilitate local glutamate release that, together with direct α1AR-mediated depolarization of pyramidal neurons, drives glutamate transmission in the NAc. The NAc then integrates the PFC-derived glutamate and VTA-derived DA signals to trigger cocaine-seeking behavior (Fig. 2.5). One limitation of our microdialysis experiment is that the rats were cocaine-naïve prior to testing. In future experiments, it will be important to confirm that these effects are maintained in cocaine-

experienced animals. In addition, direct electrophysiological evidence of α1AR-mediated mPFC pyramidal neuron excitation during reinstatement testing and accompanying changes in accumbal glutamate release are needed to confirm our model. Regardless, our data indicate an important link between NE signaling and glutamate release in the mPFC that is associated with cocaine-seeking behavior.

Devoto and colleagues have published a series of articles suggesting that increased DA transmission via the D1 receptor (D1R) in the mPFC from noradrenergic neurons, rather than decreased NE transmission, underlies the ability of DBH inhibitors to attenuate cocaine-primed reinstatement. Our findings that phenylephrine can restore cocaine-primed reinstatement to nepicastat-treated animals and that α1AR blockade in the mPFC prevents cocaine-primed reinstatement clearly implicate NE-α1AR transmission, as well. These two models are not necessarily mutually exclusive. We have shown that α1ARs and D1Rs colocalize on glutamatergic neuronal elements in the mPFC (Mitrano et al., 2014), and previous studies suggest that α1AR signaling restrains D1 signaling in mPFC pyramidal neurons (Trovero et al., 1994). Thus, supranormal D1 activation in the mPFC following DBH inhibition may result from a combination of increased DA release from noradrenergic terminals and decreased NE-α1AR transmission, thereby allowing D1 signaling to proceed unchecked. How excessive D1 activation might inhibit local cocaine-induced glutamate release and/or NAc-projecting pyramidal neuron excitability is not yet clear.

The present results have important clinical implications. A recent pilot study showed that the α1AR antagonist doxazosin decreased ratings of “like cocaine” and “likely to use cocaine if had access” in cocaine-dependent participants (Newton et al,

2012), and a larger follow-up study found that doxazosin significantly decreased cocaine use and increased the number of participants maintaining abstinence for at least two weeks (Shorter et al, 2013). α1AR blockade lacked serious adverse effects that might be present with direct glutamatergic or dopaminergic approaches (Parson et al, 2005). Combined with the preclinical data linking NE and α1ARs to cocaine responses, these studies led to several ongoing clinical trials testing α1AR antagonists for the treatment of cocaine dependence (NCT01953432, NCT01145183, NCT02538744, NCT01371851).

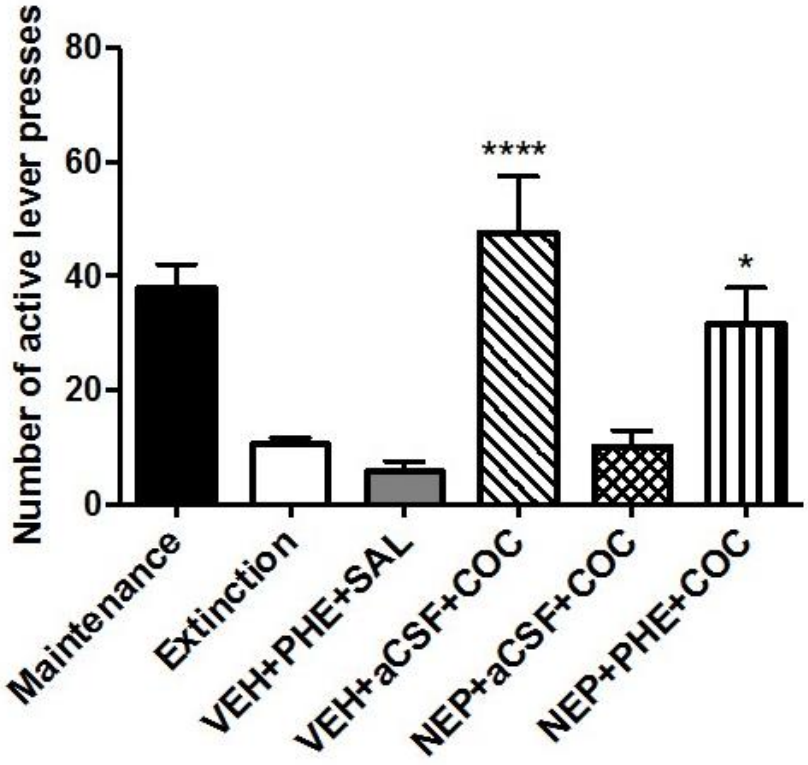

Figure 2.1. Stimulation of central α1ARs restores cocaine-primed reinstatement to NE-depleted rats. Once maintenance and extinction criteria for operant cocaine self-administration were met, rats (n=6-7 per group) were pretreated with vehicle (VEH) or nepicastat (NEP; 50 mg/kg, i.p.) 2 h prior to infusion of artificial CSF (aCSF) or the α1AR agonist phenylephrine (PHE; 10 µg/0.5 µl, i.c.v.). Rats were then primed with saline (SAL) or cocaine (COC; 10 mg/kg, i.p.), and a 2-h reinstatement session commenced. Shown are mean ± SEM active lever responses. Maintenance values reflect an average of the last 3 days of maintenance sessions, and extinction values reflect an average of the last 3 days of extinction. ****$p<0.0001$, *$p<0.05$ compared with extinction.

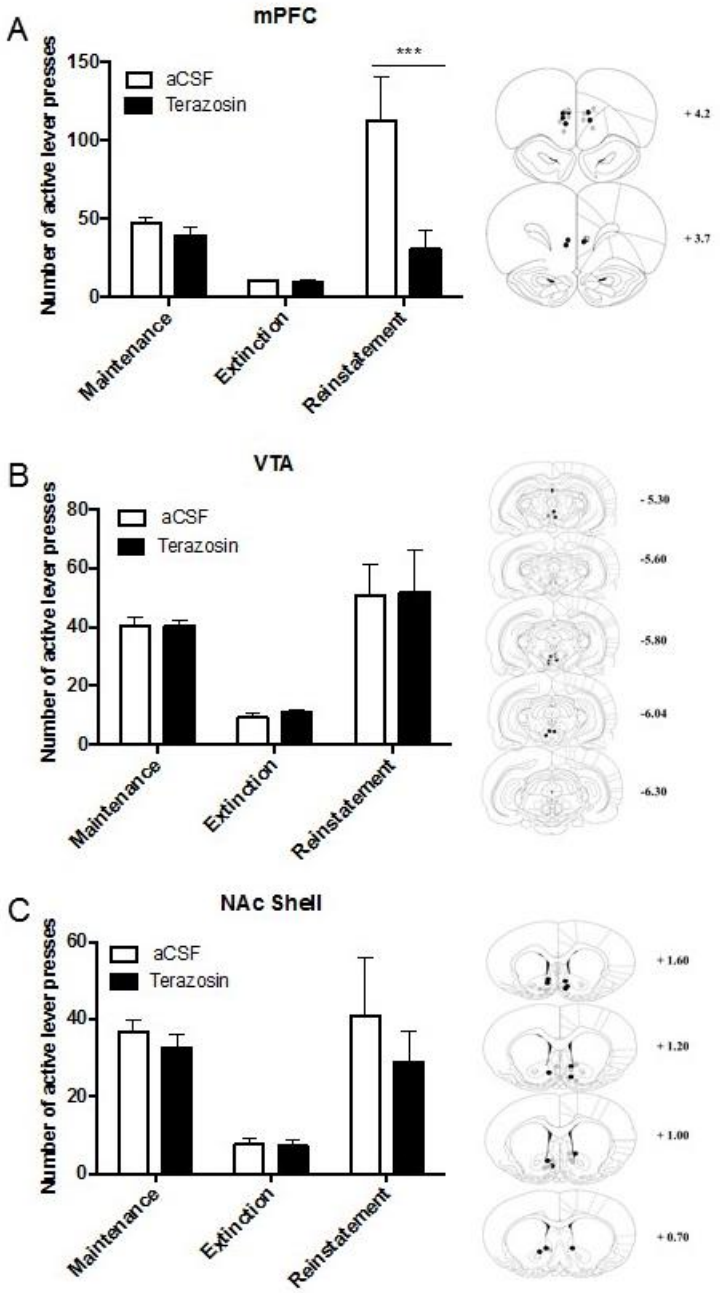

Figure 2.2. Blockade of α1ARs in the mPFC, but not the VTA or NAc shell, attenuates cocaine-primed reinstatement. Once maintenance and extinction criteria for operant cocaine self-administration were met, rats (n=5-7 per group) were infused with artificial CSF (aCSF) or the α1AR antagonist terazosin (3 µg/0.5 µl/side) into (**A**) the

medial prefrontal cortex, (**B**) the ventral tegmental area, or (**C**) the nucleus accumbens shell. Rats were then primed with cocaine (15 mg/kg, i.p.), and a 2-h reinstatement session commenced. Shown are mean ± SEM active lever responses, with probe placements to the right of each graph (aCSF, gray circles; terazosin, black circles). Maintenance values reflect an average of the last 3 days of maintenance sessions, and extinction values reflect an average of the last 3 days of extinction. ***$p<0.0001$ compared with aCSF.

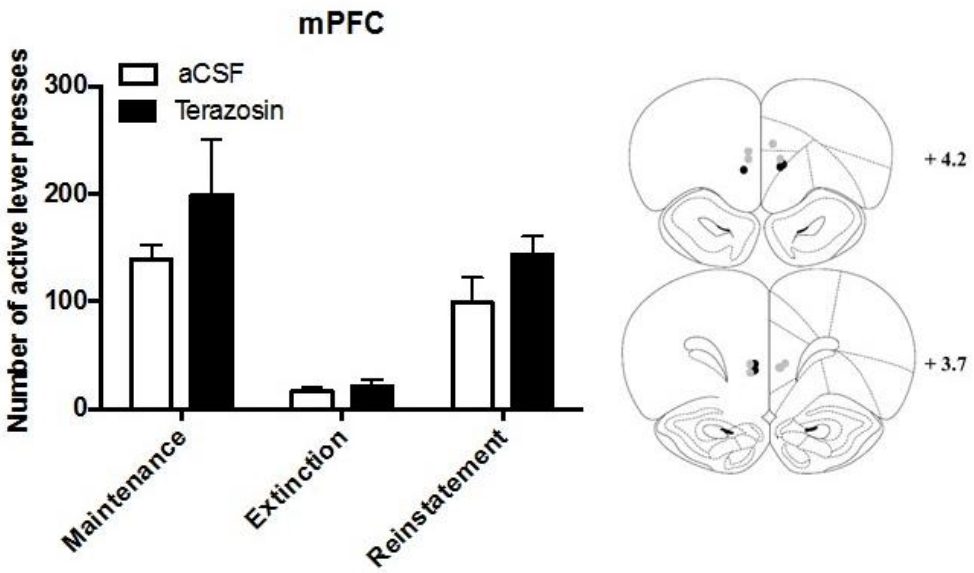

Figure 2.3. Blockade of α1ARs in the mPFC does not perturb food-primed reinstatement of food seeking. Once maintenance and extinction criteria for operant food self-administration were met, rats (n=5 per group) were infused with artificial CSF (aCSF) or the α1AR antagonist terazosin (3 μg/0.5 μl/side) into the medial prefrontal cortex, and a 2-h food-primed reinstatement session commenced. Shown are mean ± SEM active lever responses, with probe placements to the right (aCSF, gray circles; terazosin, black circles). Maintenance values reflect an average of the last 3 days of maintenance sessions, and extinction values reflect an average of the last 3 days of extinction. ***$p<0.0001$ compared with aCSF.

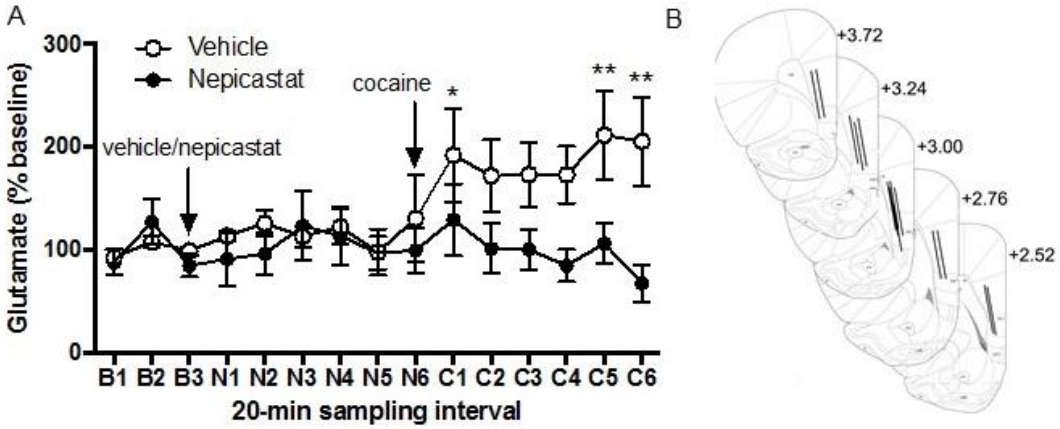

Figure 2.4. DBH inhibition attenuates cocaine-induced glutamate overflow in the mPFC. Following collection of three 20-min baseline microdialysis samples (B1-B3), rats were injected with vehicle or the DBH inhibitor nepicastat (50 mg/kg, i.p.). Six more 20-min samples were collected (N1-N6) prior to administration of cocaine (10 mg/kg, i.p.), and then six 20-min post-cocaine samples were collected (C1-C6). Shown are (**A**) mean ± SEM glutamate levels (% baseline) and (**B**) probe placements in the mPFC. *p<0.05 **p<0.01 compared to baseline (B3) for that time point.

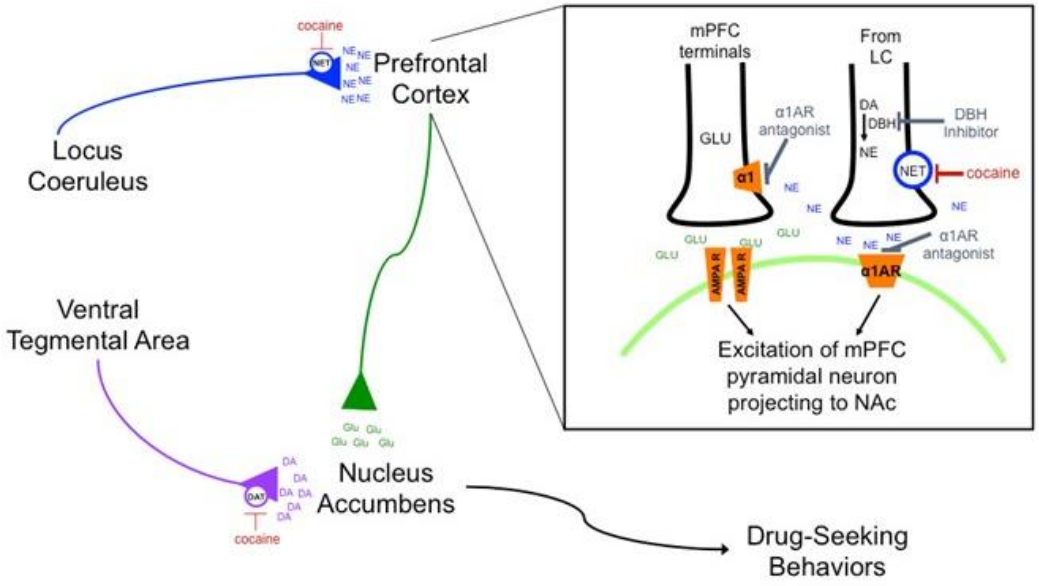

Figure 2.5. Hypothetical model for noradrenergic control of cocaine-primed reinstatement. Shown on the left is a wiring diagram summarizing the neural circuitry underlying cocaine-primed reinstatement. The nucleus accumbens integrates dopamine (DA) signaling from the ventral tegmental area and glutamate (GLU) signaling from the prefrontal cortex to drive drug-seeking behavior. In our model, cocaine activates the nucleus accumbens-projecting mPFC via norepinephrine (NE) derived from noradrenergic terminals originating from the locus coeruleus (LC). Shown on the left is a detailed picture of the mPFC environment. Cocaine increases extracellular NE by blocking its plasma membrane transporter (NET). The NE then activates mPFC glutamatergic neurons projecting to the NAc directly via postsynaptic α1ARs on these neurons, as well as indirectly via presynaptic α1ARs on mPFC glutamatergic elements that facilitate local glutamate release. Conditions of reduced NE transmission (DBH inhibition or α1AR blockade) prevent the activation of NAc-projecting pyramidal neurons and suppress cocaine-primed reinstatement.

CHAPTER III:

CONSEQUENCES OF CHEMOGENETIC LOCUS COERULEUS MODULATION DURING REINSTATEMENT OF COCAINE SEEKING

3.1 Abstract

It has been shown before that central administration of norepinephrine (NE) following cocaine self-administration and extinction training is sufficient to reinstate cocaine seeking, while blockade of NE synthesis prevents this relapse-like behavior. However, the source of the NE contributing to drug seeking has not been identified. The two most likely candidates are the locus coeruleus (LC), which supplies NE to most of the forebrain, and the A2 brainstem noradrenergic cluster in the nucleus tractus solitarius (NTS) that sends NE projections to the extended amygdala. In order to determine whether the critical NE transmission originates from LC neurons, we expressed excitatory (hM3Dq) or inhibitory (hM4Di) designer receptors exclusively activated by designer drugs (DREADDs) selectively in noradrenergic neurons in the LC of rats. We allowed animals to self-administer cocaine under two different paradigms: one that is typically used to assess stress-induced reinstatement, and one that is popular for examining cocaine-primed reinstatement. We then administered the ligand to activate the DREADDs alone or in combination with cocaine. Our preliminary results indicate that hM3Dq-mediated activation of LC neurons is sufficient to induce cocaine seeking under "stress reinstatement", but not "cocaine reinstatement" conditions, and fails to enhance subthreshold cocaine-priming doses in the latter. By contrast, inhibition of LC neurons appears to modestly reduce cocaine-primed reinstatement. These pilot results have important implications for LC-PFC interactions in cocaine-primed reinstatement and the role of the LC in stress-mediated drug seeking behaviors.

3.2 Introduction

Cocaine dependence remains a prevalent disorder with significant impacts on our economy, criminal justice system, and health care costs. Our society is slowly transitioning from thinking about and treating drug addiction as a weakness of will requiring incarceration towards an emphasis on the need to medically treat substance use disorder as a medical disease (*National Drug Control Strategy*, 2014). However, because there are no FDA-approved pharmacotherapies for cocaine dependence, treatment options are limited.

Because cocaine's primary reinforcing and euphorigenic effects result from its ability to increase dopamine (DA) neurotransmission, much research has been focused on developing drugs that target DA to treat cocaine dependence. These compounds have largely failed because they have either been ineffective or shown high abuse potential themselves (Mariani and Levin, 2012). However, studies using rodent models of cocaine abuse indicate that norepinephrine (NE) based approaches have potential for the prevention of relapse (Weinshenker and Schroeder, 2007; Schmidt and Weinshenker, 2014; Schroeder et al., 2010; 2013). In the "reinstatement" model of relapse, rats are trained to self-administer cocaine, undergo extinction training, and then cocaine seeking is induced under extinction conditions by the delivery of either a drug prime, a stressor, or cues previously associated with drug delivery. The ability of each of these stimuli to reinstate previously extinguished drug-maintained behaviors is sensitive to noradrenergic manipulations (Schroeder et al., 2010; 2013; Zhang and Kosten, 2005; Smith and Aston-Jones, 2011; Vranjkovic et al., 2014; Erb et al., 2000; Leri et al., 2002). Furthermore,

intracerebroventricular (ICV) administration of NE itself is sufficient to drive reinstatement in cocaine-experienced rats (Brown et al., 2011).

The noradrenergic system is comprised of clusters of NE-producing cells, primarily the pontine locus coeruleus (LC) and the medullary A1/A2 groups. The LC projects through the dorsal noradrenergic bundle and is the primary source of NE throughout the forebrain. The LC mediates aspects of learning, attention, and decision making. A1/A2 project through the ventral noradrenergic bundle to innervate the hypothalamus, midbrain, and extended amygdala. Activation of these cells occurs in response to stressors to initiate fight-or-flight responses.

There have been some assumptions made about the neural circuitry underlying NE's ability to drive cocaine seeking, but the definitive experiments have not been done. Because footshock-induced reinstatement can be blocked by β2 adrenergic receptor (AR) antagonism in the bed nucleus of the stria terminalis BNST or central amygdala, while intra-BNST administration of a β2AR agonist is sufficient to induce drug seeking (Erb et al., 2000; Leri et al., 2002; Vranjkovic et al., 2014), it is generally accepted that noradrenergic transmission from A1/A2 is important for the effects of stress in this model. While there is one published study using site-specific infusion of pharmacological agents and neurotoxins to show this is true for stress-induced heroin seeking (Shaham et al., 2000), it has never been tested for cocaine. By contrast, drug-primed reinstatement requires α1AR activity in the prefrontal cortex (see Chapter 2; Zhang and Kosten, 2005), which receives noradrenergic innervation primarily from the LC, but the importance of this nucleus has not been tested directly. There is essentially no information regarding the

source of the NE required for cue-induced reinstatement, but both α1ARs and βARs appear to be involved (Smith and Aston-Jones, 2011).

In order to determine the role of LC activity in cocaine reinstatement, we used LC-selective expression of designer receptors exclusively activated by designer drugs (DREADDs). DREADDs are modified muscarinic receptors that can no longer bind acetylcholine but instead respond to the physiologically inert ligand clozapine-N-oxide (CNO). When activated by CNO, DREADDs signal through G-coupled proteins to either activate, in the case of the Gq DREADD hM3Dq, or inactivate, in the case of the Gi DREADD hM4Di, the neurons that express them. We targeted DREADD expression to noradrenergic LC neurons using a viral approach driven by the PRSx8 promoter (Hwang et al., 2001; Vazey and Aston-Jones, 2014). We first expressed hM3Dq and determined whether increasing LC firing is sufficient to induce reinstatement alone or facilitate a low-dose cocaine prime. We used two different conditions to determine the role of LC activation alone on cocaine seeking: a "stress model" and a "cocaine-prime model." The stress model was designed to follow methods used in previous reports showing ICV NE or footshock causes cocaine-seeking (Brown et al., 2011; Erb et al., 2000; Leri et al., 2002). This paradigm utilizes Long Evans rats with extended cocaine training, an abstinence period in the homecage, and massed extinction sessions. The cocaine-prime model follows procedures frequently used by our lab and others in Sprague Dawley rats with limited cocaine access and once daily extinction sessions. Separate groups of rats were also tested in the latter procedure to determine whether LC activation would facilitate a low dose cocaine prime and whether hM4Di DREADD-induced LC silencing can attenuate cocaine-primed reinstatement.

3.3 Materials and Methods

Subjects

In experiment 1, we used adult, male Long Evans rats weighing 225-300 g at the time of surgery. In experiments 2 and 3, we used adult male Sprague Dawley rats weighing 225-300 g. All rats were purchased from Charles River (Willington MA), individually housed under reverse light/dark cycle (8am off/8pm on) conditions, and allowed one week following arrival to acclimate before testing. Rats had *ad libitum* access to food and water except during experimental sessions. All procedures were conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals and approved by the Emory University IACUC.

Surgery

Rats were implanted with indwelling jugular catheters and injected bilaterally with DREADD viral vectors in the LC. Rats were anesthetized with vaporized isoflurane anesthesia and administered meloxicam (1 mg/kg, s.c.) analgesia. A catheter was inserted into the right jugular vein and passed subcutaneously to exit the skin through a mount situated between the scapulas. Immediately following catheter placement, rats were placed in a stereotaxic apparatus and injections of 1.2 µL of AAV9-PRSx8-HA-hM3D (experiments 1 and 2; titer 1.12×10^{13}) or AAV9-PRSx8-HA-hM4D (experiment 3; titer 4.73×10^{12}) were targeted to the LC. Coordinates for injection were A/P -10.0; M/L -1.5; D/V -7.8 mm from bregma. Catheters were flushed with 0.1 ml heparin (30 U/ml in

saline) and 0.05 ml gentamicin (4 mg/ml) daily to maintain patency, and rats were allowed 1 week to recover from surgery before behavioral testing commenced.

Behavioral Testing Apparatus

Operant conditioning chambers (Med Associates, VT) were used. Sessions began with the illumination of a house light and extension of 2 retractable levers into the chamber. Cue lights were situated above each of the levers. One lever was designated the "active" lever, and responses on this lever were reinforced by cocaine delivery. For 20 s beginning with the activation of the infusion pump, the cue light above the active lever was illuminated and the houselight was extinguished. During this 20 s timeout period, responses on the active lever were recorded, but had no programmed consequences. Responses on the inactive lever were recorded, but had no programmed consequences.

Cocaine Self-Administration

Experiment 1: LC activation under stress-induced reinstatement conditions

Cocaine self-administration and reinstatement testing in experiment 1 were similar to those previously described for footshock-induced reinstatement (Brown et al., 2011). Rats were allowed to self-administer cocaine (0.5 mg/kg/infusion; 60 infusion maximum) during daily 3-h sessions on a fixed ratio 1 (FR1) schedule for 10 consecutive days. For the following week, rats were not tested. Rats then underwent extinction training during three 1-h sessions each day, with each session separated by 15 min. During these sessions, conditions were identical to self-administration except responses on the active lever no longer led to delivery of cocaine. Rats were extinguished for at

least three days and until the number of active lever presses were less than 25% of the average number of active lever presses during maintenance. The day following meeting extinction criteria, a 1-h session under extinction conditions was given to ensure low baseline levels of responding. Rats were then injected with CNO (0, 1, 3, or 10 mg/kg, i.p.) and returned to the chambers 20 min later for a 2-h reinstatement session under extinction conditions. Subsequent reinstatement tests were separated by at least one day of extinction testing, and animals received treatments in a counterbalanced manner. One animal was not tested at either the 3 or 10 mg/kg dose and another animal was not tested at the 10 mg/kg dose; all other animals received all treatments.

Experiment 2: LC activation under cocaine-primed reinstatement conditions

In experiment 2, cocaine self-administration and reinstatement testing were similar to cocaine-primed reinstatement methods (Stefanik et al., 2013). Rats were allowed to self-administer cocaine (0.5 mg/kg/infusion; 40 infusion maximum) during daily 2-h sessions on a FR1 schedule for 10 consecutive days. On days 11-24, rats were placed in extinction sessions during which all conditions were identical to self-administration sessions, except active lever responses no longer resulted in delivery of cocaine or activation of cues. Following extinction, rats were injected with CNO-primed (0, 1, 3, and 10 mg/kg, i.p.) 30 min prior to a 2-h reinstatement session under extinction conditions. The reinstatement sessions occurred twice per week in a counterbalanced manner, with tests separated by at least 2 days of extinction.

Following testing at each dose of CNO alone, rats were then tested with CNO (0, 1, or 10 mg/kg, i.p.) in combination with a low dose cocaine prime (5 mg/kg, i.p.). These

reinstatement tests were also separated by at least 2 days of extinction. On reinstatement days, rats were subcutaneously injected with CNO or vehicle followed 30 min later by 5 mg/kg cocaine, and then immediately placed in the testing chambers for the start of the session. Sessions lasted 2 h.

Experiment 3: LC inhibition during cocaine-primed reinstatement

Cocaine self-administration sessions were identical to those described in experiment 2, but criteria for maintenance and extinction were similar to those described previously (Schroeder et al., 2010). Daily maintenance sessions continued for at least 5 days with stable responding for 3 consecutive days (<20% variability and allocation of responses with >75% on the active lever). The day after meeting stabilization criteria, rats underwent extinction sessions during which all conditions were identical to self-administration sessions except active lever presses no longer resulted in delivery of cocaine or presentation of cues. Daily 2-h extinction sessions continued until active lever presses over 3 consecutive days were <30% of the average of the last 3 days of maintenance responding. After meeting extinction criteria, rats were injected with CNO (0 or 10 mg/kg, i.p.) 30 min prior to cocaine (15 mg/kg, i.p.) and placement in the chambers for a 2-h reinstatement session under extinction conditions.

Drugs

Cocaine hydrochloride and CNO were provided by the National Institute on Drug Abuse Drug Supply Program (Besthesda, MD). Cocaine was dissolved in sterile 0.9%

saline. CNO was dissolved in a vehicle of 2.5% DMSO, 2.5% Cremophor EL, and 95% sterile 0.9% saline.

Data Analysis

Data were analyzed by ANOVA followed by Sidak's post hoc tests, where appropriate, using Prism 6.0 for Windows.

3.4 Results

PRSx8 viral vector expresses hM3Dq selectively in noradrenergic neurons in the LC

As previously reported (Vazey and Aston-Jones, 2014), the PRSx8 viral vector system selectively expresses DREADDs in the LC (Figure 3.1). Unfortunately, at this time we are unable to visualize PRSx8-hM4Di expression because we and others have noticed an inability for the HA in this construct to be detected by immunohistochemistry (our unpublished data; E. Vazey, personal communication).

Experiment 1. LC activation under stress-induced reinstatement conditions increases cocaine seeking

ICV administration of NE or intra-BNST administration of a β2AR agonist is sufficient to drive reinstatement of cocaine seeking (Brown et al., 2011; Vranjkovic et al., 2014). The purpose of this experiment was to determine whether specific activation of the LC can also elicit relapse-like behavior. Following cocaine self-administration and extinction, rats expressing hM3Dq DREADDs exclusively in noradrenergic LC neurons received CNO (1, 3, or 10 mg/kg, i.p.) or vehicle 30 min prior to a reinstatement session.

Active lever presses trended towards a dose-dependent increase in responding (Figure 3.2). A one-way ANOVA revealed significant effect of treatment, ($F_{8, 30} = 3.152$, $p=0.01$), but post hoc tests comparing each reinstatement test to vehicle reinstatement and each reinstatement test to the extinction preceding it revealed no significant differences.

Experiment 2. LC activation under cocaine-primed reinstatement conditions fails to induce drug seeking alone or facilitate the effects of cocaine

Although it has been shown that NE transmission is necessary for cocaine-primed reinstatement (Schroeder et al, 2010, 2013; Zhang and Kosten, 2005), it is not known whether activation of the LC is sufficient to drive cocaine seeking alone or enhance the effects of cocaine. Following cocaine self-administration and extinction, rats expressing hM3Dq DREADDs exclusively in noradrenergic LC neurons received CNO (1, 3, or 10 mg/kg, i.p.) or vehicle 30 min prior to a reinstatement session. Active lever presses tended to be lower than extinction responding following vehicle and each dose of CNO (Figure 3.3). A one-way ANOVA revealed a significant main effect of treatment, ($F_{2.025, 24.30} = 18.22$, $p<0.0001$). Post hoc tests comparing reinstatement test responding to the average of the two days of extinction preceding it revealed no significant differences. CNO also failed to change active lever presses following administration of a sub-threshold dose of cocaine (5 mg/kg, i.p.) (Figure 3.4). A one-way ANOVA revealed a significant main effect of treatment, ($F_{2.190, 21.90} = 11.54$, $p=0.0003$), but post hoc tests comparing reinstatement test responding to the average of the two days of extinction preceding it revealed no significant differences. These data indicate that under cocaine-

primed reinstatement conditions, LC activation is unable to drive cocaine seeking or facilitate responding following a low dose cocaine prime.

Experiment 3: LC inhibition partially attenuates cocaine-primed reinstatement

Inhibition of NE synthesis prevents cocaine-primed reinstatement (Schroeder et al., 2010; 2013), but the source of the critical NE has not been identified. To determine whether inhibiting LC activity impairs cocaine-primed reinstatement, rats expressing the inhibitory hM4Di DREADD in the LC were injected with CNO (10 mg/kg, i.p.) or vehicle 30 min prior to a cocaine-primed (15 mg/kg, i.p.) reinstatement session (Figure 3.5). A one-way ANOVA revealed a significant main effect of treatment, ($F_{4, 33}$= 5.687, p=0.001). Post hoc analysis comparing reinstatement responding to the average of the two days of extinction preceding it revealed a significant difference between vehicle extinction and vehicle + 15 mg/kg cocaine reinstatement, but not CNO extinction and CNO + 15 mg/kg cocaine reinstatement. However, there was no significant differences between Veh + cocaine reinstatement and CNO + cocaine reinstatement. These results indicate a subtle effect of LC inhibition on reducing cocaine-primed reinstatement.

3.5 Discussion

In these preliminary data, we have shown that selective activation of the LC induces reinstatement of cocaine seeking under some, but not all, experimental conditions, and fails to enhance the effects of a subthreshold cocaine prime. Furthermore, inhibition of the LC prior to a cocaine prime modestly reduces drug-seeking behavior.

These data warrant further investigations into noradrenergic circuitry and its role in the relapse like behavior.

It is important to recognize that these preliminary studies have some limitations that must be taken into considerations when interpreting the results. First, the number of animals used in the "stress reinstatement" paradigm needs to be increased to have sufficient statistical power. Also, our inability to confirm DREADD expression in animals injected with hM4Di impacts the voracity of the results. Multiple labs using this viral vector have been working to determine another way to identify whether animals are indeed expressing DREADD, while also reexamining the vector map to determine why the HA tag is not currently being detected by immunochemistry.

Previous studies have shown that NE is sufficient to reinstate cocaine-seeking behaviors likely via activation of βARs in the BNST (Brown et al., 2011; Vranjkovic et al., 2014). We modeled our "stress reinstatement" parameters after these studies to determine whether LC activity could replicate the results found with ICV NE and local administration of βAR agonists in the BNST. Under these conditions, we found a trend towards CNO-dependent increases in drug-seeking behavior at a magnitude similar to what was reported following ICV NE (Brown et al., 2011). Although the dogma in the field is that the BNST receives noradrenergic innervation primarily from A2 (Delfs at al., 2000), and stress-induced reinstatement occurs through the ventral noradrenergic bundle originating in A1/A2 cell groups and not the LC (Brown et al., 2011; Shaham et al., 2000; Wang et al., 2001). NE release has been detected in the BNST following stimulation of the dorsal noradrenergic bundle, which originates from the LC (Park et al., 2009). To determine whether a LC-BNST projection mediates the effects we report here, further

studies locally infusing CNO into the BNST following hM3Dq expression in the LC should be performed. If these local infusion studies replicate our findings, models of stress-induced reinstatement would need to be modified to incorporate the LC.

The role of A2 activation in reinstatement of cocaine seeking has not been fully elucidated. The studies showing A2 transmission via the ventral noradrenergic bundle to the BNST affected reinstatement examined opioid, not stimulant seeking (Delfs et al., 2000; Shaham et al., 2000). To test the role of A2 in cocaine seeking, similar experiments to those performed here should be conducted, except with DREADD expression in A2 rather than the LC. Although there are some reports that phox2b in the brainstem is in glutamatergic neurons (Geerling et al., 2008; Stornetta et al., 2006), the PRSx8 lentiviral approach has achieved selective noradrenergic expression in this brain region (Duale et al., 2007). TH:Cre transgenic rats might also be an option, but the nucleus of the solitary tract that includes A2 does contain a small number of dopamine neurons that could express the DREADD and confound the results (Maley, 1996). Following hM3Dq expression in A2, rats in the stress paradigm could be injected systemically and/or locally in the BNST. Silencing these neurons via hM4Di activation prior to footshock-stress would further confirm whether these neurons are necessary for stress-induced cocaine seeking.

We have shown that under the experimental conditions optimized for cocaine-primed reinstatement, ICV administration of an α1AR agonist failed to induce drug-seeking behaviors (Chapter 2). Here we extend those data by showing that under these same conditions, activation of the LC is insufficient to induce reinstatement alone or facilitate a subthreshold cocaine prime. By contrast, either systemic or intra-PFC

blockade of α1ARs reduces cocaine-primed reinstatement (Zhang and Kosten, 2005; Chapter 2). When we suppressed LC activity with an inhibitory DREADD prior to cocaine prime, we also observed a reduction in drug seeking, albeit modest. We predict that the ability of hM4Di-mediated LC inhibition to attenuate cocaine-primed reinstatement is due to a downstream decrease in α1AR activation in the PFC. Again, further tests locally administering CNO in the PFC following hM4Di expression in LC neurons will be required to test this hypothesis.

Interestingly, using the "stress reinstatement", but not the "cocaine-primed reinstatement", parameters we were able to see an effect of LC activation. The strain of rat is likely the simplest explanation; Long-Evans rats used in the stress model are more aggressive and perhaps more sensitive to stressors than Sprague-Dawley rats (Faraday, 2002; Blanchard et al., 1984). Another potential explanation is differences in the total amount of cocaine intake; rats in the stress model are allowed more infusions per day and usually self-administer for more days than the "cocaine-prime" animals. Finally, the week of undisturbed abstinence between the maintenance and extinction sessions in the "stress" group could allow for an incubation of craving effect (Grimm et al., 2001; Pickens et al 2011), in which drug seeking occurs at higher magnitudes following prolonged abstinence from drug intake. We have found previously that footshock stress fails to reinstate cocaine seeking without each of these methodological alterations (unpublished observations). Because these two approaches are quite distinct and optimized for drug seeking responses to different types of stimuli (drug vs stress), we chose to test LC activation using both. Our results suggest that the increase of NE

transmission elicited by stress, but not cocaine, is sufficient by itself to drive drug-seeking behavior.

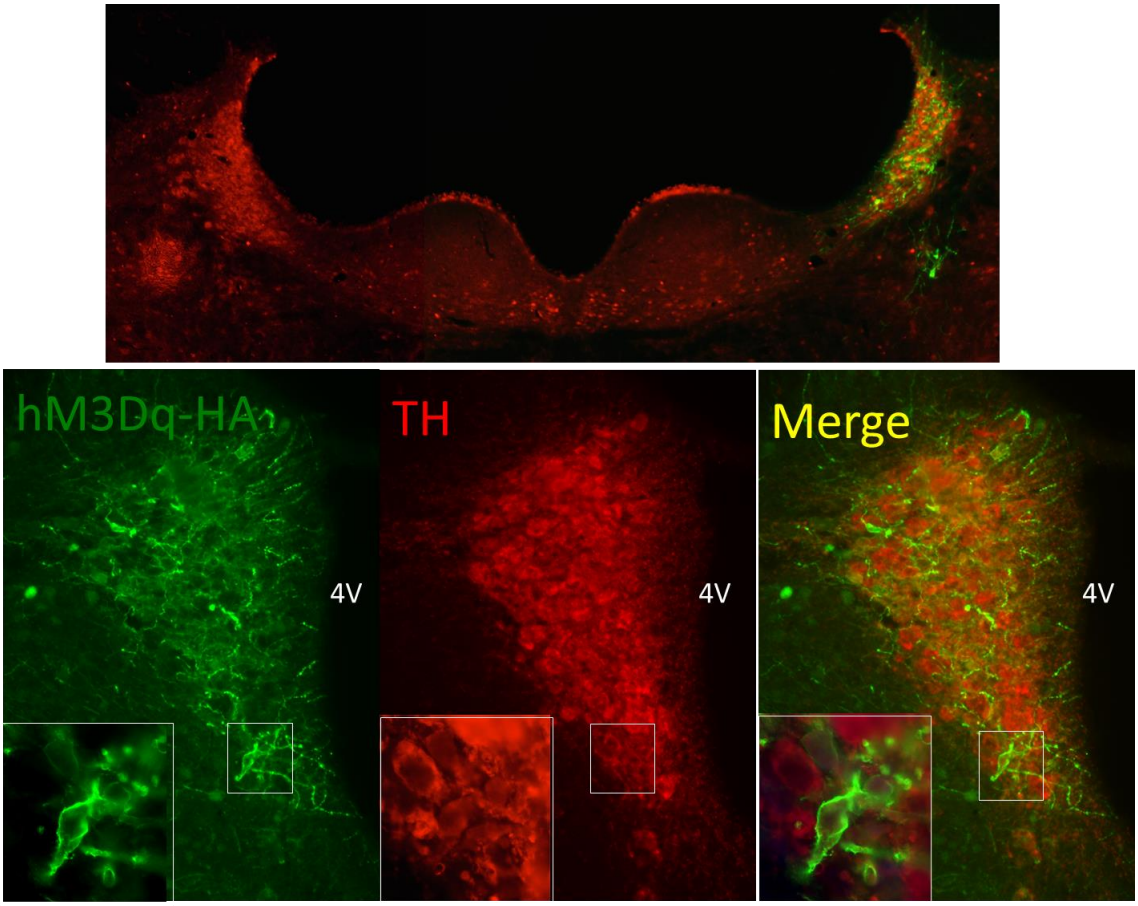

Figure 3.1. Selective hM3Dq expression in the locus coeruleus. Rats were unilaterally injected with PRSx8-hM3Dq AAV targeting the locus coeruleus (LC). Immunohistochemistry was performed to label DREADD expression via HA-tag (green) and noradrenergic neurons via tyrosine hydroxylase staining (red). Merge shows colocalization of the HA and TH stains. Top row shows bilateral LC with unilateral DREADD expression at 5x magnification. Bottom row images are shown at 10x with inset (bottom left) showing 20x magnification of selected area within the white box.

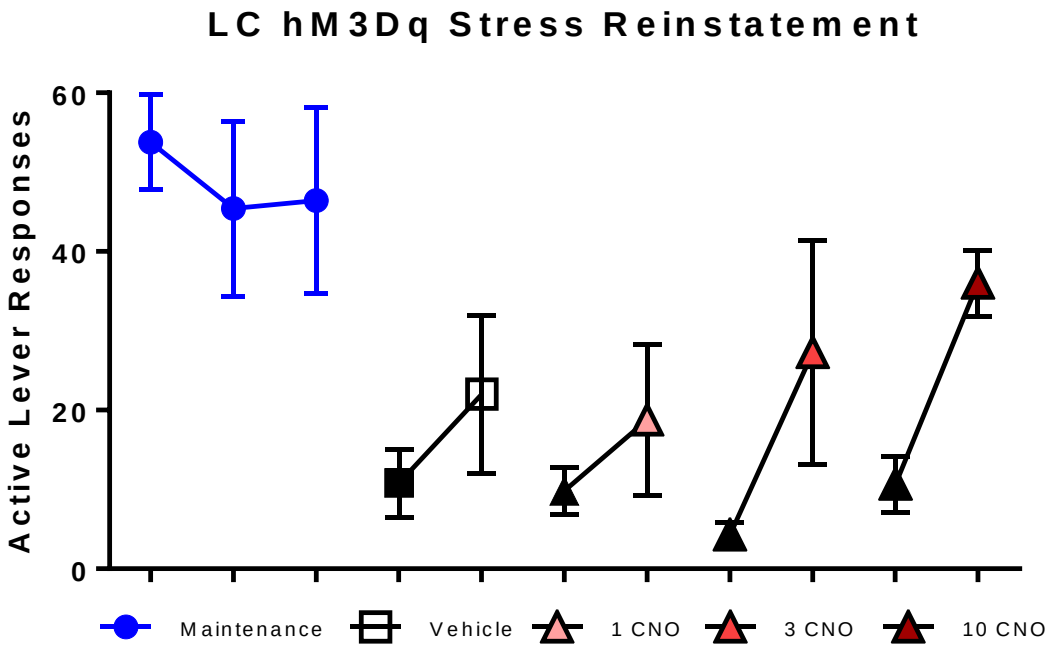

Figure 3.2. Trend for hM3Dq induced activation of the locus coeruleus to increase reinstatement responding when tested with a stress-induced reinstatement paradigm. Mean ± SEM active lever responding during the final three maintenance sessions (blue circles), extinction sessions prior to reinstatement tests (black symbols), vehicle reinstatement (white square), or increasing doses of CNO (triangles). n = 3-5 per group.

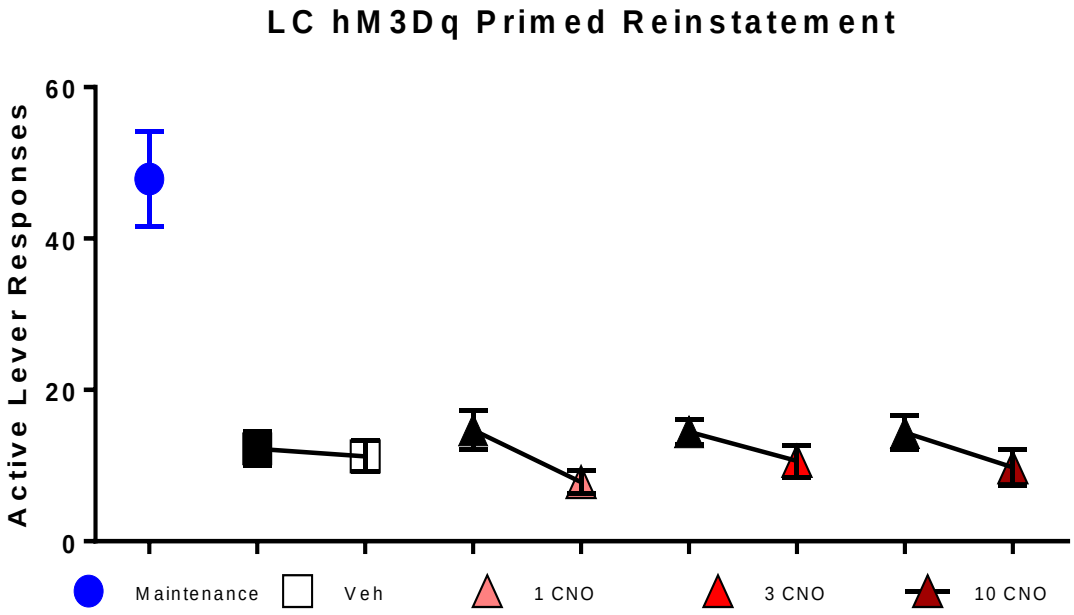

Figure 3.3. Locus coeruleus activation fails to reinstate cocaine seeking behavior when tested with a drug-primed reinstatement paradigm. Mean ± SEM active lever responding of stabilized maintenance sessions (blue circle), extinction sessions prior to reinstatement tests (black symbols), vehicle reinstatement (white square), or increasing doses of CNO to activate hM3Dq DREADDs expressed on locus coeruleus neurons (triangles). n = 13.

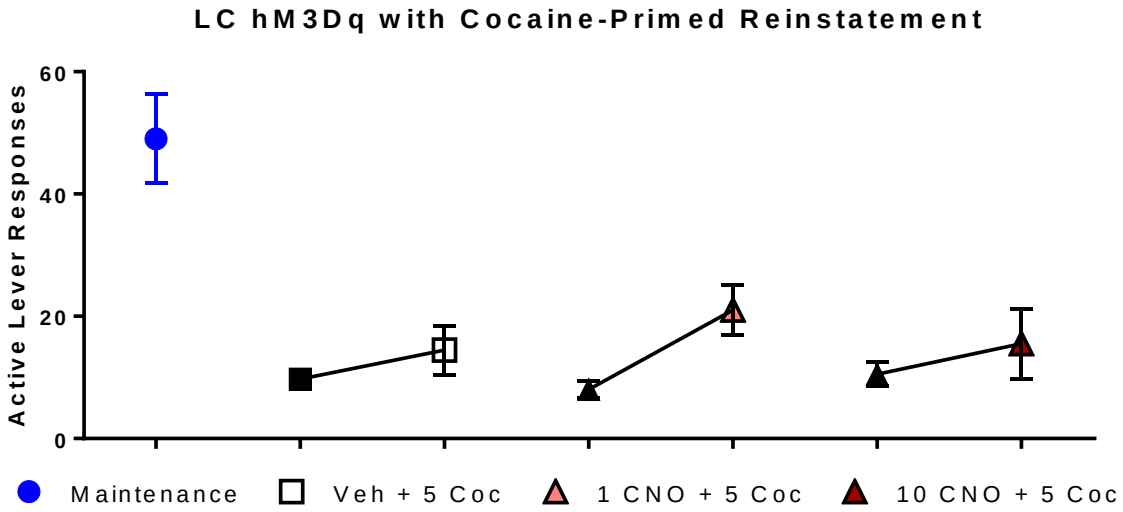

Figure 3.4. Locus coeruleus activation fails to enhance reinstatement by a low-dose cocaine prime. Mean ± SEM active lever responding of stabilized maintenance sessions (blue circle), extinction sessions prior to reinstatement tests (black symbols), vehicle and 5 mg/kg cocaine reinstatement (white square), or increasing doses of CNO to activation hM3Dq DREADDs expressed on locus coeruleus neurons and 5 mg/kg cocaine (triangles). n = 13.

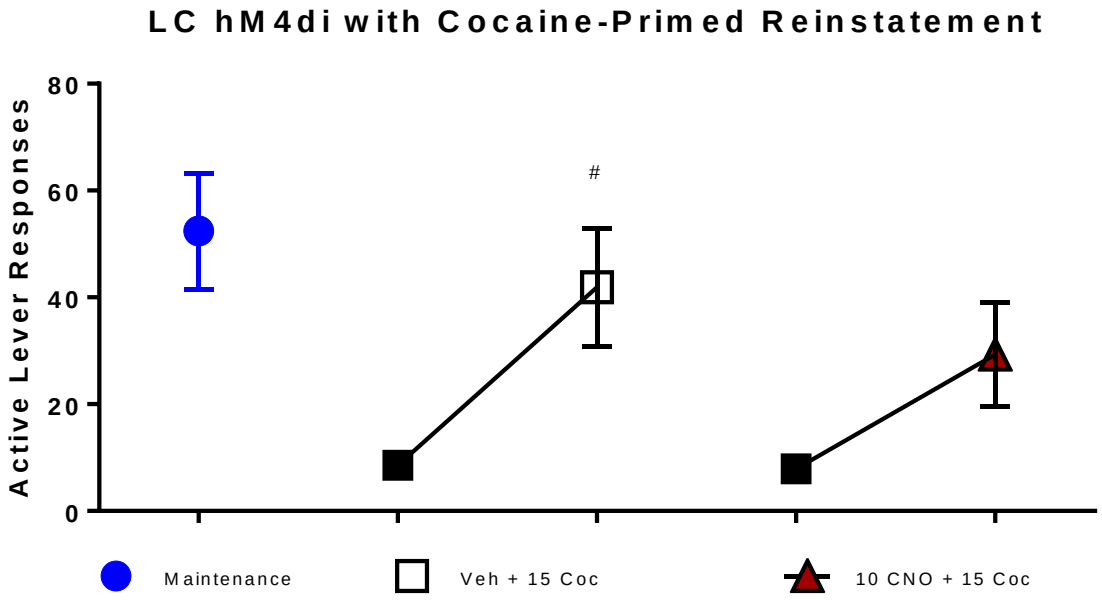

Figure 3.5. Inhibition of the locus coeruleus with hM4Di subtly inhibits cocaine-primed reinstatement. Mean ± SEM active lever responding of stabilized maintenance sessions (blue circle), extinction sessions prior to reinstatement tests (black symbols), vehicle and 15 mg/kg cocaine reinstatement (white square), or 10 mg/kg CNO and 15 mg/kg cocaine (triangles). # indicates significantly different than preceding extinction. n = 7-8.

CHAPTER IV:

OPTOGENETIC ACTIVATION OF THE LOCUS COERULEUS AS A PRIMARY REINFORCER

4.1 Abstract

It is generally accepted that dopamine (DA) is the primary neurotransmitter of the brain's reward system. While accumulating data indicates that norepinephrine (NE) may also be important for some forms of reward and reinforcement, there are conflicting reports from the literature. For example, some groups found that electrical stimulation of the noradrenergic locus coeruleus (LC) could maintain operant behaviors, while others obtained the opposite result. Because these intracranial self-stimulation (ICSS) studies were limited by issues of electrode placement, could not discriminate between stimulation of LC cell bodies or fibers of passage, and likely resulted in collateral stimulation of non-noradrenergic cells in the area, a clear interpretation of these studies is elusive. We are taking an optogenetic approach to overcome these technical obstacles and conclusively determine how the LC fits into the brain's reward circuitry and whether noradrenergic activity can function as a reinforcer. We set out to selectively express the light-sensitive cation channel channelrhodopsin (ChR2) in noradrenergic cell groups and assess whether their activation could support lever pressing behavior. We have tested the selective expression of ChR2 in the LC using two different viral systems: a Cre-dependent AAV-DIO in TH:Cre transgenic rats and a lentivirus containing a LC-specific, phox2b-dependent promoter (PRSx8). Because the PRSx8 drove stronger expression in the LC, we have been using this system for our behavioral experiments. Our pilot results suggest that optogenetic stimulation of the LC can serve as a reinforcer to maintain lever-pressing behavior, implicating this noradrenergic nucleus as a component of the brain's reward system.

4.2 Introduction

In 1954, Olds and Milner published a fundamental paper describing how electrodes placed in specific locations in the brain could alter behavior. This discovery began the search for the regions and neurochemicals involved in reinforcement, defined as the increase of behaviors resulting from the consequences of those behaviors. Prior to the development of tools to distinguish norepinephrine (NE) from dopamine (DA), the catecholamine hypothesis of reward prevailed with the notion that certain neurons were responsible for encoding reinforcement signals and an essential element of this reward circuit signaled through catecholamine transmission (Wise, 1978). At this time, intracranial self-stimulation (ICSS) used electrode placement to determine brain regions involved in reward. As studies progressed, evidence including dopaminergic influence over medial forebrain bundle stimulation (Redgrave, 1978; Zarevics and Setler, 1979; Broekkamp et al., 1975) and self-administration of dopaminergic compounds (Yokel and Wise, 1978; Baxter et al., 1974) accumulated indicating dopamine as the neurotransmitter responsible for reward. By contrast, interest in NE and reinforcement dwindled as conflicting reports on the ability of the locus coeruleus (LC) to support ICSS, with some studies affirming (Crow et al., 1972; Ritter and Stein, 1973; Dresse, 1966) and others refuting (Amaral and Routtenberg, 1975; Simon et al., 1975) that LC stimulation could serve as a reinforcer. Even today, interpretation of these mixed reports is difficult because of significant technical challenges inherent in the ICSS technique including the precision of electrode placement in the small nucleus, stimulation of non-noradrenergic neurons in and near the LC, and the potential for stimulating fibers of passage and terminals with electrical stimulation (Wise, 1978). Furthermore, the firing patterns encoding the

potential reward signal remains unknown because electrical stimulation parameters (~100 Hz) used in these studies does not replicate physiologically relevant firing frequencies of LC neurons (1-15 Hz).

Advances in pharmacology and neurotransmitter detection have updated the catecholamine hypothesis of reward to identify DA as the primary neurotransmitter responsible for reinforcement signaling. Whereas LC stimulation produced mixed results, stimulation of the dopaminergic neurons in the ventral tegmental area (VTA) consistently reinforced operant behavior (Phillips and Fibiger, 1976; Wise, 1978; Olds and Fobes, 1981; Corbett and Wise, 1980). When DA neurotransmission was pharmacologically manipulated, changes in ICSS responding were observed (Redgrave, 1978; Zarevics and Setler, 1979). The final evidence supporting DA as responsible for reward came from studies of drugs of abuse which act as exogenous activators of the brain reward system. Commonly abused drugs all share the ability to increase DA concentrations in the nucleus accumbens (Di Chiara and Imperato, 1988). Furthermore, self-administration of stimulants requires DA (Roberts et al., 1977). In mice with modified DA transporters that lack a cocaine binding site, cocaine self-administration and reward are abolished (Thompson et al., 2009). Similar manipulations of noradrenergic systems failed to show an ability for NE to modulate the primary reinforcement effects of drugs of abuse (Weinshenker and Schroeder, 2007).

Although it is now generally accepted that primary reinforcement occurs through DA, NE may still have a role in appetitive behavior. Several lines of evidence support this hypothesis. First, noradrenergic neurons project both directly and indirectly to, and drive the activity of, mesolimbic DA neurons (Jones et al, 1977; Simon et al, 1979;

Grenhoff et al., 1993; Grenhoff and Svensson, 1993; Liprando et al., 2004). Second, NE is required for some forms of reward that do not appear to require DA (Olson et al., 2006; Hnasko et al., 2005; Pettit et al, 1984). Lastly, NE influences the reinstatement of drug seeking, an operant behavior that may reflect addiction relapse (Schmidt and Weinshenker, 2014).

With the advance of optogenetics, we now have the ability to specifically delineate the role of the LC in reinforcement. Optogenetics uses light-sensitive proteins and fiber optics to regulate cellular activity. This approach overcomes the limitations of previous studies because unlike pharmacological attempts using self-administration of noradrenergic compounds, optogenetics stimulates the LC at physiologically relevant firing frequencies. Moreover, unlike electrical ICSS, optogenetics selectively activates targeted neurons without electrical spread to nearby neurons or fibers of passage. The importance of physiologically relevant firing patterns should not be overlooked because LC neurons discharge at both tonic and phasic rates with corresponding changes in attention and responsiveness to stimuli (Aston-Jones et al., 1999; Devilbiss and Waterhouse, 2011). The LC has been observed to fire at low tonic frequencies (1-2 Hz) during periods when task engagement is low, e.g. sleep and grooming (Aston-Jones et al., 1999). When these tasks are interrupted by relevant stimuli requiring attention and/or behavioral responses, the LC responds with a phasic burst. In particular, these phasic bursts (10-20 Hz) of two or three spikes in less than 500 ms occur in response to stimuli that signal the availability of reward (Rajkowski et al., 1994; Aston-Jones and Bloom, 1981). In addition to NE, LC neurons firing phasically are thought to co-release neuropeptides such as galanin and neuropeptide Y that may have independent effects on

behavior (Karhunen et al., 2001; Verhage et al., 1991; Devilbiss and Waterhouse, 2011). Finally, LC neurons can fire at a high tonic rate (3-5 Hz) that appears to decrease the likelihood of phasic bursts and correlates with distractibility and behavioral errors (Aston-Jones et al., 1999). Optogenetic activation at this high tonic frequency (3-5 Hz) in mice induced a conditioned place aversion and avoidance behaviors (McCall et al., 2015), which are unlikely to reflect the positive valence typically associated with reward. Therefore, we have selected to focus on the potential for phasic activation of the LC to serve as a reinforcer.

Thus far, the application of optogenetics to ICSS paradigms has largely focused on DA circuitry. Optogenetic activation of VTA DA neurons functions as a reinforcer in mice (Adamantidis et al., 2011; Kim et al., 2012; McCall et al., 2013; Ilango et al., 2014), rats (Witten et al., 2011; Steinberg et al., 2014), and macaques (Stauffer et al., 2016). In rodents, the stimulation parameters that supported operant behavior ranged from a single 200 ms pulse (Kim et al., 2012) to a 1 s train of pulses from 20-25 Hz (Witten et al., 2011; Adamantidis et al., 2011; McCall et al., 2013; Steinberg et al., 2014). The DA neurons of primates were only capable of following pulse trains up to 10 Hz (Stauffer et al., 2016). Optogenetic activation of excitatory inputs to the VTA can also maintain operant responding at similar stimulation parameters (Steidl and Veverka, 2015; Barbano et al., 2016). Furthermore, activation DA neurons in the substantia nigra also served as reinforcers (Rossi et al., 2013). Only one report has found optogenetic self-stimulation in systems other than DA or outside the VTA; this study indicated that activation of the glutamatergic projection from the basolateral amygdala to the nucleus accumbens is reinforcing (Stuber et al., 2011).

In order to establish a role of LC activity as a reinforcer, we expressed channelrhodopsin (ChR2) selectively in the LC of rats and allowed them to self-stimulate. We predicted that driving phasic activity in the LC would serve as a reinforcer and facilitate lever-pressing behavior. This pilot study shows that following food training, operant responding is maintained by 10 Hz stimulation of the LC and VTA. Continued examination of the LC's role in reinforcement is warranted.

4.3 Materials and Methods

Subjects

Adult male Long Evans rats weighing 250-350 g at the time of surgery were used. Transgenic rats on a Long-Evans background expressing Cre-recombinase under a tyrosine hydroxylase (TH) promoter were used to target ChR2 to catecholaminergic neurons as previously reported (Witten et al., 2011). A colony of these rats was maintained at Emory. Wild-type Long Evans rats ordered from Charles River (Wilmington MA) were used for the PRSx8 expression system. All rats were individually housed in a temperature controlled environment on a reverse light/dark cycle (8am off/8pm on). Rats had *ad libitum* access to food and water except during the experimental sessions. All procedures were conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals and approved by the Emory IACUC.

Stereotaxic Surgery

To achieve anatomical and cell-type selective expression of ChR2, rats underwent stereotaxic surgery to inject either a Cre-dependent AAV or a PRSx8 lentivirus. Rats

were anesthetized with isoflurane and administered meloxicam (1 mg/kg, s.c.) analgesia. A Cre-recombinase dependent AAV (EF-1α-DIO-hChR2-eYFP) was injected in the LC or VTA at a volume of 1.0 µL/injection. To target the LC, injections were made at the anterior and posterior limits at A/P -9.6 & -10.5; M/L -1.5; D/V -7.75 mm from bregma. To target the VTA, injections were made at A/P -5.4 & -6.2; M/L +0.7; D/V -8.0 mm from bregma. Because the EF-1α-DIO-hChR2-eYFP virus failed to achieve adequate expression in the TH-Cre rats, we turned to the phox2b-dependent PRSx8 promoter lentiviral system. Phox2b is required for LC neuron specification and maintenance of the noradrenergic phenotype, and is expressed exclusively in LC neurons in the pons region (Hwang et al., 2001; Vazey and Aston-Jones, 2014). For this virus, we employed a single injection of 1.2 µL targeting A/P -10.0; M/L -1.5; D/V -7.8. Behavioral testing commenced at least 5 weeks following virus injection to allow time for optimal ChR2 expression.

One month following viral injection, rats undergoing behavioral testing underwent a second surgery to implant a fiber optic ferrule (Doric Lenses, Quebec). Rats were anesthetized with isoflurane and administered meloxicam analgesia as described above. Fiber optic ferrules were implanted 1 mm dorsal to viral target region. Coordinates for LC implantations were A/P -9.8; M/L -1.4; D/V -6.8 while VTA coordinates were A/P -5.6; M/L -0.7; D/V -7.0. Rats were allowed one week to recover from this surgery before behavioral testing.

Operant Conditioning

Rats were allowed to self-stimulate on a FR-1 schedule during daily 2-h sessions, similar to previous descriptions (Witten et al., 2011). Rats were connected to 473 nm lasers (OEM Laser Systems, UT) via fiber optics cables (ThorLabs, NJ) and placed in operant conditioning chambers (Med Associates, St Albans, VT). At the beginning of the session, two levers extended into the chamber and a house light on the wall opposite the levers was illuminated. Presses on the lever assigned as "active" led to a 1 s activation of the laser, extinguishing of the house light, and illumination of cue lights directly over each of the levers. Presses on the "inactive" lever had no programmed consequences. During initial testing in VTA animals, laser delivery was programmed to illuminate at 20Hz (5ms pulse width; ~1.5mW at fiber tip). The stimulation parameters were chosen to match previous reports showing optogenetic VTA self-stimulation in these rats (Witten et al., 2011). In subsequent tests, the frequency was reduced to 10Hz to match physiologically relevant patterns of LC activity.

To verify the stimulus control of the laser reinforcer, a within-session extinction test was performed. During this test, responding during the first 30 min activated the laser, followed by 60 min with the laser deactivated (min 30-90), and then the laser was turned on again for the final 30 min of the session (min 90-120).

Food Training

A separate group of animals underwent surgeries as described and following recovery were food trained for 1-2 days on an FR-1 schedule to facilitate acquisition of lever pressing. During these sessions, responses on the "active" lever resulted in delivery of one 45 mg grain chow pellet (BioServ) until either 100 reinforcers were earned or 6 h

elapsed. In order to meet food training criteria, rats were required to earn 100 reinforcers with > 75% of responses allocated to "active" lever. All rats were able to achieve these criteria in 1 or 2 sessions. Once food training criteria were met, rats progressed to laser-maintained operant conditioning as described above.

Immunohistochemistry

To verify expression of ChR2, double label immunohistochemistry (IHC) was performed to identify tyrosine hydroxylase (TH)-positive LC neurons and ChR2 expression. Rats were transcardially perfused with PBS and 4% paraformaldehyde, and brains were removed and sectioned with a cryostat to collect 40 micron slices. In order to visualize ChR2 expression in the cre-dependent AAV injected animals, mouse anti-GFP (1:1000; Invitrogen) and rabbit anti-TH (1:1000; Millipore) primary antibodies were used. In the PRSx8 injected animals, rabbit anti-DsRed (1:500; Clontech) and mouse anti-TH (1:1000; ImmunoStar) primary antibodies were used to visualize ChR2 expression. For all IHC, AlexaFluor 488 anti-mouse (1:400) and AlexaFluor 594 (1:400) anti-rabbit secondary antibodies were used.

Data Analysis

Data were analyzed by ANOVA using Prism 6.0 for Windows. Sidak's or Tukey's post hoc tests were performed where appropriate.

4.4 Results

Channelrhodopsin2 Expression in the VTA and LC Using the Cre-dependent AAV and PRSx8 Systems.

5 weeks following Cre-dependent AAV injection, we observed expression of ChR2 in the VTA and LC of TH-Cre transgenic rats, as described previously (Witten et al., 2011). Figure 4.1 shows a representative section containing VTA and substantia nigra dopaminergic neurons (TH; red) and expression of ChR2 (green). As seen in the merged panel, ChR2 expression was limited to TH-positive areas and highly expressed in the VTA. ChR2 expression in the LC was also achieved using this system (Fig. 4.1 lower panel), but the magnitude was very weak, with only a few neurons ChR2-positive neurons per sample. 5 weeks following PRSx8 lentiviral injection, we observed much more robust expression of ChR2 in the TH-positive LC neurons that was comparable to that achieved in the VTA using the Cre-dependent system (Fig. 4.2). Based on these results, we used the Cre-dependent AAV to drive ChR2 expression in the VTA and PRSx8 lentivirus to drive ChR2 expression in the LC for the behavioral studies.

Activation of VTA Dopamine Neurons at 20 Hz Functions as a Reinforcer

Optogenetic activation of the VTA was able to serve as a reinforcer (Fig. 4.3). Across 6 sessions, responses were allocated to the active lever that led to activation of VTA neurons. Furthermore, during the extinction test, responding ceased soon after the laser was turned off, but returned when the laser was reactivated, demonstrating that the operant behavior was laser-dependent.

Activation of LC or VTA Neurons Maintains Operant Behavior

Following food training, animals were returned to the operant conditioning chamber under one of three conditions: extinction conditions with no stimulation, 1 s at 10 Hz stimulation of the VTA DA neurons, or 1 s at 10 Hz stimulation of the LC (Fig. 4.4). A two-way repeated measures ANOVA of the "No Stimulation" group revealed significant main effects of Day ($F_{5, 48} = 21.77$, $p < 0.001$) and Lever ($F_{1, 48} = 55.18$, $p < 0.001$) with a significant interaction ($F_{5, 48} = 9.643$, $p < 0.001$). Post hoc tests indicate no differences between any day of inactive lever responding, but significant differences between day 1 and all other days and a significant difference between days 2 and 6 on the active lever. Thus, animals in the no stimulation condition increased active lever responding on the first day in a pattern reminiscent of an extinction burst. Their active lever responding then decreased on each subsequent day until reaching levels similar to their inactive lever response by day 6. Two-way repeated measures ANOVAs on responding maintained by 1 s stimulation at 10 Hz in the VTA and LC reported significant main effects of lever ($F_{1, 32} = 21.84$, $p < 0.001$ and $F_{1, 6} = 9.033$, $p = 0.024$, respectively), but no significant effects of day or interaction. The sustained VTA and LC response patterns indicates that under these conditions, activation of either of these nuclei can maintain previously established behaviors.

Reinforcer Efficacy of Optogenetic ICSS is Frequency Dependent

In order to compare our stimulation parameters with published reports of optogenetic self-stimulation of the VTA (Witten et al., 2011; Steinberg et al., 2014), we also tested animals at 20 Hz. When lever presses were reinforced by 1 s at 20 Hz, responding was increased throughout the session compared to when the reinforcer was 1

s at 10 Hz (Fig. 4.5). Two-way ANOVA show significant main effects of session time ($F_{119,3480} = 2.564$, $p<0.0001$) and frequency ($F_{1,3480} = 891.8$, $p<0.0001$) with a significant interaction ($F_{119,3480} = 1.389$, $p=0.004$). Post hoc test reveal significantly higher responding in the 20 Hz group after the 84th minute of the session.

4.5 Discussion

Here we report that optogenetic activation of noradrenergic LC neurons at 10 Hz can maintain operant behavior at consistent levels similar to identical stimulation parameters of VTA dopamine neurons. Furthermore, we were able to extend previous literature by showing that the reinforcing efficacy of optogenetic ICSS in the VTA is frequency-dependent.

We were able to achieve selective expression of ChR2 in noradrenergic neurons of the LC and dopaminergic neurons of the VTA. The expression profile using the TH-Cre system was similar to previous reports using this system in both the VTA (Witten et al., 2011). However, we were unable to achieve reliable, high expression in the LC using this system in rats, a limitation that has been reported by others in the field (E. Vazey and G. Aston-Jones, personal communication). Therefore, we also tested ChR2 expression in the LC using the PRSx8 promoter. As previously reported with DREADDs (Vazey and Aston-Jones, 2014), the PRSx8 system drove robust and selective expresses in noradrenergic neurons of the LC.

Although Cre-dependent viral expression in the LC of TH-Cre rats is weak, this approach works very well in TH:Cre mice (Carter et al., 2010; McCall et al., 2015). The difference between rats and mice that underlie both the ability to process viral vectors and

the response to stimulation requires further research. The paper reporting the development of the TH:Cre rats found that LC expression required a AAV10 serotype (Witten et al., 2011), so perhaps mouse and rat LCs differentially respond to viral coat proteins.

Although responding was maintained over 6 sessions in both our VTA- and LC-targeted groups, the behavioral output for the VTA was substantially lower than the responding seen in previous optogenetic ICSS experiments (Witten et al., 2011; Steinberg et al., 2014). This decreased responding was likely an effect of the 10 Hz firing frequency, as we observe a 4-fold increase in lever pressing when stimulation frequency was raised to 20 Hz reinforcer. We did not increase the firing rate in the LC beyond 10 Hz because we wanted to remain in physiologically relevant frequencies (Berridge and Waterhouse, 2003; Aston-Jones and Cohen, 2005), and driving beyond 15 Hz prevented behavioral output in mice (Carter et al., 2010). Our lower response rates could also be explained by our use of levers as the operant manipulandum, which typically generate fewer responses than the nosepokes strategy used in previous optogenetic ICSS experiments (Clemens et al., 2010; Witten et al., 2011; Steinberg et al., 2014).

Whereas our data indicate that LC stimulation can be reinforcing, others have demonstrated that LC activation induces anxiety-like aversion (McCall et al., 2015). There are a few notable differences between our study design and theirs. First, they used mice which, as already discussed, may have different responses to LC activation than rats. Second, the firing frequency and pattern that induced aversion was in the high tonic range (5 Hz) whereas we chose phasic parameters. Under phasic frequencies, the Bruchas group saw no change in preference/aversion behaviors. This distinction between tonic

and phasic firing is critical as LC firing under each condition signal different responses to environmental stimuli and behaviors (Aston-Jones et al., 1999; Devilbiss and Waterhouse, 2011). Three distinct sets of firing frequencies have been described for LC neurons, with each correlating with behavioral profiles. During periods of LC low tonic activity (1-2 Hz), animals are engaged in highly repetitive behaviors that require low levels of attention, e.g. grooming, feeding, or sleeping (Aston-Jones et al., 1999). This low tonic activity can be interrupted by phasic bursting behavior when the LC fires with two or three spikes (10-20 Hz). These bursts occur in response to behaviorally relevant stimuli and increase orientation to those stimuli. The frequencies reported by the Bruchas group to induce aversion fall in the high tonic range (3-5 Hz). These high tonic frequencies inhibit the ability of the LC to burst fire and correlates with distractibility and behavioral errors (Aston-Jones et al, 1999). Our data show activation of the LC is able to maintain pre-established operant behavior, but not initiate responding prior to other operant training. We were unable to show that LC activation could serve as a reinforcer to acquire a behavior (data not shown). The difference between the acquisition of behavior and maintenance of ongoing ICSS has not been studied extensively, but is reminiscent of differences in noradrenergic influence during various phases of stimulant self-administration (Schmidt and Weinshenker, 2014). During the acquisition and maintenance phases of stimulant self-administration, noradrenergic signaling plays, at best, a minimal role, whereas DA is critical. However, the reinstatement of drug seeking following extinction can be influenced by adrenergic signaling. Also, it has been suggested that prior operant training might be the explanation as to why some groups were able to achieve electrical ICSS in the LC and others were not (Wise, 1978). Perhaps

the acquisition of operant behavior requires a threshold of dopaminergic activity that food-training or medial forebrain bundle ICSS provides, but once trained, LC activation is sufficient to support these already learned behaviors.

While technical limitations of maintaining fiber optic implants across repeated testing limited the number of animals that were able to complete this study, we have shown the ability of LC activation to serve as a reinforcer under certain conditions. These data are congruent with some clinical reports from patients with electrodes placed in the LC who describe activation as “warm”, “relaxing”, and “pleasant” (Libet and Gleason, 1994). Thus, we believe that further investigations assessing the role of the LC in brain reward circuitry are warranted.

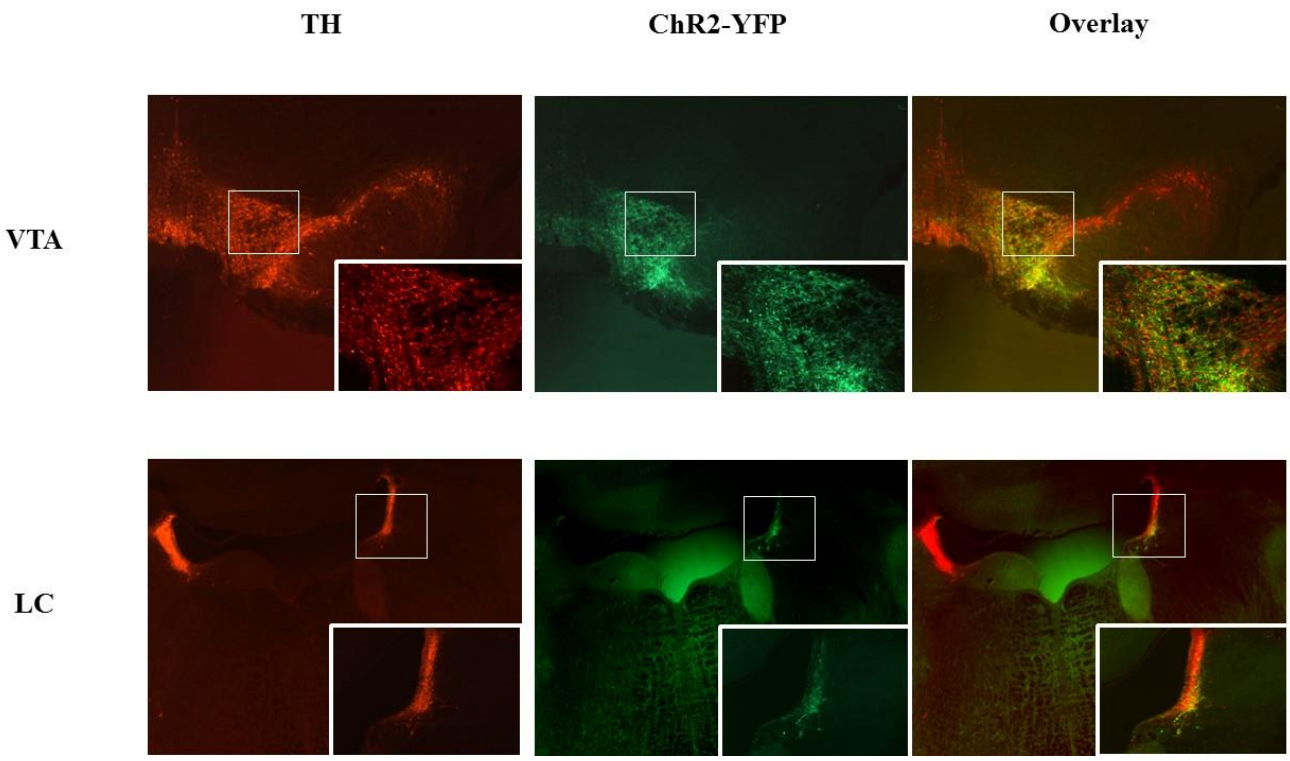

Figure 4.1. Channelrhodopsin expression in the ventral tegmental area and locus coeruleus using a Cre-dependent expression system. Cre-dependent AAV containing ChR2 was injected into the VTA (top row) or LC (bottom row) of TH-Cre transgenic rats, and expression was assessed 5 weeks later by immunohistochemistry. Tyrosine hydroxylase (TH)-positive neurons are labeled in green, ChR2 expressing neurons are labeled in red, and yellow indicates neurons expressing both TH and ChR2 in the overlay. 5x magnification images showing expression in the region. Inset shows 10x magnification of area within white box.

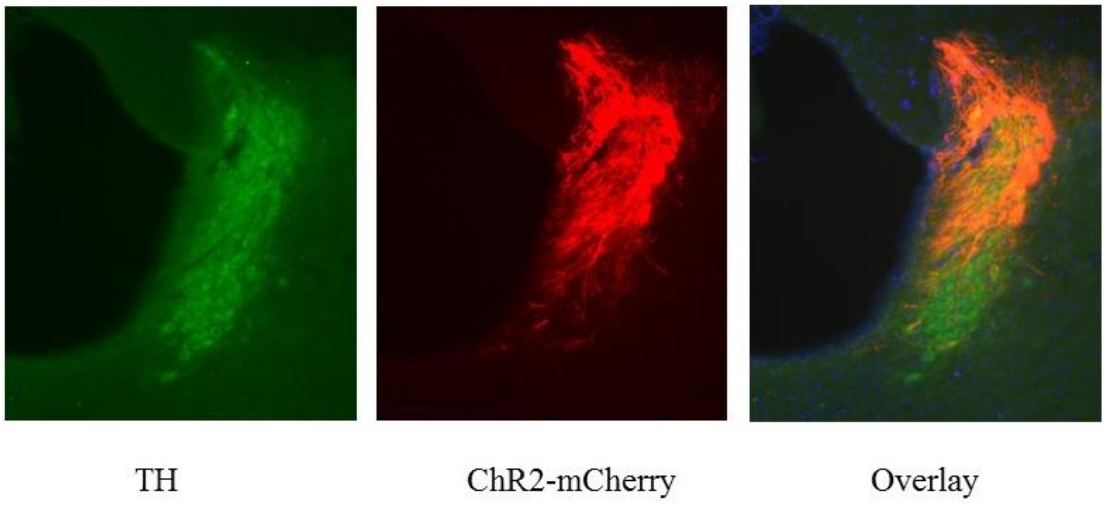

Figure 4.2. Channelrhodopsin2 expression in the locus coeruleus using the PRSx8 expression system. PRSx8 lentivirus containing ChR2 was injected into the LC of rats, and expression was assessed at least 5 weeks later by immunohistochemistry. Tyrosine hydroxylase (TH)-positive neurons are labeled in green, ChR2 expressing neurons are labeled in red, and yellow indicates neurons expressing both TH and ChR2 in the overlay. 10x magnification is shown.

A B

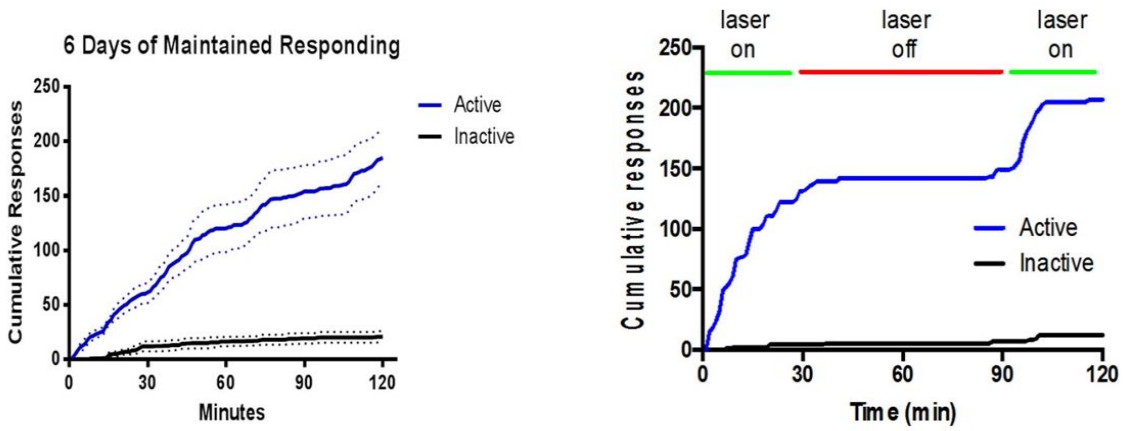

Figure 4.3. Optogenetic stimulation of TH-positive neurons of the VTA serves as a reinforcer. Cumulative records of responding during 120-min sessions. A) The mean (solid lines) ± SEM (dotted lines) of 6 days of responding for the active (blue) and inactive (black) levers. n = 1. B) Within-session extinction test when the laser was on during the first 30 min, off from 30 to 90 min, and reactivated during 90 to 120 min. n = 1.

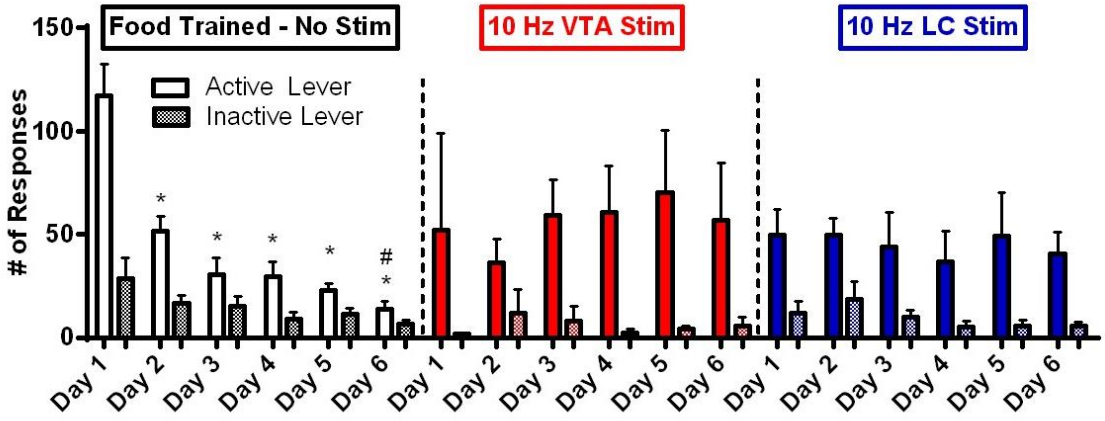

Figure 4.4. Optogenetic self-stimulation of the VTA or LC maintains operant behavior. Mean ± SEM responses during daily 120 min sessions in which responses on the active lever were reinforced by 1 sec of 10 Hz LC stimulation + light cue, 10 Hz VTA stimulation + light cue, or light cue alone. * indicates significant Active Lever differences compared to Day 1 Active Lever responding. # indicates significant Active Lever responding compared to Day 2 Active Lever. n = 4-5 per group.

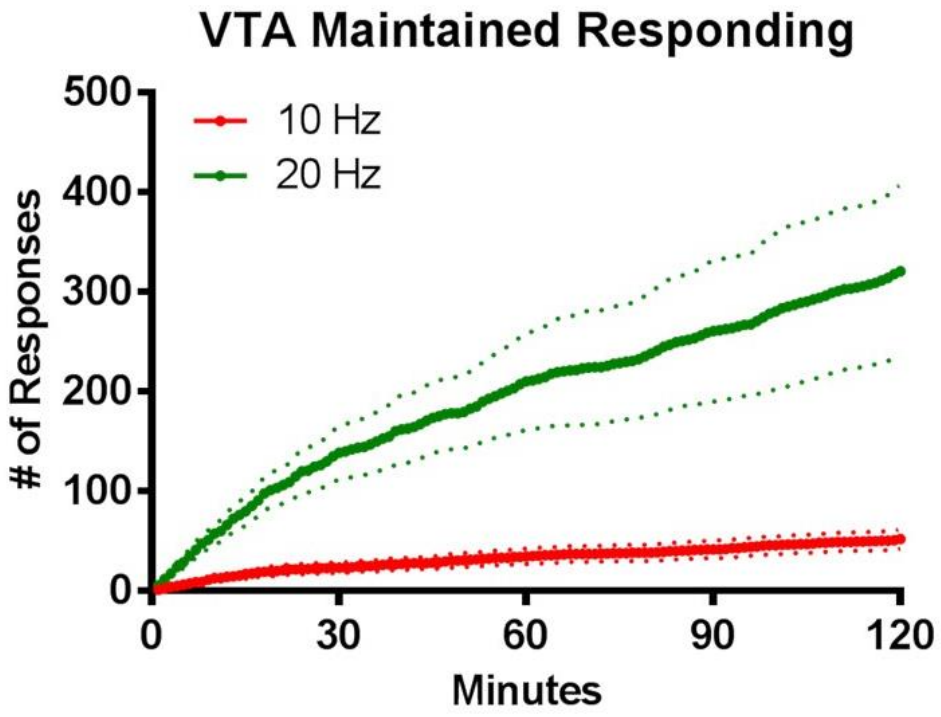

Figure 4.5. Optogenetic ICSS of the VTA is frequency-dependent. Shown are the mean (solid lines) ± SEM (dotted lines) cumulative response patterns during 6 daily 120 min sessions in which responses were reinforced by either 10 Hz stimulation (red) or 20 Hz stimulation (green) of the VTA. n = 2-3 per group.

CHAPTER V:

DISCUSSION

5.1 Introduction

The experiments described in this dissertation address the norepinephrine (NE) circuitry and receptors mediating the reinstatement of cocaine seeking and reinforcement. Blockade of α1 adrenergic receptors (α1AR) in the medial prefrontal cortex (mPFC), but not ventral tegmental area (VTA) or nucleus accumbens (NAc), reduces drug-seeking behaviors. Previous neuroanatomical studies indicate that noradrenergic fibers and terminals in the mPFC likely originate in the brainstem locus coeruleus (LC) cell group. Pilot studies suggest that chemogenetic silencing of the LC reduces cocaine-primed reinstatement, yet activation of these neurons or α1ARs alone fails to initiate drug seeking unless tested under conditions optimized for stress-induced reinstatement. Furthermore, we have presented some preliminary evidence that selective optogenetic activation of the LC maintains previously learned operant behaviors, similarly to equivalent activation of VTA dopaminergic neurons.

5.2 NE and reinstatement of cocaine seeking

We have shown previously that inhibition of NE synthesis prevents cocaine seeking across reinstatement modalities, i.e. drug-primed, cue-induced, and stress-induced (Schroeder et al., 2010, 2013). The mechanism of action underlying these effects had not been directly tested, but was hypothesized that α1AR activity is required for drug-primed reinstatement effect, βAR activity is required for stress-induced reinstatement, and both α1AR and βARs contribute to cue-induced reinstatement (Zhang and Kosten, 2005; Leri et al., 2002; Vranjkovic et al., 2014; Smith and Aston-Jones, 2011). The primary goal of my work was to characterize the role of NE in cocaine-

primed reinstatement; the stress reinstatement circuitry has been worked out by other groups, while the noradrenergic contribution to cue-induced reinstatement remains mostly unstudied.

The idea that dopamine β-hydroxylase (DBH) inhibitors block cocaine-primed reinstatement *because* they decrease α1AR transmission was a matter of debate. In support of that hypothesis, systemic administration of an α1AR antagonist attenuates cocaine-primed reinstatement (Zhang & Kosten, 2005). However, DBH inhibitors prevent the conversion of dopamine (DA) into NE, and thereby increase DA release from noradrenergic terminals. Devoto and colleagues have proposed that this increased DA release, specifically in the PFC, suppress cocaine seeking by "overstimulating" D1 receptors (Devoto et al., 2012; 2014; 2016). Here, we have shown that restoration of α1AR signaling overcomes DBH inhibition to restore cocaine-primed reinstatement, adding support to the reduced α1AR transmission model. In order to determine the neuroanatomical location of these critical α1ARs, we blocked α1ARs locally in the mPFC, VTA, and NAc, and showed that blockade of α1ARs in the mPFC decreased cocaine-primed reinstatement. Although these receptors are necessary for cocaine-primed reinstatement, they seem to be insufficient on their own, as neither α1AR agonists nor chemogenetic activation of the LC could induce reinstatement in the absence of a cocaine-prime.

Taking all of the data into account, we propose a model of the neural circuits required for cocaine-primed reinstatement (Fig. 5.1). The VTA, dorsal PFC, NAc core, and ventral pallidum (VP) are well established as critical regions for cocaine-primed reinstatement; inhibition of any of these regions via local infusion of GABA agonists

prevents drug seeking (McFarland and Kalivas, 2001). We have specifically highlighted the noradrenergic influences on these regions. In particular, cocaine increases extracellular DA and NE in the prefrontal cortex, which signals through D1 receptors and α1ARs, respectively, on glutamate projection neurons terminating in the NAc core. D1 receptors and α1ARs colocalize on these glutamatergic elements in the mPFC (Mitrano et al., 2014). How these receptors might interact to excite glutamate neurons is not clear. Either D1 receptor antagonism or stimulation can reduce drug-seeking behavior (Devoto et al., 2016; Capriles et al., 2003; Sun and Rebec, 2005), suggesting that too little or too much D1 transmission prevents reinstatement. Importantly, our delivery of α1AR antagonists in the mPFC targeted the prelimbic cortex in the dorsomedial PFC, not the ventromedial infralimbic cortex. These neighboring regions have distinct projections to the NAc with the prelimbic cortex projecting to the NAc core while the infralimbic cortex projects to the NAc shell. The prelimbic-NAc core is thought to be active during reinstatement and high behavioral output, whereas the infralimbic-NAc shell is believed to be a brake on the system and active during extinction learning (Peters et al., 2008; Gourley and Taylor, 2016; West et al., 2014). Furthermore, glutamate release in the NAc core is required for cocaine-primed reinstatement, and this signal is transmitted to motor output regions via the VP (McFarland and Kalivas, 2001). Although we showed that depletion of NE decreases cocaine-induced glutamate release in the PFC, whether the same is true in the NAc still needs to be tested in cocaine-experienced rats. Neurochemical confirmation that our blockade of α1AR signaling in the mPFC decreases cocaine-induced glutamate in the NAc core would be the missing connection between cocaine's monoaminergic action and the necessity of glutamate in relapse-like behaviors.

Our results allow a number of new research questions to be asked regarding cocaine-primed reinstatement. Because terazosin has equal affinity at each α1AR subtype, determining whether α1a, α1b, or α1d mediate the effects would further direct therapeutic development. The α1bAR is the most likely candidate because prazosin loses its ability to alter stimulant-induced locomotor activity in α1b KO mice (Auclair et al., 2002; 2004; Drouin et al., 2002; Villegier et al., 2003). α1d KO mice have similar decreased locomotor responses to stimulants, but also have reduced wheel running indicating a potential nonspecific effect on motor activity (Sadalge et al., 2003). Furthermore, α1bARs have been localized to unmyelinated axons, dendrites, spines, and axon terminals in the PFC (Mitrano et al., 2012). Unfortunately, no α1bAR-selective antagonists currently exist, but there are selective antagonists for α1a and α1d (Zhong and Minneman, 1999). To identify the receptor subtype involved in cocaine-primed reinstatement, local infusion of the α1a receptor antagonist 5-methylurapilil or the α1d receptor antagonist BMY 7378 into the mPFC prior to a cocaine-primed reinstatement test could potentially rule out those subtypes when compared to terazosin infusion. Alternatively, intra-mPFC infusion of siRNA viral vectors to block the expression of each receptor would allow all three subtypes to be examined. These α1ARs signal intracellularly through Gq pathways (Fig. 5.2), yet targeting these second messenger systems in the mPFC in the context of relapse has not been conducted. Activation of α1ARs stimulates phospholipase C activity to increase levels of inositol triphosphate (IP3) and diacylglycerol (DAG) which in turn activates protein kinase C (PKC). PKC can phosphorylate GluR1 and GluR2 AMPAR subunits (40, 30). PKC phosphorylation following α1AR activation increases AMPAR trafficking and signaling (Luo et al.,

2014). Furthermore, α1AR activation increases glutamate release via presynaptic PKC interacting with N-type-Ca^{2+} channels and glutamate release machinery (Luo et al., 2015). Combined, these α1AR-mediated presynaptic and postsynaptic signaling pathways have the potential to increase excitability of mPFC glutamatergic projection neurons, thus facilitating the release of glutamate in the NAc to drive drug-seeking behavior. To determine how these intracellular signaling mechanisms impact reinstatement, local infusions of PKC inhibitors into the mPFC prior to cocaine prime need to be performed. The brains from these experiments could then be harvested to examine the amount of membrane bound AMPARs following cocaine prime alone or cocaine prime combined with PKC inhibition.

The subtype of adrenergic receptor and neuroanatomical region required for stress-induced reinstatement are distinct from those regulating cocaine-primed reinstatement. The central amygdala and bed nucleus of the stria terminalis (BNST) act as a central hub to coordinate stress and reward signals during reinstatement (McFarland et al., 2004). Administration of βAR antagonists into the BNST or central amydgala prevents shock-induced reinstatement (Leri et al., 2002), while direct activation of β2ARs in the BNST alone is sufficient to induce cocaine seeking (Vranjkovic et al., 2014). These receptors activate a corticotropin releasing factor (CRF) pathway that activates the VTA and mesocorticolimbic circuitry required for reinstatement (Vranjkovic et al. 2014, McFarland et al., 2004). The BNST also expresses the immediate early gene c-Fos following i.c.v. administration of NE that induces reinstatement (Brown et al., 2011). The source of NE that activates these βARs is believed to originate in A1/A2 and proceed through the ventral noradrenergic bundle (Mantsch et al., 2016). However, we show here

that chemogenetic activation of the LC can reinstate when tested under similar conditions as used in these stress-induced reinstatement paradigms. Release of NE has been detected in the vBNST following stimulation of the dorsal noradrenergic bundle from the LC, but it may be via indirect projections (Park et al., 2009; Fox et al., 2016). Perhaps chemogenetic activation of the LC releases NE in the BNST, activating βARs on neurons that in turn release CRF and lead to downstream drug-seeking behaviors. Alternatively, we know that a PFC-NAc pathway is critical for footshock-induced reinstatement. Thus, it is possible that LC firing in response to stress activates adrenergic receptors in the PFC, which causes excitation of glutamate projections to the NAc to drive drug seeking. Although D1 receptors are thought to be responsible for the PFC-NAc connection necessary for footshock-induced reinstatement (Capriles et al., 2003; Mantsch et al., 2016), we cannot rule out a noradrenergic contribution given the interactions between D1Rs and α1ARs in the PFC (Trovero et al, 1994; Mitrano et al., 2014).

We have summarized the circuits mediating stress-induced drug-seeking behaviors with a focus on noradrenergic influences in Fig. 5.3. Inactivation of the central amygdala, BNST, VTA, dorsal PFC, NAc, and VP blocks footshock-induced reinstatement of cocaine seeking (McFarland et al., 2004). Central responses to stress begin in the A1/A2 noradrenergic nuclei, which project via the ventral noradrenergic bundle to the central amygdala and BNST. Activation of βARs in the central amygdala initiates the release of CRF in the BNST (Erb et al., 2001). As discussed above, BNST βAR activation facilitates CRF transmission in the VTA, which in turn activates the dorsal PFC (Vranjkovic et al., 2014). Similar to cocaine-primed reinstatement, dorsal PFC pyramidal neurons release glutamate in the NAc to activate motor outputs circuits

leading to drug-seeking behaviors. However, the experiments measuring glutamate overflow in the NAc following noradrenergic manipulations have not yet been performed and offer a new avenue to assess noradrenergic regulation of stress induced glutamate release in addiction.

Lastly, cue-induced reinstatement requires noradrenergic activity at either α1 and β ARs (Smith and Aston Jones, 2011). Decreasing NE release via α2AR agonism is also sufficient to reduce cue-induced drug seeking. Further information regarding the location of these receptors and circuitry required for cue-induced reinstatement remains to be tested. Perhaps the same noradrenergic pathways mediating stress-induced and cocaine-primed reinstatement can account for the actions of α1 and β ARs on cue-induced reinstatement. To test this hypothesis, dual infusions of α1AR antagonist targeting the PFC and β2AR antagonists targeting the BNST need to be performed prior to a cue-induced reinstatement session. Other brain areas indicated as possibly mediating cue-induced reinstatement include the lateral septum, NAc shell, NAc core, basolateral amygdala, and ventral subiculum of the hippocampus (Zhou et al., 2014; Mahler and Aston-Jones 2012; Stefanik and Kalivas, 2013; McGlinchey et al 2016).

5.3 Clinical implications

The results presented in this dissertation have the potential to inform the development of new therapies for treatment of cocaine dependence. As there are currently no FDA approved pharmacotherapies for stimulant addiction, the need for new treatments is high. However, I also advise caution because our hypotheses require further

testing and there are reasons to think that the findings cannot be directly translated to human drug dependence.

The preclinical reinstatement model uses administration of the drug itself, cues previously associated with drug availability or delivery, or stress to invigorate previously extinguished drug-seeking behaviors. One strength of this model is that these same stimuli have been reported to cause relapse in humans (de Wit, 1996; Childress et al., 1993; Sinha, 2001). However, this has been called into question because human studies using these stimuli often report increased ratings of drug craving, but do not show that those feelings of craving lead to relapse behaviors (Epstein et al., 2006). Furthermore, the difference between a drug lapse leading to full-blown binging and an experimenter-administered drug prime leading to drug-seeking behaviors might be more significant than currently appreciated. Perhaps a better drug-primed reinstatement session would deliver cocaine I.V. contingent on only the first lever response of the session to invigorate responding. Regarding stress-induced reinstatement, using psychosocial stressors to reinstate cocaine-responding might more closely model the stress conditions that cause craving in humans than the physical or chemical stressors currently used (Manvich et al., 2016). Furthermore, in the reinstatement model, animals are trained for several extinction sessions to learn that the operant behavior that once delivered drugs is no longer reinforced. This is done to reduce operant behavior so that reinstatement responding can be more easily detected over a low baseline. Even in drug treatment programs, humans do not typically have this experience to disassociate drug-seeking behaviors from the drugs they produce.

Assessing the reinstatement model as a therapeutic screen for psychostimulant addiction pharmacotherapies is difficult because (1) there is no positive control, and (2) few compounds have been tested to prevent relapse in humans (Epstein et al., 2006). One particularly challenging aspect of this research is that clinical trials frequently use treatment-seeking drug users, with decreases in ongoing drug intake as a primary measure of treatment efficacy (Vocci and Ling 2005). With regards to noradrenergic therapeutics, noradrenergic manipulations have little to no effect on ongoing drug intake in animal models, yet significantly impact cocaine reinstatement (Ecke et al., 2012; Schroeder et al., 2010; 2013; Schmidt and Weinshenker, 2014), except during models of binging with prolonged access during which α1AR antagonists prevented escalated drug intake (Zhang and Kosten, 2007; Wee et al., 2008). Therefore, careful considerations need to be made when designing clinical studies to test the efficacy of noradrenergic manipulations on cocaine dependence.

Previous clinical studies of noradrenergic compounds offer a cautionary tale when translating rodent models to nonhuman primates and human therapeutics. In rats, NE inhibitors attenuate all three modalities of the reinstatement model: drug-prime, drug-associated cues, and stress (Schroeder et al., 2010; 2013). However, when these compounds were tested in squirrel monkeys, they had no effect on cocaine-primed reinstatement (Cooper et al, 2014). Furthermore, while the selective inhibitor nepicastat had no effect on its own and blocked cocaine-primed reinstatement in rats, it actually induced squirrel monkey drug-seeking behavior in the absence of a cocaine-prime. When taken to clinical trials, results NE synthesis inhibitors on cocaine dependence have been

mixed (Carroll et al., 1998; Grassi et al., 2007; Carroll et al., 2012; Oliveto et al., 2011; Biotie Therapies, 2015, http://www.biotie.com/product-portfolio/syn117.aspx).

With this in mind, new reports are accumulating indicating beneficial effects of α1AR antagonists in cocaine-dependent populations. The α1AR antagonist doxazosin reduces ratings of "like cocaine" and "likely to use cocaine if had access" in cocaine-dependent participants while reducing cocaine use and improving abstinence rates (Newton et al., 2012; Shorter et al., 2013). Doxazosin was selected for these clinical trials because its half-life is approximately seven times longer than prazosin and is independent of age, renal function, and dose (Shorter et al., 2013; Rubin et al., 1981; Jaillon, 1980). Furthermore, doxazosin acts similarly to prazosin to prevent cocaine sensitization in rodents (Haile et al., 2012). Several clinical trials are following up on these human laboratory studies to determine the effect of α1AR antagonists for the treatment of cocaine dependence (NCT01953432, NCT01145183, NCT02538744, NCT01371851). Preliminary results from these trials indicate that doxazosin is an efficacious treatment and may show pharmacogenetic differences depending on α1AR polymorphisms (T. Kosten, personal communication).

5.4 Chemogenetic and optogenetic tools to study the noradrenergic system and reinstatement

Here we have also shown new technological advances to study the role of the noradrenergic system in motivated behavior. Whereas chemogenetics and optogenetics both offer the ability to control specific populations of neurons, each technique has strengths and weaknesses when applied to studies of cocaine seeking. DREADD

manipulations are particularly useful for determining circuitry mediating behaviors because a single systemic injection can activate or inactivate an entire cell population, such as the LC. DREADDs are advantageous in the reinstatement paradigm because they allow continued activation or inactivation of select neurons throughout a long (several hours) test session. Furthermore, CNO can be locally infused to isolate specific pathways (e.g. LC-PFC) and their roles in reinstatement. However, because DREADD alterations in cellular activity last hours, this approach would not be ideal for determining whether brief, acute activation of a particular cell population would be reinforcing because operant behavior is driven by rapid stimulus-response contingencies. Turning neurons on or off for hours would not support this type of behavior. To determine the reinforcing properties of neuronal activation, optogenetics would be a better approach, as we discussed in Chapter 4. Optogenetics also allows subsecond control of activation patterns via programming laser light pulses to drive neuron firing at physiologically relevant frequencies for brief epochs. This consideration of firing rate is especially important for the LC, which is active at both tonic and phasic frequencies to signal various behavioral responses. One particular challenge to applying optogenetics to operant behavioral tests is the technical difficulty maintaining fiber optics to deliver light during ongoing behaviors. For instance, in order to use optogenetics during intravenous self-administration sessions, one would need to determine how to prevent the drug-delivery tubing and fiber optic cable from tangling. To solve this issue, wireless optogenetic strategies are in development (McCall et al., 2013) and could be used to determine how various neuronal activity patterns modify drug self-administration and reinstatement.

The use of optogenetics and chemogenetics to understand the neural circuits of reinstatement has already led to treatment development. Based on human literature showing that continued drug use correlates with decreased PFC activity (Goldstein and Volkow, 2011; Jentsch and Taylor, 1999), Chen et al used a "compulsive drug seeking" model in which they identified rats that continued self-administration despite a 30% likelihood that drug delivery would coincide with footshock, which is reminiscent of continued drug use in human addicts despite negative consequences (2013). In the rats resistant to the aversive effects of shock, optogenetic stimulation of the pyramidal neurons in the PFC reduced cocaine seeking. To translate these data to the clinic, Terraneo et al. targeted transcranial magnetic stimulation (TMS) to activate the dorsolateral PFC in treatment-seeking people meeting criteria for cocaine use disorder (2016). TMS treatment decreased cocaine-positive urines and cocaine craving. With further characterization of the circuits mediating cocaine dependence and relapse, new targets for pharmacotherapies or TMS could be assessed.

5.5 Conclusion

Our data provide a new, previously underappreciated role for the LC in drug-motivated behaviors including reinstatement of cocaine seeking and operant self-stimulation. In our model, which is based on the present results as well as reports by other groups in the literature, activation of the LC increases βAR signaling in the BNST and α1AR signaling in the mPFC. The activation of βARs drives downstream circuits leading to a stress-like reinstatement of cocaine seeking. Whereas αAR activation is insufficient on its own to drive drug seeking under conditions optimized for cocaine-primed

reinstatement, it acts in a permissive role to facilitate cocaine-primed reinstatement. Furthermore, activation of the LC via optogenetics is sufficient to maintain previously learned operant behaviors.

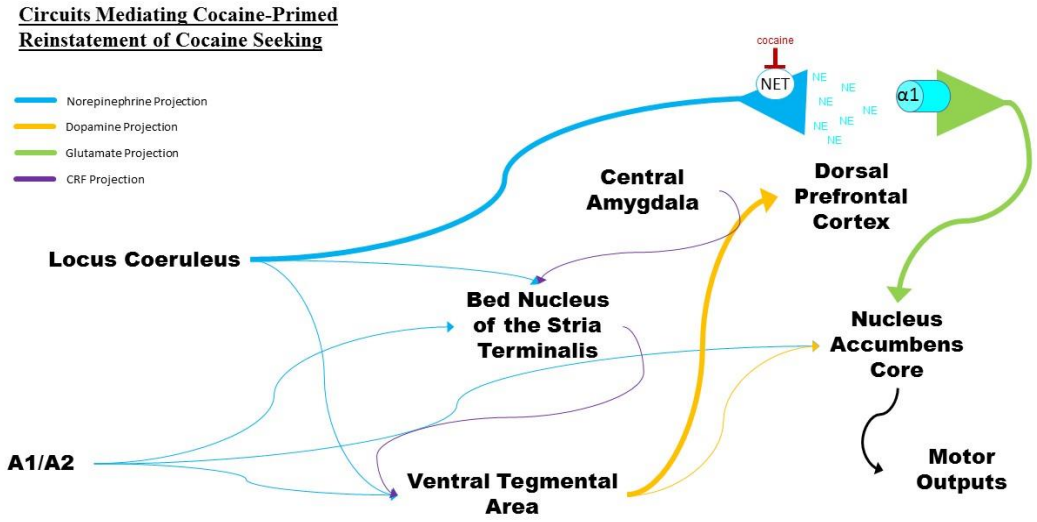

Figure 5.1. Circuits mediating cocaine-primed reinstatement of cocaine seeking. Following a cocaine prime, increased extracellular norepinephrine (NE; cyan) in the prefrontal cortex activates α1ARs on glutamatergic (lime) neurons projecting to the nucleus accumbens. Lines indicate connections between key regions involved in reinstatement of cocaine seeking, with thicker lines indicating key roles in cocaine-primed reinstatement.

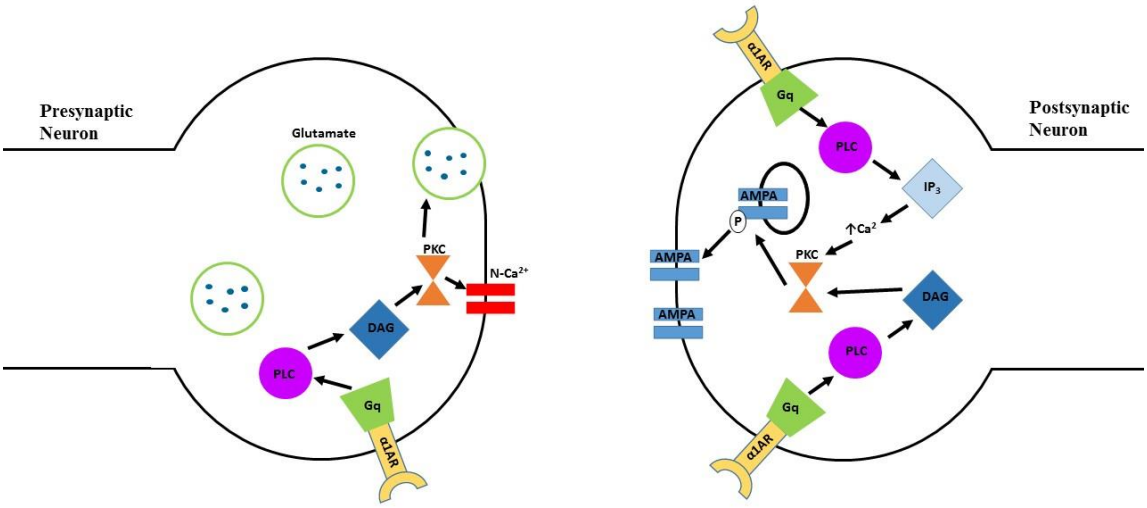

Figure 5.2. Intracellular signaling mechanisms of α1ARs in the prefrontal cortex. α1ARs act presynaptically and postsynaptically to increase glutamate signaling. α1ARs signal through Gq (green) to increase phospholipase C (PLC; fuchsia) which increases levels of inositol triphosphate (IP3; light blue) and diacylglycerol (DAG; dark blue) which in turn activates protein kinase C (PKC; orange). Presynaptically PKC interacts with N-type-calcium channels (red) to increase glutamate release. Postsynaptically, PKC phosphorylates AMPA receptors (blue) to enhance trafficking to the membrane.

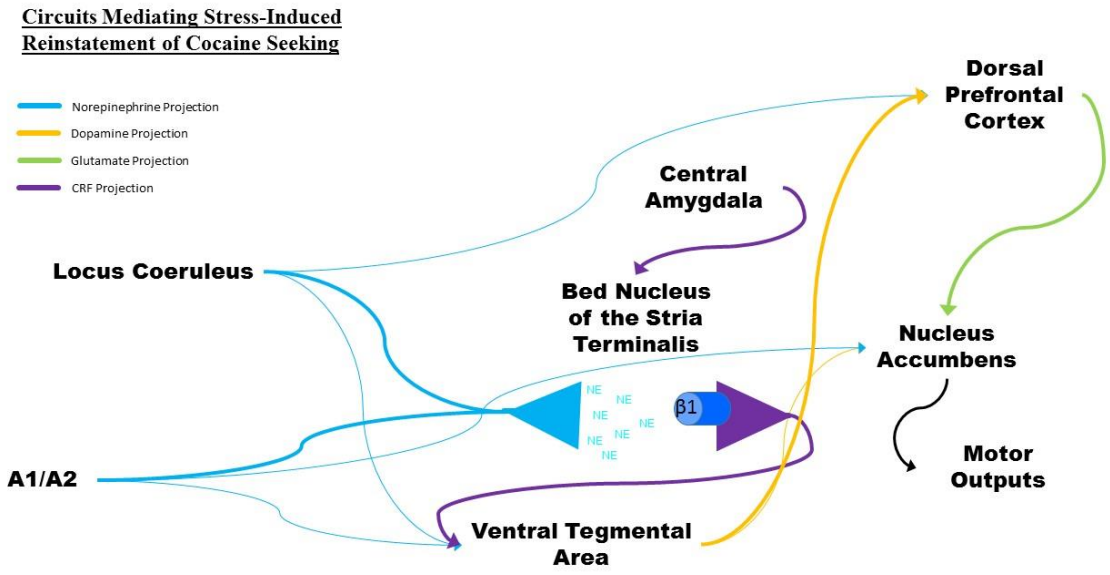

Figure 5.3. Circuits mediating stress-induced reinstatement of cocaine seeking. Following stress, increased extracellular norepinephrine (NE; cyan) in the bed nucleus of the stria terminalis activates β1AR on CRF (lilac) neurons. Activation of these CRF neurons leads to downstream activation of VTA dopamine neurons, prefrontal cortex pyramidal neurons, and the nucleus accumbens, which initiates motor outputs for drug seeking. Lines indicate connections between key regions involved in reinstatement of cocaine seeking, with thicker lines indicating key roles in stress-induced reinstatement.

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