2016

Roles of Accumbal and Septal Circuitry in Stimulus-Induced Reinstatement of Cocaine Seeking

Ellen Morgan McGlinchey
Medical University of South Carolina

Roles of Accumbal and Septal Circuitry in Stimulus-Induced Reinstatement of Cocaine Seeking

by

Ellen Morgan McGlinchey

A dissertation submitted to the faculty of the Medical University of South Carolina in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the College of Graduate Studies.

Department of Neuroscience

2016

Approved by:

Chair: Gary Aston-Jones

Advisory Committee:

Jacqueline McGinty

Howard Becker

Thomas Jhou

Colleen Hanlon

ACKNOWLEDGEMENTS

To Gary:
Thank you for allowing me to pursue projects based on my interests and supporting me throughout graduate school. Your scientific imagination is infectious. I know you'll do great things for the neuroscience program at Rutgers.

To my committee members:
Thank you for helping mold my projects, providing valuable input, and ensuring my progression throughout graduate school.

To Mom and Dad:
Thank you for all your love and support throughout my life. You are always there when I need you the most. I am forever grateful for everything you've taught me. You've encouraged me to pursue what makes me happiest, taught me how to persevere when times are rough, and stand up for myself. Sometimes I feel like an ant lost in the woods, but you've always taught me to march on. ;) You are exemplary role models in every sense of the word.

To Brian, Colleen, and Jake:
Thanks for picking up the tab for me for the last 5 years on gifts for holidays, birthdays, special occasions, etc. You guys are awesome. I'm glad I was forced to have you as my siblings. **To Rufus**: Thanks for being adorable and always cheering me up even when you didn't know it.

To Bill:
We've come a long way from the deli line at the local Piggly Wiggly and eating grilled cheeses together all those years ago. Graduate school would not be *at all* the same without you. I am incredibly happy I met you. I'm looking forward to our many adventures to come!

To the GAJ lab members: past and present, MUSC & Rutgers:
You guys are seriously the coolest, smartest, most helpful people I know. You made the work environment so enjoyable! Thank you for all the input you've given me on my projects throughout the years. You are all great friends. Special thanks to Steve Mahler: thank you for getting me up and going in the lab and letting me bombard you with questions on a daily basis. Special thanks to Elena Vazey: it made all the difference that you moved to Rutgers with the lab. You seem to just know everything. You are an amazing person all around and I know you'll be an amazing mentor. Special thanks to Benjamin Zimmer: thank you for letting me talk your ear off constantly and always providing excellent advice. Thank you all!

To my graduate school crew:
Thank you for the camaraderie during graduate school. You guys are amazing and I can't wait to keep in touch post-grad school!

TABLE OF CONTENTS

LIST OF TABLES

CHAPTER 2

CHAPTER 3

LIST OF FIGURES

CHAPTER 1

CHAPTER 2

CHAPTER 3

ELLEN MORGAN MCGLINCHEY. Roles of accumbal and septal circuitry in stimulus-induced reinstatement of cocaine seeking. (Under the direction of GARY ASTON-JONES).

ABSTRACT

Chronic relapse is a tremendous obstacle in battling addiction. Animal models of relapse (e.g. reinstatement of extinguished lever pressing) are critical for uncovering the neural substrates of addiction. Understanding the neurocircuitry of relapse may provide potentially viable targets to reduce relapse rates and treat addiction. Elucidating the circuits involved in stimulus-induced reinstatement is of particular importance because drug-associated stimuli (e.g. discrete or contextual drug-associated cues) evoke drug craving and perpetuate drug seeking. Nucleus accumbens (NAc) and lateral septum (LS) are two subcortical structures known to contribute to drug addiction and relapse behaviors. However, NAc and LS afferents recruited during stimulus-induced reinstatement of extinguished cocaine seeking have not been fully characterized.

Here, we used self-administration paradigms combined with retrograde tracers, Fos immunohistochemistry, pharmacology, and chemogenetics to elucidate NAc and LS afferents involved in stimulus-induced reinstatement of cocaine seeking. In the first set of experiments, we found that neurons in the prelimbic cortex, basolateral amygdala, and ventral subiculum that project to NAc core, but not to NAc shell, showed enhanced neural activation during cue-induced reinstatement compared to extinguished cocaine-seeking behavior. However, only activation of the prelimbic-NAc core pathway positively correlated with cocaine seeking behavior. Furthermore, activation of these circuits was specific to cocaine-, but not sucrose-seeking.

In the second set of experiments, we found that dorsal hippocampus, LS, and dorsal hippocampal inputs to LS showed enhanced neural activation during context-

induced compared to cue-induced reinstatement of cocaine seeking. In support of these results, chemogenetic-mediated inhibition of the dorsal hippocampal-LS pathway attenuated context-, but not cue-induced reinstatement. However, pharmacological inhibition of LS attenuated both context- and cue-induced reinstatement. We then examined if ventral hippocampal neurons may drive cue-induced reinstatement via LS. We found that ventral hippocampal inputs to LS were activated during context- and cue-induced reinstatement, but chemogenetic-mediated inhibition of the circuit did not attenuate either reinstatement modality.

Together these data elucidate multiple excitatory inputs to NAc or LS during stimulus-induced reinstatement of cocaine seeking, and indicate distinct neural circuits mediating various reinstatement modalities. The results provide greater insight into the neurocircuitry of relapse that may lead to new therapeutics to treat drug addiction.

CHAPTER 1: INTRODUCTION

Drug addiction: a public health epidemic

Drug abuse and addiction is rising as an ever more prevalent disease and major public health concern in the United States. The Diagnostic and Statistical Manual of Mental Disorders (DSM-5) characterize a substance use disorder as when a substance user exhibits impaired control, social impairment, risky use, and pharmacological criteria including tolerance and withdrawal symptoms (American Psychiatric Association, 2013). A 2013 survey by the Substance Abuse and Mental Health Services Administration estimated 24.6 million Americans (ages 12 and older) have used an illicit drug in the past month—equivalent to 9.4% of the U.S. population. These staggering statistics are only trending upward. Death rates from drug overdose have substantially increased—up to 600%—between 2001 and 2014 (National Institute on Drug Abuse, 2015). The rise of drug addiction is highly linked with other public health concerns such as HIV/AIDS where sharing needles and unprotected sex increase the risk of transmitting and contracting the disease. Given these numbers, drug abuse and addiction also cost significant economic burdens. Substance abuse disorders cost the nation more than $600 billion annually ($193 billion for illicit drugs) due to crime, loss of work productivity, and healthcare costs (National Institute on Drug Abuse, 2012). One of the most trafficked, illegal drugs that contributes to the health and economic concerns of addiction is cocaine.

Cocaine addiction

Cocaine is a powerful psychostimulant that exists naturally in the leaves of the coca plant, *Erthyroxylon coca*, indigenous to South America (Ciccarone, 2011, National Institute on Drug Abuse, 2016). Pharmacologically, cocaine blocks monoamine reuptake

at transporters and transiently increases extracellular dopamine, norepinephrine, and serotonin in the synapse (Ritz et al., 1990; Volkow et al., 2000). Changes in extracellular dopamine levels lead to increased energy and euphoria immediately following acute cocaine use. However, acute cocaine also causes increased heart rate, blood pressure, and body temperature, and constricted blood vessels (Crandall et al., 2002, National Institute on Drug Abuse, 2016). These symptoms increase the likelihood of cardiac arrest and stroke among cocaine users, a prevalent outcome with overdose (Hollander et al., 1995; Auer et al., 2001). Of the total population of drug users, an estimated 1.5 million Americans are cocaine users and approximately 71,500 Americans have died from cocaine overdose between 2001 and 2014 (National Institute on Drug Abuse, 2015). Whether smoked, snorted, or injected, cocaine users tend to use cocaine in a binge pattern that increases the likelihood of chronic use and dependence.

The persistent desire for the pleasurable effects of drugs (e.g. positive reinforcement; Wise, 1980) and the need to relieve or avoid withdrawal symptoms (e.g. negative reinforcement; Koob et al., 1989; Koob and Volkow, 2010) exacerbate persistent drug use in addicts. However, these concepts of positive and negative reinforcement do not address the propensity, and frequency, at which recovering addicts relapse long after acute withdrawal symptoms have subsided.

Relapse

One of the greatest obstacles in effectively treating addiction is the propensity of relapse. Drug craving can powerfully influence addicts to relapse. Clinical studies have shown that in cocaine-dependent individuals, an intravenous dose of cocaine increases drug “wanting” and craving (Jaffe et al., 1989). Stress and life crises perpetuate drug use and evoke relapse in human addicts (Khantzian, 1985; Kosten et al., 1986). Cues

associated with drugs provoke craving that elicit profound arousal-like physical changes in addicts (Childress et al., 1988), and videos of cocaine-associated cues stimulate cerebral blood flow in multiple limbic regions in cocaine-dependent individuals (Childress et al., 1999). Also, addicts who successfully extinguished psychological and physiological conditioned responses to drug-associated cues in the clinic show high relapse rates upon returning home (Childress et al., 1993). These studies demonstrate that drug-priming, stress, discrete and contextual (environmental) cues associated with drug use have powerful effects that motivate addicts to seek drugs.

Drug-associated stimuli are of particular interest because everyday objects or environments associated with drug use are difficult to avoid and frequently provoke relapse in recovering addicts. One theory for persistent relapse is the enhanced salience of drug-associated stimuli (Kalivas and Volkow, 2005; Hyman et al., 2006). These stimuli become associated with drugs through conditioned learning. Addicts often experience repeated pairings of an unconditioned stimulus (e.g. paraphernalia, house) with a rewarding stimulus (e.g. cocaine) resulting in a conditioned association between the paraphernalia (discrete drug cue) or drug-taking environment (drug context) and the drug reward. Exposure to these two types of drug-associated stimuli can elicit drug craving and consequently induce drug seeking.

Clinical studies demonstrate that drug craving is an extremely difficult obstacle to overcome and thus often leads to relapse. Reducing the propensity of relapse in addicts would be a major accomplishment in treating addiction.

Animal models of addiction

Addiction hijacks the brain's reward circuitry and provokes synaptic and neurochemical adaptations and altered neurocircuitry. Animal models of addiction allow

researchers to elucidate the neurobiology and neurocircuitry of relapse behavior to test viable therapeutic targets that might prevent relapse in human addicts. The three most commonly used models to study addiction in animals are locomotor sensitization, conditioned place preference, and self-administration.

Locomotor sensitization

Locomotor sensitization is a behavioral addiction model where rodents demonstrate increased motor activity with repeated, intermittent drug exposure (Robinson and Berridge, 1993; Stewart and Badiani, 1993). This behavioral outcome persists for months (Paulson et al., 1991) and has been shown in multiple drugs of abuse including cocaine (Post et al., 1992), amphetamine (Robinson and Becker, 1986), morphine (Joyce and Iversen, 1979), heroin (Morrison et al., 2011), nicotine (Benwell and Balfour, 1992), and ethanol (Cunningham and Noble, 1992). This rodent model is rapid, inexpensive, and useful for studying drug-induced neuroplasticity, but it lacks construct validity. Drug injections for locomotor sensitization studies are given non-contingently, which does not represent the human condition. Clinical studies also were unable to show sensitization in cocaine-dependent individuals (Volkow et al., 1997; Martinez et al., 2007), indicating a less-than-ideal model for understanding neural circuit adaptations in addiction pathology.

Conditioned Place Preference (CPP)

Conditioned place preference (CPP) is another rodent model to study addiction behaviors. During CPP, a particular environment is associated with a drug through repeated pairings that leads to an increased time spent in that environment even in the absence of the reward (Bardo and Bevins, 2000; Harris et al., 2005). CPP has been

demonstrated with multiple drugs of abuse including cocaine (Harris and Aston-Jones, 2003), morphine (Mucha et al., 1982), heroin (Hand et al., 1989), amphetamine (Spyraki et al., 1982), ethanol (Reid et al., 1985), and nicotine (Fudala et al., 1985). The CPP procedure is a simple and rapid method to evaluate the rewarding properties of drugs, but there are some methodological issues that do not match the human situation. Similar to the locomotor sensitization model, CPP involves non-contingent, systemic drug injections. As addicts require an action to obtain drugs, an instrumental task is a better representation of the human condition. Also, with passive injections, a ceiling effect of the drug makes it difficult to establish a dose-response curve in this model (Bardo and Bevins, 2000). These drawbacks indicate that CPP is not the best-suited model for studying addiction pathology.

Self-administration

The self-administration and reinstatement (relapse) model addresses all these concerns, is a better representation of the human condition, and is the best-characterized and accepted animal model to assess drug-taking and relapse-like behaviors. The model shows both high face and construct validity (Epstein et al., 2006). During self-administration, rats perform a voluntary behavior (e.g. press on an active lever) to receive an intravenous drug infusion. These active lever presses and drug infusions are often paired with stimuli (contingently or non-contingently depending on the procedure) that become associated with drug use. Light and tone stimuli represent discrete drug cues associated with drug taking, and surrounding environmental stimuli within the self-administration chamber signify contextual cues. After repeated pairings and learned behavior, rats undergo extinction where lever pressing does not yield drug infusions or light and tone cues. After lever pressing is extinguished, reinstatement of

lever pressing (e.g. relapse of drug seeking) can be assessed. Drug-primed, stress-induced, and stimulus-induced (discrete cue-induced or context-induced) reinstatements are the most commonly studied triggers of relapse (Shaham et al., 2003). After a brief description of each reinstatement procedure, we'll focus mostly on stimulus-induced reinstatement—e.g. context-induced and cue-induced reinstatement of drug seeking, as these stimuli frequently provoke craving and relapse.

Reinstatement (an animal model of relapse)

Following self-administration and extinction training, reinstatement of extinguished drug seeking can be induced by drug priming injections, stressors, discrete drug cues, or contextual drug cues. As mentioned previously, similar triggers provoke relapse in human addicts. As such, these different modalities for inducing reinstatement of drug seeking each reflect synonymous human conditions of relapse.

In the animal model of cocaine-primed reinstatement, a small dose of cocaine is given prior to a reinstatement test under conditions similar to extinction, which elicits robust responding on the lever associated with cocaine infusions during self-administration (McFarland and Kalivas, 2001). In an animal model of stress-induced reinstatement, various forms of stressors including footshock (Shaham and Stewart, 1995; Shaham et al., 2003; Mantsch et al., 2016), food deprivation (Shalev et al., 2003), and pharmacological induction of stress-like states with yohimbine or corticotropin-releasing hormone (Erb et al., 2006; Feltenstein and See, 2006) all induce drug-seeking behavior.

However, this dissertation focuses on reinstatement behavior induced by stimuli that are conditionally associated with drug usage: discrete cue-induced (Meil and See, 1997) and context-induced (Crombag and Shaham, 2002) reinstatement. These two

forms of relapse are particularly important to understand because managing conditioned responses elicited by drug-associated objects and surrounding environments are likely the first challenges recovering addicts face when discharged from rehab.

Cue-induced reinstatement

In a conditioned stimulus (CS+) reinstatement procedure, active lever presses during reinstatement result in the presentation of the CS+; often a discrete light and tone cue previously paired with drug infusions. The presentation of this CS+ elicits robust lever responding (e.g. drug seeking) even in the absence of drug infusions (Meil and See, 1997). In a discriminative stimulus (DS+) reinstatement procedure, reinstatement of extinguished lever pressing is elicited by re-exposure to non-contingent stimuli originally presented during reinforced drug taking. These stimuli signify drug availability (even in the absence of drug) and thus evoke robust lever responding. In both procedures, the amount of lever responding is assessed to determine the degree of cue-induced reinstatement of drug seeking. This dissertation focuses on a CS+ procedure to examine cue-induced reinstatement of cocaine seeking.

Cue-induced reinstatement behavior has been established with multiple drugs of abuse including cocaine (Meil and See, 1997; Smith et al., 2009; Mahler et al., 2014), heroin (LaLumiere and Kalivas, 2008; Lai et al., 2013), methamphetamine (Koseki et al., 2014; Parsegian and See, 2014), morphine (Grasing et al., 2005), nicotine (Gipson et al., 2013b), and ethanol (Bäckström and Hyytiä, 2004). Many brain regions also have been implicated in cue-induced reinstatement of cocaine seeking (discussed in the following sections). Different variations of this procedure have been developed to answer specific scientific questions, as seen in Chapters 2 and 3 of this dissertation.

Context-induced reinstatement

To test for context-induced reinstatement, rats learn to self-administer drug in one environmental context, extinguish the behavior in an alternative context, and then are re-exposed to the environment originally paired with the drug—which provokes reinstatement behavior (Crombag and Shaham, 2002). Drug-associated contextual stimuli act as 'occasion setters' for drug craving and usage (Crombag et al., 2008). This type of procedure, termed an ABA renewal design, was first characterized in fear conditioned learning tasks (Bouton and Bolles, 1979). It has shown repeated effectiveness in reinstatement of cocaine (Crombag and Shaham, 2002; Fuchs et al., 2005; Kearns and Weiss, 2007; Fuchs et al., 2008), heroin (Bossert et al., 2011; 2012), methamphetamine (Rubio et al., 2015), alcohol (Burattini et al., 2006; Hamlin et al., 2007; Moorman et al., 2016), and nicotine (Diergaarde et al., 2008) seeking.

Multiple variations of this procedure have been conducted with (Crombag and Shaham, 2002) and without (Fuchs et al., 2005) response-contingent discrete cues during self-administration and extinction training, but none to our knowledge have performed context- and discrete cue-induced reinstatement tests separately within the same animals. In Chapter 3 of this dissertation, we will use a modified self-administration-extinction-reinstatement procedure to allow for a within-subjects examination of context- and cue-induced reinstatement. This paradigm is designed to elucidate neural circuits involved in different forms of stimulus-induced reinstatement of drug seeking within the same subjects.

Nucleus accumbens and lateral septum: subcortical regions mediating addiction

Elucidating the neurocircuitry involved in stimulus-induced relapse is crucial to understanding how to reduce the likelihood of relapse. As such, experiments in this

dissertation focused on identifying neurocircuits recruited during relapse to discrete and/or contextual cues. Two subcortical brain structures—the nucleus accumbens and lateral septum—offer promising therapeutic targets for addiction.

Nucleus Accumbens

Nucleus accumbens (NAc) is a canonical structure in reward circuitry (Kalivas and Volkow, 2005; Barker et al., 2015; Volkow and Morales, 2015). Increased dopamine transmission from the ventral tegmental area (VTA) to NAc facilitates the addictive properties of drugs (Anderson et al., 2006; Schmidt et al., 2006). NAc is thought to function as a link between stimulus-reward associations and response output systems (Pennartz et al., 1994). However, NAc is not a heterogeneous structure—it is comprised of both the nucleus accumbens core (NAcC) and nucleus accumbens shell (NAcSh). These two sub-structures differ in anatomical connectivity (Zahm, 2000; Voorn et al., 2004), neurochemical properties (Meredith, 1999; Zahm, 1999; Goto and Grace, 2008), electrophysiological responses (O'Donnell and Grace, 1993; Pennartz et al., 1994) and behavioral functions (Jongen-Rêlo et al., 2003; Cassaday et al., 2005; Ito and Hayen, 2011). Importantly, NAcC and NAcSh play contrasting roles in drug abuse (Ito et al., 2000; Kalivas and McFarland, 2003; McFarland et al., 2003; Fuchs et al., 2004; Peters et al., 2008a; Owesson-White et al., 2009; Chaudhri et al., 2010; Shiflett and Balleine, 2010; Fischer et al., 2013). For example, inhibition of NAcC, but not NAcSh, attenuates cue-induced reinstatement of cocaine seeking (Fuchs et al., 2008), whereas NAcSh is necessary for context-induced reinstatement of heroin seeking (Bossert et al., 2006; 2007; Marchant et al., 2015).

Nucleus accumbens neurons are characterized as medium spiny neurons that release the inhibitory neurotransmitter gamma-aminobutyric acid (GABA; >95% of cells).

NAc medium spiny neurons have hyperpolarized (intrinsically less excitable) potentials and do not fire spontaneous action potentials (Floresco, 2015). Therefore, excitatory glutamatergic inputs arising from cortical regions strongly influence the activity and subsequent functions of the nucleus accumbens.

Glutamate transmission in NAc is critical in driving drug-seeking behavior, and dysregulated glutamate transmission and glutamate homeostasis in NAc are theorized to mediate perpetual relapse (Kalivas and Volkow, 2005; Kalivas, 2009). AMPA, NMDA, or mGluR5 glutamate receptor antagonists systemically administered or microinjected into NAcC attenuate discrete cue-induced reinstatement of cocaine seeking (Bäckström and Hyytiä, 2007; Kumaresan et al., 2009). Additionally, inhibition of AMPA receptors in NAcC or NAcSh attenuates context-induced reinstatement of cocaine seeking (Xie et al., 2012). Glutamate transmission in NAc is also necessary for heroin (LaLumiere and Kalivas, 2008), alcohol (Sinclair et al., 2012), and nicotine (Liechti et al., 2007; Gipson et al., 2013b) seeking. The major sources releasing glutamate into NAc are the medial prefrontal cortex, basolateral amygdala, and ventral hippocampus (Britt et al., 2012). Specific regions that release glutamate into NAcC or NAcSh drive only certain reinstatement modalities or are recruited only with particular drugs of abuse (Fuchs et al., 2008; Marchant et al., 2015). Therefore, it is imperative to dissociate circuits involved in addiction to identify potential targets for future therapeutics. Experiments in Chapter 2 examine glutamatergic inputs to NAcC or NAcSh that are activated during cue-induced reinstatement of cocaine seeking.

Lateral septum

Much less is known about lateral septum (LS) in reward and addiction compared to NAc. This is interesting given that there are neurochemical, electrophysiological, and

anatomical similarities between NAc and LS (Zahm et al., 2013). LS neurons strongly resemble NAc neurons; they are medium-sized spiny neurons that have radiating dendrites with extensive branching (Risold and Swanson, 1997; Paxinos, 1995) and use GABA as their neurotransmitter (Paxinos, 1995). LS and NAc are anatomically adjacent structures divided by the island of Calleja magna (Paxinos, 1995). Similarly to NAc, LS is a heterogeneous structure. LS is divided into rostral/intermediate, caudal/dorsal, and ventral regions based on differences in the sizes and densities of their neurons and their connections with other brain structures (Risold and Swanson, 1997; Paxinos, 1995). Differences in connectivity will be addressed in the sections discussing LS afferents.

Despite the similarities between NAc and LS, relatively little has been reported concerning the role of LS in addiction. This is in spite of the fact that LS was the first brain region to show robust intracranial electrical self-stimulation in rodents (Olds and Milner, 1954). In this historic study, rats with electrodes implanted in LS spent 75-92% of their time responding for electrical stimulations; the highest responding rodent self-stimulating 7500 times in 12 hours (up to 742 responses per hour). During non-stimulating periods, responding was nearly zero. Stimulation of cingulate, tegmentum, and caudate did not initiate responding nearly to the same degree as LS. In 1963, electrodes were implanted into the septal area of two human patients (one with chronic narcolepsy and the other with chronic epilepsy). Both reported that septal stimulation caused pleasurable and rewarding feelings compared to stimulators implanted in other brain regions (Heath, 1963).

Lateral septum has been relatively neglected in past decades, but reward and addiction research involving LS is re-emerging. Inactivation of LS, and of LS inputs to orexin neurons in lateral hypothalamus, reduces the preference for the cocaine-paired side in conditioned place preference (Sartor and Aston-Jones, 2012). Additionally, rostral

(but not caudal) LS neurons that project to lateral hypothalamus express more Fos cells in cocaine-conditioned compared to saline-conditioned rats (Sartor and Aston-Jones, 2012). LS neurons that project to VTA express more Fos cells during reinstatement of cocaine seeking behavior relative to multiple control conditions (Mahler and Aston-Jones, 2012), and pharmacological inhibition of that circuit attenuates reinstatement of cocaine seeking (Luo et al., 2011). LS has also been implicated in drugs of abuse other than cocaine. LS neurons express Fos during methamphetamine seeking (Cornish et al., 2012) and with high doses of administered methamphetamine (Tomita et al., 2013), and rats self-administer morphine directly into LS (Le Merrer et al., 2007). These studies all signify the potential importance of LS in drug addiction.

Similarly to NAc, anatomical studies have revealed several glutamatergic regions that project to LS. One recent study used optogenetic and chemogenetic tools to examine ventral hippocampal inputs to LS in the regulation of feeding behavior (Sweeney and Yang, 2015). However, unlike NAc literature, no studies to our knowledge have examined brain regions that drive relapse-like behaviors via LS. Experiments in Chapter 3 will examine glutamatergic inputs to LS involved in reinstatement behavior.

Glutamatergic inputs to nucleus accumbens or lateral septum involved in drug addiction

Prefrontal cortex

The medial prefrontal cortex (mPFC) is a cortical structure involved in many higher order cognitive processes including attention, executive function, working memory, and behavioral flexibility (Kane and Engle, 2002; Floresco, 2015). Functional knowledge of the prefrontal cortex originally stemmed from an accident in 1848 when a railroad worker named Phineas Gage was impaled through the frontal skull with an iron railroad rod (Van Horn et al., 2012). He survived the accident, but it resulted in

significant changes to his personality including deficits in emotional processing and rational decision-making (Damasio et al., 1994; Thiebaut de Schotten et al., 2015). Clinical observations of Phineas Gage laid the groundwork for the study of prefrontal cortical dysfunction in multiple neuropsychiatric disorders, including addiction. Relatively recent research has focused on elucidating cortical inputs to downstream subcortical regions that modulate behavioral responses. Here, we focused on mPFC inputs to NAcC or NAcSh. Although there is substantial literature in the addiction field about the mPFC-NAc circuit, only subsets of mPFC neurons (based on their projection targets) are involved in particular drugs of abuse, reinstatement types, or behavioral paradigms (West et al., 2014; Moorman and Aston-Jones, 2015). It is important to decipher these subcircuits to fully characterize the circuitry of addiction pathology.

The mPFC contains the prelimbic (PL) and infralimbic (IL) cortices (also anterior cingulate). PL and IL cortices send predominantly glutamatergic projections and differently innervate NAcC and NAcSh. Anterograde tracers show that PL densely labels both NAcC and NAcSh with a slight tendency of more PL fibers in NAcC than NAcSh (Vertes, 2004). Infralimbic cortex also projects to NAcC and NAcSh with IL more heavily innervating NAcSh (Vertes, 2004). In a similar manner, retrograde tracers into NAcC heavily label dorsal and ventral PL and less robustly labeled IL, whereas NAcSh injections densely labeled IL and moderately labeled ventral PL (Brog et al., 1993).

PL, IL, and their projections to NAcC or NAcSh have differential involvement in reinstatement behavior. Inhibition of PL attenuates context-, cue-, and drug prime-induced reinstatement of cocaine seeking (McFarland et al., 2003; McLaughlin and See, 2003; Fuchs et al., 2005). Optogenetic or pharmacological inhibition of PL inputs to NAcC attenuates cocaine-primed and cocaine- plus cue-induced reinstatement of cocaine seeking (McFarland et al., 2003; Stefanik et al., 2013; 2016) and synaptic and

electrophysiological adaptations occur in NAcC following inactivation of PL (Gipson et al., 2013a). In contrast, inhibiting IL, and IL projections to NAcSh augments reinstatement of cocaine seeking (Peters et al., 2008a; 2008b), but also attenuates context-induced reinstatement of heroin seeking (Bossert et al., 2011; 2012). The heterogeneity of mPFC structures and mPFC inputs to NAc in drug addiction behaviors indicate that it is necessary to elucidate all avenues of the circuit to gain further insight to its involvement in drug addiction.

In contrast to the mPFC-NAc circuit, mPFC projections to LS are substantially less dense. Of these projections, IL projects more heavily to LS than PL (Vertes, 2004). Retrograde tracer injections in LS label cells ipsilaterally in IL, and bilaterally (but preferentially ipsilaterally) in PL (Gabbott et al., 2005). Similarly, anterograde tracers injected into PL or IL show minimal labeling in LS (Sesack et al., 1989). It is unknown if mPFC inputs to LS play a role in addiction behaviors.

Basolateral Amygdala

The amygdala is a critical structure for emotion and affective processing. Clinical knowledge about amygdaloid function stemmed from epileptic patients who received implantations of chronic stimulating electrodes into their amygdala. In these patients, amygdala stimulation produced a state of fantasy and imaginative experiences (Delgado et al., 1968; Gloor et al., 1982), indicating amygdala involvement in altering affective states. Animal lesion and inactivation studies also provided knowledge into amygdala function. Preclinical studies identified that the basolateral amygdala (BLA) is particularly important for establishing positive and negative conditioning (Weiskrantz, 1956; McDonald, 1998; Baxter and Murray, 2002), assigning reward value (Floresco, 2015), and processing drug-related cues during reinstatement (Buffalari and See, 2010).

Basolateral amygdala is predominantly a glutamatergic structure and has one of its densest projections to NAc. Anatomy studies reveal that BLA projects to both NAcC and NAcSh. Retrograde tracers injected into medial NAcSh label ipsilateral neurons in the posterior BLA and accessory basal nuclei (Brog et al., 1993), whereas retrograde tracers into NAcC label ipsilateral neurons in the rostrocaudal extent of BLA and lesser so in accessory basal nuclei (Brog et al., 1993). In regards to reinstatement of drug seeking, excitotoxic lesions or transient inhibition of BLA attenuates cue-induced (Meil and See, 1997; Stefanik and Kalivas, 2013) and context-induced reinstatement of cocaine seeking (Fuchs et al., 2005); and at the circuit-level, inhibition of BLA inputs to NAcC blocks cue-induced reinstatement of cocaine seeking (Di Ciano and Everitt, 2004a; Stefanik and Kalivas, 2013).

In contrast to the BLA-NAc pathway, BLA sparsely projects to LS (Canteras et al., 1992; 1995). Furthermore, very few studies have investigated amygdala inputs to LS in any behavioral function. One study indicated that olfactory sensory information is processed in amygdala and sent to LS (Nelson and Trainor, 2007); however, amygdala inputs to LS have not been examined in addiction behaviors.

Hippocampus

Hippocampus is required for higher order cognitive processing of memory. A patient named H.M. confirmed this function after receiving surgery to remove his medial temporal lobe (including the hippocampus) to suppress chronic seizures. The surgery resulted in anterograde and some retrograde amnesia (Scoville and Milner, 1957). Memory integration, storage, and recall including associative memory processing and spatial and contextual learning all require hippocampal activity (Squire, 1992; Fanselow and Dong, 2010). For example, context-conditioned fear learning requires dorsal

hippocampal activity (Holland and Bouton, 1999) and lesions of the dorsal hippocampus cause deficits in spatial memory tasks including radial arm maze (Pothuizen et al., 2004) and Morris water maze (Morris et al., 1982; Moser et al., 1995).

The hippocampus is also important for addiction behaviors. Inhibition of dorsal hippocampus attenuates context-, but not cue-induced reinstatement of cocaine seeking (Fuchs et al., 2005). Transient inactivation of hippocampus also impairs the acquisition and expression of conditioned place preference for cocaine (Meyers et al., 2006). Moreover, inactivation of dorsal CA3 (Cornu Ammonis 3) region of the hippocampus attenuates lever responding during context-induced reinstatement of cocaine seeking (Luo et al., 2011).

The densest output of hippocampus is LS via the fornix (Risold and Swanson, 1997; Sheehan et al., 2004). Pyramidal neurons of the CA3 of dorsal hippocampus project bilaterally and selectively to the caudal/dorsal area of LS (Risold and Swanson, 1996; 1997) and these projections predominantly synapse on dendritic spines of LS neurons (Risold and Swanson, 1997; Paxinos, 1995). A study from our lab characterized a novel circuit from the dorsal CA3 region of the hippocampus to the VTA via the caudal/dorsal region of LS. In this study, CA3 theta stimulation induced both inhibitory and excitatory responses in VTA neurons, whereby transient inactivation of the caudal/dorsal LS blocked these responses. Additionally, pseudo rabies virus injections into VTA labeled caudal/dorsal LS neurons at 36 hours, and dorsal CA3 neurons at 48 hours after injection, indicating a dorsal CA3-LS-VTA circuit (Luo et al., 2011). Given the implications of dorsal hippocampus in addiction and the densest output of the hippocampus being LS, it is somewhat surprising that no studies have investigated if hippocampal neurons drive drug addiction behaviors via LS.

In contrast to the dorsal hippocampus, which only innervates a relatively small

area of LS (caudal/dorsal), ventral hippocampus innervates a much larger portion of LS. Retrograde tracer injections into the rostral/intermediate and ventral subregions of LS predominantly label the CA1 and subiculum regions of ventral hippocampus (with scattered cells in ventral CA3) (Risold and Swanson, 1996; 1997). These projections are ipsilateral, which differ from the bilateral projections from dorsal hippocampus to LS. In regards to addiction studies, inactivation of ventral hippocampus decreases cue-induced, context-induced and cocaine-primed reinstatements of cocaine seeking (Sun and Rebec, 2003; Rogers and See, 2007; Lasseter et al., 2010) and context-induced reinstatement of heroin seeking (Bossert and Stern, 2012). However, the involvement of ventral hippocampal neurons that project to LS has not been characterized in addiction behaviors.

Ventral hippocampus also densely innervates NAc. Retrograde tracer injections into the medial NAcSh densely label the ventral subiculum and CA1 regions of the hippocampus (Brog et al., 1993). Ventral subiculum and CA1 projections also extend to the medial NAcC (Groenewegen et al., 1987; Brog et al., 1993; Groenewegen et al., 1999; Britt et al., 2012). A few studies have investigated ventral hippocampal projections to NAc in addiction behaviors. For example, ventral hippocampal neurons that project to NAcSh express Fos during context-induced reinstatement of heroin seeking, and inhibition of the circuit decreases context-induced heroin seeking (Bossert et al., 2016). However, to our knowledge, projections from ventral hippocampus to NAc during cue-induced reinstatement of cocaine seeking have not been investigated. Chapter 2 examines projections from ventral hippocampus to NAcC or NAcSh activated during cue-induced reinstatement of cocaine seeking.

Techniques to decipher circuits

Neuronal tracers

Neuronal tracers elegantly decipher anatomically connected regions by means of axonal transport. There are two types of neuronal tracers: anterograde and retrograde tracers. An anterograde tracer injected into a particular brain region transports from the cell bodies towards the axonal terminals. A retrograde tracer injected into a particular brain region transports from the axon terminals to the cell bodies.

Neuronal tracers can precisely identify neuronal pathways (Oztas, 2003) and can be used in conjunction with other immunohistochemistry methods including Fos expression (the protein of the immediate early gene *c-fos*). Fos protein is rapidly and transiently expressed in the nucleus of neurons after synaptic stimulation. As such, Fos protein signifies enhanced activity of neurons during a (relatively) focal period of time (Müller et al., 1984). When combined with tracers, Fos can metabolically map activation of specific circuits at specific periods of time or functions.

However, there are some technical issues to consider with neuronal tracers. Uptake by passing fibers (Chen and Aston-Jones, 1995), bidirectional or collateral-collateral labeling (Gerfen and Sawchenko, 1984; Chen and Aston-Jones, 1998), the boundaries of the injection site (Brog et al., 1993), and selectivity of uptake are technical concerns with this technique. Fortunately, evidence indicates bidirectional-labeling shows some visually distinguishable characteristics, and the “fringe” area surrounding the central injection site minimally contributes to tracer labeling (Gerfen and Sawchenko, 1984; Brog et al., 1993). Nonetheless, it is necessary to compare results from neuronal tracers to other anatomical studies and incorporate other methods to decipher true connections.

Overall, neuronal tracing is a sophisticated technique to examine projections to

or from a region of interest. In conjunction with Fos immunohistochemistry, it can be a powerful tool to characterize activated neural circuits with (some) temporal specificity during a variety of different behaviors.

Neuropharmacology

One of the most widely used and classic methods to examine regional and circuit functions is neuropharmacology. Neuropharmacology uses drugs to investigate receptor signaling during specific behaviors. There are several advantages to this technique. Firstly, pharmacological drugs target known receptors that have relatively known functions (caveats to this mentioned below). Secondly, the drugs act on receptors that are part of normal physiology in contrast to the newer techniques utilized to decipher circuits. Thirdly, pharmacological methods can be performed relatively immediately as there is no need for the time required for viral transduction, and lastly, results can be easily compared to previous studies. Pharmacology can also be used to understand functions in connected circuits. This is done using an asymmetric pharmacological disconnection procedure. This involves pharmacologically manipulating a brain region in one hemisphere and a projection target on the contralateral hemisphere (Gaffan et al., 1993; Marchant et al., 2015). Although advantageous for deciphering neurocircuitry, the pharmacological disconnection procedure is not ideal for certain circuits (discussed below).

Neuropharmacology has disadvantages as well. One caveat is that many pharmacological drugs have off-target binding sites, which can make interpreting results ambiguous. Additionally, receptors are often expressed on multiple cell types; therefore, there is minimal cell-type specificity when using pharmacology. Also, the location/spread of the drug action can be uncertain. Lastly, an asymmetric pharmacological

disconnection procedure does not work for every circuit. Many projections that are mainly ipsilateral also have less dense contralateral projections, which are often neglected with this procedure. It must be demonstrated that a single intact hemisphere can maintain behaviors (Marchant et al., 2015). This method does not allow dense bilateral projections (in which both circuits may have a role) to be manipulated (e.g. dorsal hippocampus to LS circuit).

Overall, neuropharmacology is a necessary technique to elucidate brain regions and circuits not well characterized in the literature, but new tools help expand the scope of scientific questions and knowledge.

Viral Transduction

Optogenetics and chemogenetics (Designer Receptors Exclusively Activated by Designer Drugs; DREADDs) are two new techniques that are transforming neuroscience research. Both these techniques use viral transduction to insert novel genes into cell-type specific neurons. These techniques are used to manipulate specific cell types within particular brain regions or circuits for greater specificity of effects. This is advantageous over methods like neuropharmacology where cell-type specificity is more difficult to obtain (Zhu and Roth, 2015).

In the case of optogenetics, a gene for a light-gated protein is virally transduced into neurons, then fiber optics are implanted, and light at a specific wavelength is used to activate the light-sensitive proteins. Light-activation of these proteins manipulates cell-type specific neuronal activity (Boyden et al., 2005; Deisseroth, 2011). The greatest advantage of optogenetics is precise temporal control (millisecond scale) of neurons (Boyden et al., 2005; Aston-Jones and Deisseroth, 2013). However, optogenetics is limited clinically for several reasons. Firstly, it requires an implanted source of light

delivery. Secondly, sufficient light needs to be transmitted through the tissue to alter neural activity (Williams and Denison, 2013), and lastly, the exogenous genes do not use physiologically natural signaling.

On the other hand, DREADDs (e.g. chemogenetics) are designer G-protein coupled receptors that are activated solely by synthetic ligands (Armbruster et al., 2007; Roth, 2016). The designer receptors are engineered from kappa-opioid or human muscarinic receptors (Armbruster et al., 2007; Vardy et al., 2015). In the presence of clozapine-*N*-oxide (CNO) or salvinorin B—the designer ligands for the designer muscarinic or kappa-opioid receptors, respectively—DREADDs can manipulate (excite or inhibit) specific neuronal populations (Armbruster et al., 2007; Rogan and Roth, 2011; Vardy et al., 2015). The designer receptors use physiologically conserved GPCR signaling pathways—Gi/o, Gq, or Gs—to influence neural activity. The hM4Di DREADD (used in experiments in Chapter 3) is a Gi/o-coupled receptor engineered from the human M4 muscarinic receptor that silences neurons (in the presence of CNO) by decreasing cyclic adenosine monophosphate (cAMP) signaling and activating inwardly rectifying potassium channels (Figure 1-1; Armbruster et al., 2007). The hM4Di DREADD has no constitutive activity and CNO is biologically inert (Armbruster et al., 2007). When the hM4Di gene is inserted into a viral vector driven by a specific neuronal promoter, it can be expressed in select neuronal populations and terminals and used to selectively inhibit activity of specific neurons or neuronal circuits.

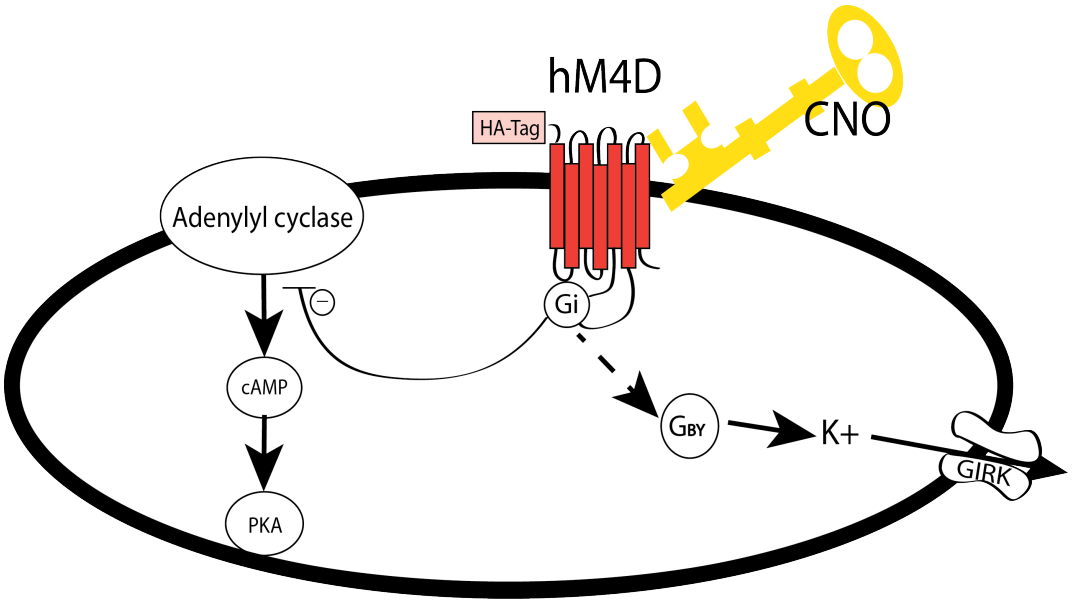

Figure 1-1. Schematic of DREADD mechanism. The hM4Di-DREADD receptor (red) is a 7-transmembrane receptor modified from a human muscarinic receptor. In the presence of clozapine-*N*-oxide (CNO; yellow key), the ligand can "unlock" the synthetic receptor to activate it. Activation of the hM4Di receptor uses physiologically conserved GPCR (Gi) signaling pathways to modulate neuronal activity. DREADD-mediated inhibition of neuronal firing occurs by decreasing adenylyl cyclase, cyclic adenosine monophosphate (cAMP), and protein kinase A (PKA) and by activating potassium and stimulating G-protein coupled inwardly retifying potassium channels (GIRKs). Fused to the hM4Di receptor is a hemagglutinin (HA) tag that can be used to localize hM4Di receptors in neurons. Diagram adapted from Rogan and Roth, 2011.

There are several advantages (and potentially greater clinical implications) to using DREADDs over optogenetics. DREADDs do not require specialized equipment beyond routine laboratory equipment and they act on normal GPCR signaling cascades. This is important as DREADDs may have more naturalistic effects on neuronal activity (Smith et al., 2016). Also, the designer drug, CNO, is an orally available agonist—and a much less invasive method to manipulate neurons than optical fiber implantation. Additionally, DREADDs act on a slower time-scale (on the order of minutes), which can be either advantageous or disadvantageous depending on the behavioral manipulation (Aston-Jones and Deisseroth, 2013).

DREADDs have been shown to be effective for characterizing the role of select neuronal populations and pathways in a variety of behaviors. DREADDs have been effectively used to study addiction (Mahler et al., 2014; Pina et al., 2015; Marchant et al., 2016), feeding (Sweeney and Yang, 2015), epilepsy/seizures (Kätzel et al., 2014), social behavior in autism models (Peñagarikano et al., 2015), Down syndrome models (Fortress et al., 2015), anesthesia and sleep (Sasaki et al., 2011; Vazey and Aston-Jones, 2014), and memory (Zhu et al., 2014; Stefanelli et al., 2016).

Both optogenetics and chemogenetics still require additional research to gain further insight into their mechanisms. Currently, it can be difficult to interpret unexpected or inconsistent results when compared to other methods. Overall though, optogenetics and chemogenetics are powerful tools to elucidate specific neuronal populations and circuits; however, DREADDs may provide greater clinical potential.

Significance of the current research

Reducing the likelihood of relapse with therapeutics could be a valuable method of intervention for treating drug addiction (O'Brien, 2005), however, currently there are no FDA-approved treatments for the disease. This may be partly due to uncharacterized circuits involved in addiction pathology. Elucidating brain structures and connectivity in diseases has been a focal objective in neuroscience research for decades. The government-sponsored Brain Initiative to map the human brain is evidence that the government believes understanding brain connectivity and its dysregulation in disease is an incredibly important issue. The current studies aim to understand the neurocircuitry involved in drug addiction by addressing the following goals:

Chapter 2:

1. Examine activation of prefrontal cortex, amygdala, and hippocampal neurons that project to NAcC or NAcSh during cue-induced reinstatement of cocaine- or sucrose-seeking.
2. Draw comparisons between projections to NAcC versus NAcSh afferents.
3. Investigate if neurocircuits are activated specifically to cues associated with cocaine versus natural rewards (sucrose).
4. Determine if the degree of activation in neurons that project to NAcC or NAcSh correlate with cue-induced cocaine- or sucrose-seeking behavior.

Chapter 3:

5. Develop a novel behavioral paradigm to dissociate two established triggers of relapse (context- and cue-induced reinstatement) within the same subjects to better interpret relapse behaviors.
6. Confirm a causal role for LS in reinstatement of cocaine seeking.
7. Determine activation of LS neurons, hippocampal neurons, and LS afferents during context- or cue-induced reinstatement of cocaine seeking.
8. Characterize inputs to LS functionally necessary for context- and/or cue-induced reinstatement of cocaine seeking.

To aid in achieving these goals, the current studies used a combination of tract-tracing, Fos immunohistochemistry, pharmacological inactivation, and chemogenetic inactivation. This work also developed a novel, within-subjects self-administration-extinction-reinstatement paradigm to examine uncharacterized neurocircuitry during context- and cue-induced reinstatement. Results from these experiments will provide a better understanding of NAcC and NAcSh circuitry in addiction, and are the first to

elucidate brain regions that drive relapse via lateral septum. Overall, these studies provide greater insight into the neurocircuitry involved in drug relapse and identify potential therapeutic targets, which can facilitate the development of future therapeutics for the disease.

CHAPTER 2: DETERMINING ACTIVATION OF NUCLEUS ACCUMBENS CORE AND SHELL AFFERENTS DURING CUE-INDUCED REINSTATEMENT OF COCAINE OR SUCROSE SEEKING.

Introduction

Nucleus accumbens (NAc) is an integral structure in reward circuitry (Mogenson et al., 1980; Pennartz et al., 1994; Kalivas and Volkow, 2005; Kalivas et al., 2005; Goto and Grace, 2008; Britt et al., 2012; Floresco, 2015). NAc receives multiple glutamatergic inputs, with major sources from medial prefrontal cortex (mPFC), basolateral amygdala (BLA), and ventral subiculum of the hippocampus (vSub). Studies show that these structures, and their inputs to NAc, are involved in conditioned drug seeking. Pharmacological or optogenetic inhibition of the prelimbic (PL) region of mPFC, or PL terminals in NAc, reduces conditioned cocaine seeking behavior (McFarland et al., 2003; McLaughlin and See, 2003; Stefanik et al., 2013). Similarly, pharmacological or optogenetic inhibition of the BLA to NAc projection reduces cue-induced reinstatement of cocaine seeking (Di Ciano and Everitt, 2004a; Stefanik and Kalivas, 2013). Likewise, inactivation of vSub neurons reduces cue-induced reinstatement of cocaine seeking (Sun and Rebec, 2003), and theta burst stimulation of vSub elicits reinstatement of cocaine seeking (Vorel et al., 2001).

However, recent evidence indicates that only subpopulations of neurons within brain structures drive drug-seeking behaviors (Bossert et al., 2011; Cruz et al., 2013; 2014; Moorman and Aston-Jones, 2015). The importance of these neural subpopulations for drug-seeking behavior may depend on their projection targets. For example, neurons in mPFC, BLA, and vSub activate during cue-induced cocaine seeking behavior (as measured by *c-fos* mRNA or Fos protein; markers of neural

activity) (Kufahl et al., 2009), but neurons in these regions that project to ventral tegmental area (VTA), a fundamental structure in addiction-related behavior, are not activated (Mahler and Aston-Jones, 2012). These findings indicate heterogeneous activity within these regions based on specific neural projection targets.

Using anatomical tract-tracing and Fos immunohistochemistry, we examined the extent to which subpopulations of neurons in PL cortex, infralimbic cortex (IL), BLA and vSub that project to NAc are activated by discrete cues associated with cocaine or a natural reinforcer (sucrose). We distinguished NAc core (NAcC) and shell (NAcSh) sub-regions as they show dissociable roles in drug abuse (Parkinson et al., 1999; Fuchs et al., 2004; Ito et al., 2004; Bossert et al., 2007; Chaudhri et al., 2010). NAcC, as well as glutamatergic afferents to NAcC, are well characterized as critical nodes in cue- and cocaine-induced reinstatement of cocaine seeking (McFarland et al., 2003; Fuchs et al., 2004; Bäckström and Hyytiä, 2007; Di Ciano et al., 2008; Stefanik et al., 2013) whereas NAcSh, or NAcSh glutamatergic afferents, show variable outcomes in drug-seeking depending on the drug of abuse, manipulation, or behavioral paradigm (Bossert et al., 2007; Di Ciano et al., 2008; Peters et al., 2008a; 2009; Bossert et al., 2012; LaLumiere et al., 2012; Cruz et al., 2014). Therefore, we predicted that mPFC, BLA, and vSub neurons that project to NAcC would be preferentially activated during cue-induced cocaine seeking behavior compared to neurons within the same regions that project to NAcSh.

Methods

Subjects

A total of 93 male Sprague Dawley rats (arrival weight 275-325 g; Charles River, Raleigh, NC) were used in these experiments. Rats were single housed under a reverse

12:12 h light cycle in a temperature- and humidity-controlled animal facility with ad libitum access to food and water. All procedures were approved by the Institutional Animal Care and Use Committees of Medical University of South Carolina and Rutgers University, and were conducted according to specifications of the Guide for the Care and Use of Laboratory Animals (National Research Council, 2011).

Intravenous catheter surgery

Rats were anesthetized with ketamine/xylazine (56.5/8.7 mg/kg, i.p., respectively) and given an analgesic (meloxicam 1 mg/kg or rimadyl 5 mg/kg, s.c.). Rats conditioned to cocaine were implanted with a chronic indwelling intravenous catheter (made with a modified 22 ga cannula; Plastics One) into the right jugular vein that exited the body via a biopsy hole on the back. Catheters were flushed with cefazolin (0.1 ml; 100mg/ml) and heparin (0.1 ml; 100 U/ml) immediately after surgery, daily beginning 3 d after surgery, and continuing throughout self-administration training. Rats were allowed to recover for 1 week after surgery prior to beginning self-administration training.

Injection of Retrograde Tracer

Immediately following catheter surgery, rats received a unilateral microinjection of cholera toxin *β* subunit (CTb, ~40 nl; 0.5% dissolved in 0.1M phosphate buffer, Sigma) into NAcC (n=52; 1.3 mm rostral to bregma; 1.8 mm from midline; 7.8 mm ventral to skull) or NAcSh (n=41; 1.3 mm rostral to bregma; 1.0 mm from midline; 7.8 mm ventral to skull) using a glass micropipette (15-20 μm diameter tip) and a pneumatic pressure source (Picospritizer; General Valve, Inc.). Pressure injections were made over a 2 min period, and pipettes remained in place for 10-15 min to minimize diffusion along the pipette track.

Cocaine or Sucrose Self-administration and Extinction

The self-administration procedure was similar to previously published studies from our laboratory (Smith et al., 2009; Mahler and Aston-Jones, 2012; Cason and Aston-Jones, 2013; Mahler et al., 2014). Rats were handled daily beginning 3 d prior to cocaine or sucrose self-administration training. Self-administration (SA) sessions (2 h/d) occurred in operant chambers within sound-attenuating boxes, and controlled by Med-PC IV software (Med-Associates, St. Albans, VT). Operant chambers were equipped with two retractable levers with cue lights above each lever and a red house light on the opposite wall. Responses on the active lever (fixed ratio-1 schedule) resulted in cocaine infusions (0.2 mg/50 µl infusion; cocaine HCl dissolved in 0.9% sterile saline, NIDA) or sucrose pellets (45mg, Test Diet, Richmond, IN, USA) paired with discrete cues (a 78 dB, 2.9 Khz tone and a white cue light above the active lever) during self-administration. After each reward, a 20 s timeout occurred (signaled by turning off the house light) when additional presses did not yield rewards or cues. Presses on the inactive lever were recorded, but had no programmed consequences.

After 10 d of criterion self-administration performance (at least 10 infusions or pellets/session) rats were subjected to extinction training, when responding on either lever had no consequence. Rats received extinction training for a minimum of 7 d and until they met the criteria of <25 active lever presses for 2 or more consecutive days.

Reinstatement and Extinction Tests

Rats were randomly assigned to either extinction (cocaine: n=14, sucrose: n=9) or reinstatement groups (cocaine n=20, sucrose n=15). Rats selected for the extinction groups were given an additional 2 h extinction test the day after reaching extinction criteria. Rats in the reinstatement groups were given a single cue-induced reinstatement

test on the session after reaching extinction criteria, in which pressing the active lever yielded the cues previously paired with cocaine infusions or sucrose pellets, but no reward. All rats were euthanized immediately following their 2 h extinction or reinstatement session. As maximal Fos induction typically occurs at ~90-120 min following neuronal activation (Müller et al., 1984; Young et al., 1991), Fos labeling here should reflect neural activity near the beginning of the test session (e.g. ~0-30 min of the test session; see Figure 2-1a), the period of peak active lever pressing (Smith et al., 2009; Mahler et al., 2014). Rats that did not acquire criteria performance in behavioral training or reinstatement were excluded from analyses (n=6).

Tissue Preparation for Immunohistochemistry

Immediately following extinction or reinstatement testing, rats were deeply anesthetized with a ketamine/xylazine mixture and transcardially perfused with 0.9% saline followed by 4% paraformaldehyde. Brains were removed and postfixed for 24 h in 4% paraformaldehyde, then transferred into a 20% sucrose-azide solution and stored at 4°C. Prior to sectioning, brains were frozen with dry ice, and then cut into 40µm coronal slices on a cryostat. Brain slices were collected in 0.1 M phosphate buffered saline with 1% sodium azide (PBS-Az).

Fos & CTb immunohistochemistry

Fos and CTb dual-labeling immunohistochemistry procedures were similar to previously published methods from our laboratory (Mahler and Aston-Jones, 2012; Sartor and Aston-Jones, 2012; Cason and Aston-Jones, 2013). Sections were quenched with 0.3% hydrogen peroxide for 15 min, washed 3X in PBS, and then blocked in a solution of 2% normal donkey serum (NDS) and PBS with Triton (PBST) for 2 h. The

tissue was then incubated in an NDS-PBST mixture with rabbit anti-Fos primary antibody (1:2000-1:2500, Santa Cruz Biotechnology, 1:10,000 CalBioChem) at room temperature for 16-17 h. Sections were washed in PBST, transferred to biotinylated donkey anti-rabbit secondary antibody (1:500, Jackson ImmunoResearch Laboratories) for 2 h, washed, then incubated in avidin-biotin complex (ABC, 1:500) for 1.5 h, followed by 2 PBST, and one 0.05 M Tris buffer washes. Sections were then incubated with 3,3'-diaminobenzidine (DAB, Sigma), 0.0002% H_2O_2, and 0.6% nickel ammonium sulfate in Tris buffer solution to produce a black Fos nuclear stain. Tissue was transferred to Tris buffer to stop the reaction, washed, and transferred to PBS-Az for 45 min.

For subsequent CTb staining, the same tissue was processed with goat anti-CTb primary antibody (1:20,000, List Biological Laboratories) followed by biotinylated donkey anti-goat secondary antibody (1:500, Jackson ImmunoResearch Laboratories). Sections were then incubated in DAB solution without nickel ammonium sulfate to produce the brown CTb cytoplasmic stain. Brain sections were mounted on glass slides, washed and dehydrated through alcohol and xylenes, and coverslipped with DPX (Sigma).

Fos & CTb Quantification

Mounted tissue sections were photographed using Leica microscopes and Stereoinvestigator (MBF Bioscience) or OpenLab (Improvision) software. CTb injection sites in the NAcC and NAcSh were photographed using a 5x objective and compared to rat brain atlas sections (1.92 mm to 1.08 mm anterior to bregma) (Paxinos and Watson, 2007). Cases in which CTb injections were >20% in the adjacent accumbal region (n=20), e.g. spread into NAcSh for NAcC injections, or in which injections were not in the NAc (n=8), were excluded from analyses. Photographs were also taken of NAcC and NAcSh afferent regions including mPFC (4.20 mm to 2.52 mm anterior to bregma), BLA

(1.80 mm to 3.24 mm posterior to bregma), and vSub (5.40 mm to 6.36 mm posterior to bregma). Three tissue sections per rat representing rostral, intermediate, and caudal levels for each brain region (mPFC, BLA, and vSub) in each group (cocaine extinction, cue-induced cocaine reinstatement, sucrose extinction, and cue-induced sucrose reinstatement) were photographed with a 10x objective and stitched together either automatically using Stereoinvestigator Virtual Slice module, or manually using Adobe Illustrator. Sections for each structure were taken at similar levels from bregma to ensure the quantified surface areas were similar across rats. Structure borders were defined based on the rat brain atlas (Paxinos and Watson, 2007).

An observer blind to experimental conditions counted the total number of CTb-labeled neurons (with or without Fos) within the borders of each NAcC or NAcSh afferent structure (mPFC, BLA, and vSub) for each of the three tissue sections per rat. Regions ipsilateral to the CTb injection site were quantified as they tend to have more retrogradely labeled neurons than contralateral connections (Brog et al., 1993). The percentage of CTb+ cells that co-expressed Fos was determined for each tissue section. Means of these percentages were computed for each rat, and these per rat means were averaged across behavioral groups for each brain region. One rat was excluded for data >2.5 standard deviations from group means in multiple structures.

Data analyses

Unpaired samples *t*-tests were used to analyze all projections to NAcC, or NAcSh. We compared the percentages of CTb+ neurons that were also Fos+ for extinction versus reinstatement for cocaine-trained rats, and separately for sucrose-trained rats. Correlations between the percentages of CTb neurons that were also Fos+ and active lever presses during the first 30 min of reinstatement test sessions were

obtained using a Pearson's correlation coefficient test. A Mann-Whitney test was used to compare extinction vs. reinstatement, and cocaine vs. sucrose lever pressing during the first 30 min of the test sessions, due to unequal variance between groups.

Given the established role of PL and BLA projections to NAcC in cocaine seeking (Di Ciano and Everitt, 2004; Kufahl et. al., 2009; Stefanik and Kalivas, 2013; Zhou et. al., 2014; Martín-García et al., 2014; Stefanik et al., 2016), we predicted *a priori* that Fos activation of NAcC-projecting PL neurons during cue-induced reinstatement would correlate positively with cocaine seeking. Therefore, we used one-tailed Pearson tests to measure correlations between behavior and Fos expression in PL and BLA neurons that project to NAcC. In all other cases, two-tailed Pearson tests were used. Graphpad Prism v6 was used for all statistical analyses, and an alpha value of 0.05 was adopted for all statistical tests.

Results

Self-administration and extinction training

All rats showed stable self-administration and extinction learning. For behavioral analyses, we combined self-administration data for NAcC- and NAcSh-injected rats as the number of rewards and lever presses for rewards did not differ during training (2-way repeated measures ANOVA, structure x day; cocaine infusions: $F_{(1,32)}=0.1863$, $p=0.6689$); sucrose pellets: ($F_{(1,22)}=0.6332$, $p=0.4347$); lever presses for cocaine: $F_{(1,32)}=0.05438$, $p=0.8171$); lever presses for sucrose: $F_{(1,22)}=0.07915$, $p=0.7811$). Both cocaine- and sucrose-trained rats decreased lever pressing over days during extinction training (Figures 2-1b,c; repeated measures ANOVA; Tukey post-hoc across cocaine extinction days; $F_{(6,33)}=78.35$, $p<0.0001$; across sucrose extinction days; $F_{(6,23)}=89.83$, $p<0.0001$).

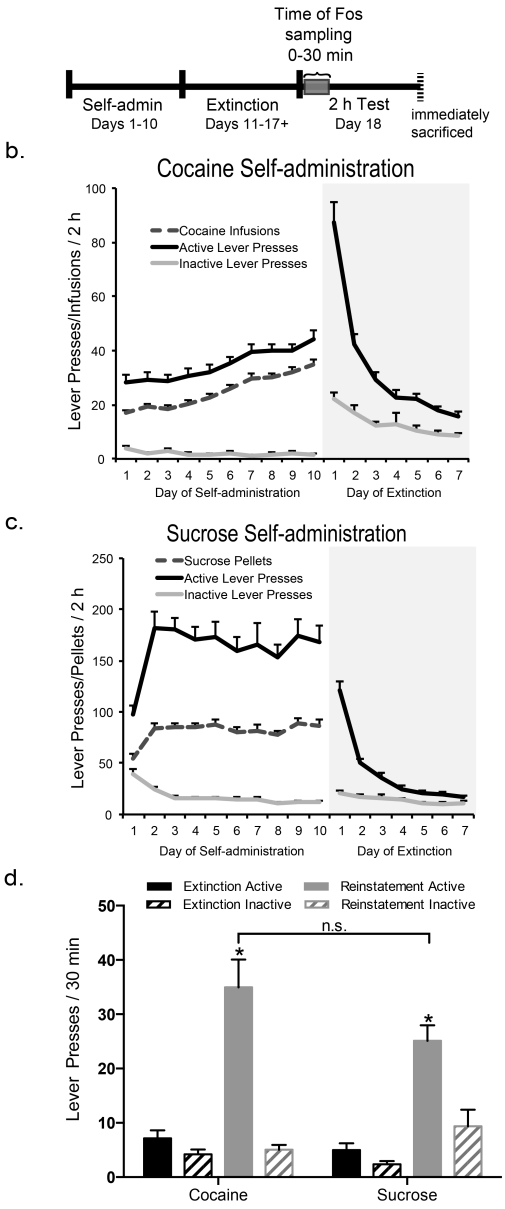

Figure 2-1. Cocaine and sucrose self-administration, extinction, and reinstatement behavior in rats. (a) Timeline for behavioral procedure. Rats were trained to self-administer cocaine and extinguish this behavior, then tested for 2 h in either an additional extinction session or a cue-induced reinstatement session. Rats were euthanized immediately after testing to stain for Fos, which reflects neural activity at the beginning of the session (~0-30 min). (b) Behavioral performance during cocaine self-administration and extinction training. (c) Behavioral performance during sucrose self-administration and extinction training. (d) Mean active and inactive lever presses of rats during the first 30 min of extinction and cue-induced reinstatement test sessions. Active lever responding during cue-induced reinstatement of cocaine and sucrose seeking did not differ at the time Fos was sampled ($p=0.2257$). Reinstatement active lever responses were significantly greater than the respective extinction session responses (cocaine: $p<0.0001$; sucrose: $p<0.0001$). Error bars represent standard error of mean (SEM).

Reinstatement and extinction tests

Rats showed reliable cue-induced reinstatement behavior. Both cocaine- and sucrose-trained rats increased pressing during the reinstatement test session compared to the average of their prior 2 extinction days (paired t-test; cocaine: t_{19}=5.612, p<0.0001; sucrose: t_{14}=9.869, p<0.0001). Similarly, rats subjected to cue-induced reinstatement of cocaine or sucrose seeking showed significantly greater active lever responding than rats that received an extinction test session instead (Mann Whitney, cocaine: U=12.50, n_1=14, n_2=20, p<0.0001; sucrose: U=5, n_1=13, n_2=16, p<0.0001). Active lever pressing was statistically similar in cocaine and sucrose rats during the first 30 min of extinction (unpaired t-test, t_{25}=1.105, p=0.2797) or reinstatement sessions (the time of maximal drug seeking, corresponding to Fos measurements; Mann Whitney, U=121.5, n_1=20, n_2=16, p=0.2257; Figure 2-1d). This indicates that differences in Fos induction among groups (reported below) were not confounded by differences in motor activity. Reinstatement pressing was significantly greater on the active (reward-associated) lever compared to the inactive lever (paired t-test; cocaine: t_{19}=6.045, p<0.0001; sucrose: t_{15}=4.074, p=0.0010).

CTb injection sites

The center of the injection sites for NAcC (Figures 2-2a,b) and NAcSh (Figures 2-2c,d) ranged between 1.92 mm and 1.08 mm anterior to bregma. NAcC injections were located in the central NAcC and generally surrounded the anterior commissure, spreading ~1 mm anteroposteriorly. NAcSh injections were located in the medial portion of the structure and spread ~2 mm dorsoventrally and ~1 mm anteroposteriorly.

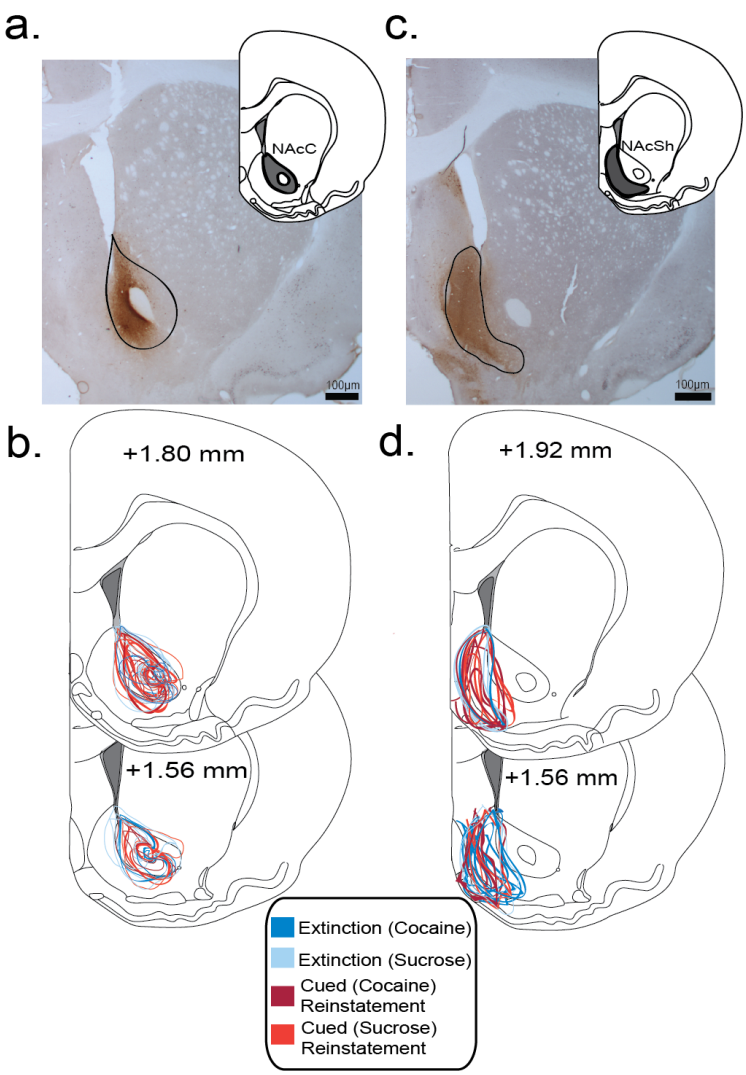

Figure 2-2. Photomicrographs of retrograde tracer injection sites. (a) Representative retrograde tracer (CTb) injection in NAcC (brown stain). Black outer line indicates the approximate outer edge of NAcC on the coronal section. Calibration bar represents 100 µm. Inset shows the location of NAcC in the rat atlas at 1.80 mm anterior to bregma (Paxinos and Watson, 2007). (b) NAcC injection sites plotted across all rats. Colored outlines represent the outer border of the center of the injection sites for rats in the cocaine extinction group (dark blue lines), cocaine cue-induced reinstatement group (dark red lines), sucrose extinction group (light blue lines), and sucrose cue-induced reinstatement group (light red lines) (c) Representative NAcSh CTb injection. Black outer line indicates the approximate outer edge of NAcSh on the coronal slice. Calibration bar represents 100 µm. Inset shows the location of NAcSh in the rat brain atlas at 1.92 mm anterior to bregma. (d) NAcSh injection sites across all rats, illustrated as in panel b.

Patterns of NAcC and NAcSh afferents

The relative strengths of PL, IL, BLA, and vSub projections to NAcC or NAcSh (as revealed by the mean ± SEM of retrogradely labeled neurons; Table 2-1) were consistent with previous findings (Groenewegen et al., 1987; Brog et al., 1993; Groenewegen et al., 1999; Vertes, 2004). As shown in Table 2-1, there were relatively more projections from PL and BLA to NAcC than to NAcSh, and more projections from vSub to NAcSh than to NAcC (PL: t_{56}=3.05, p=0.0035; BLA: t_{56}=4.16, p=0.0001; vSub: t_{56}=5.67, p<0.0001). There were no differences in the number of IL neurons that project to NAcC compared to NAcSh (t_{56}=0.769, p=0.445). Figure 2-3 includes representative brain sections for CTb+ (projections to NAcC, brown stain), Fos+ (black nuclear stain), and doubly labeled neurons (Fos+CTb; arrows).

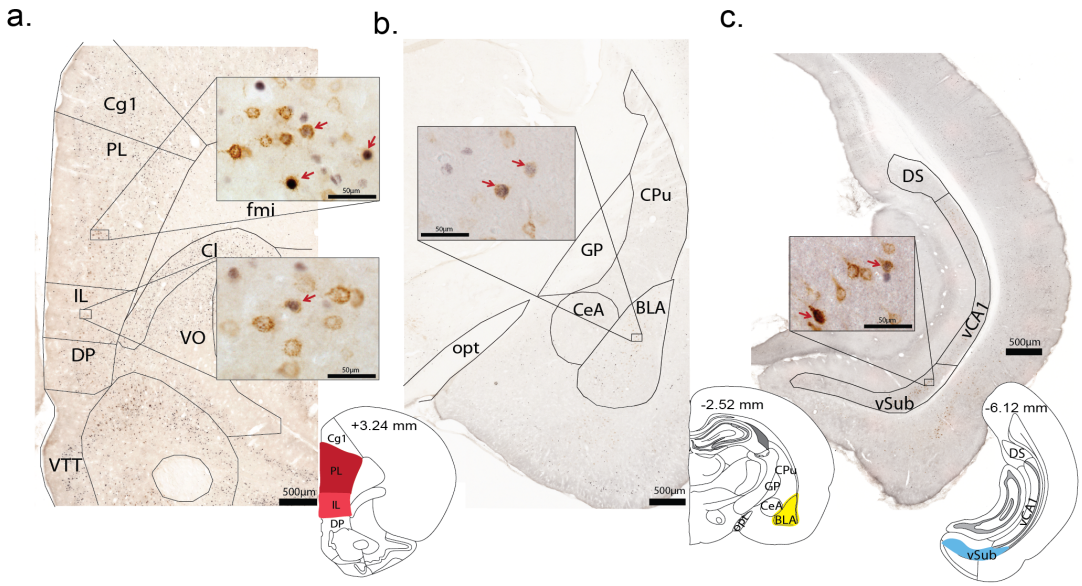

Figure 2-3. Photomicrographs of retrogradely labeled neurons from NAcC. (a) mPFC brain section with magnified photographs (figure insets) of CTb+ neurons retrogradely labeled from NAcC (brown stain), Fos+ neurons (black nuclear stain), and dual labeled (Fos+/CTb+) cells (red arrows) in prelimbic (PL) and infralimbic (IL) cortices. Rat atlas coronal section inset shows the location of PL and IL cortices (highlighted in red) at 3.24 mm anterior to bregma as seen in Paxinos & Watson, 2007 edition. Abbreviations include Cg1, cingulate cortex; PL, prelimbic region of the medial prefrontal cortex; IL, infralimbic region of the medial prefrontal cortex; DP, dorsal peduncular cortex; VTT, ventral tenia tecta; fmi, forceps minor of the corpus callosum; Cl, claustrum; VO, ventral orbital cortex. (b) BLA brain section with magnified photographs (figure insets) of CTb+ neurons retrogradely labeled from NAcC, Fos+ neurons, and dual-labeled (Fos+/CTb+) cells illustrated as in panel a. Rat atlas coronal section inset shows the location of BLA (highlighted in yellow) at 2.52 mm posterior to bregma. Abbreviations include CPu, caudate putamen; GP, globus pallidus; CeA, central amygdala; BLA; basolateral amygdala; opt, optic tract (c) vSub brain section with magnified photographs (figure insets) of CTb+ neurons retrogradely labeled from NAcC, Fos+ neurons, and dual-labeled (Fos+/CTb+) cells illustrated as in panel a. Rat atlas coronal section inset shows the location of vSub (highlighted in blue) at 6.12 mm posterior to bregma. Abbreviations include vSub, ventral subiculum; vCA1, ventral CA1 region of the hippocampus; DS, dorsal subiculum. Calibration bars for mPFC, BLA, and vSub coronal sections represent 500 µm. Calibration bars for all magnified insets represent 50 µm.

Table 2-1. Mean ± SEM CTb-labeled cells per slice for PL, IL, BLA, and vSub projections to NAcC and NAcSh. Values represent the mean number (± standard error of mean; SEM) of neurons in PL, IL, BLA, and vSub that are retrogradely labeled from either NAcC or NAcSh. PL, prelimbic region of the medial prefrontal cortex; IL, infralimbic region of the medial prefrontal cortex; BLA, basolateral amygdala; vSub, ventral subiculum region of the hippocampus; NAcC, nucleus accumbens core; NAcSh, nucleus accumbens shell. *p<0.05 represents the stronger projection target (either NAcC or NAcSh) of PL, IL, BLA, or vSub structures using a multiple t-test correction (PL: t_{56}=3.05, p=0.0035; IL: t_{56}=0.77, p=0.4451; BLA: t_{56}=4.16, p=0.0001; vSub: t_{56}=5.67, p<0.0001). Each projection to NAcC: n=31 rats (3 brain sections per rat averaged). Each projection to NAcSh: n=27 rats (3 brain sections per rat averaged).

Table 2-1. Afferents to NAcC and NAcSh

	CTb cells/slice			
	NAcC		NAcSh	
Structure	Mean	SEM	Mean	SEM
PL	93 *	14	42	8
IL	72	12	61	6
BLA	200 *	23	86	12
vSub	65	13	291 *	40

Activation of prelimbic cortex inputs to NAcC during cocaine seeking.

Many neurons in mPFC express Fos during cue-induced reinstatement, but these activated neurons do not project to VTA (Kufahl et al., 2009; Mahler and Aston-Jones, 2012). Here, we found that many of these neurons instead project to NAcC. In particular, a greater percentage of PL neurons retrogradely-labeled from NAcC expressed Fos during cue-induced reinstatement of cocaine seeking compared to extinction of cocaine self-administration (Figure 2-4a; t_{15}=2.774, p=0.0142). Similar analyses for sucrose-trained rats revealed no differences between reinstatement and extinction in the percentage of Fos+ PL neurons retrogradely-labeled from NAcC (Figure 2-4b; t_{12}=1.278, p=0.2255). Further, the percentages of CTb+ neurons in PL that were also Fos+ correlated positively with active lever pressing during the first 30 min of cue-induced reinstatement of cocaine seeking (Figure 2-5a; Pearson correlation, n=10,

r=0.60, p=0.0334), but not with the same period of cue-induced reinstatement of sucrose seeking (Figure 2-5b; Pearson correlation, n=9, r=0.08, p=0.84).

In contrast, the percentages of NAcC-projecting IL neurons that were also Fos+ did not differ between cue-induced reinstatement and extinction of cocaine seeking (Figure 2-4a; t_{15}=0.236, p=0.8167) or between cue-induced reinstatement and extinction of sucrose-seeking (Figure 2-4b; t_{12}=1.913, p=0.0799). Additionally, there were no correlations between the percentage of Fos+ IL neurons retrogradely-labeled from NAcC and active lever pressing during the first 30 min of reinstatement for cocaine- or sucrose-trained rats (Pearson correlations; Table 2-2).

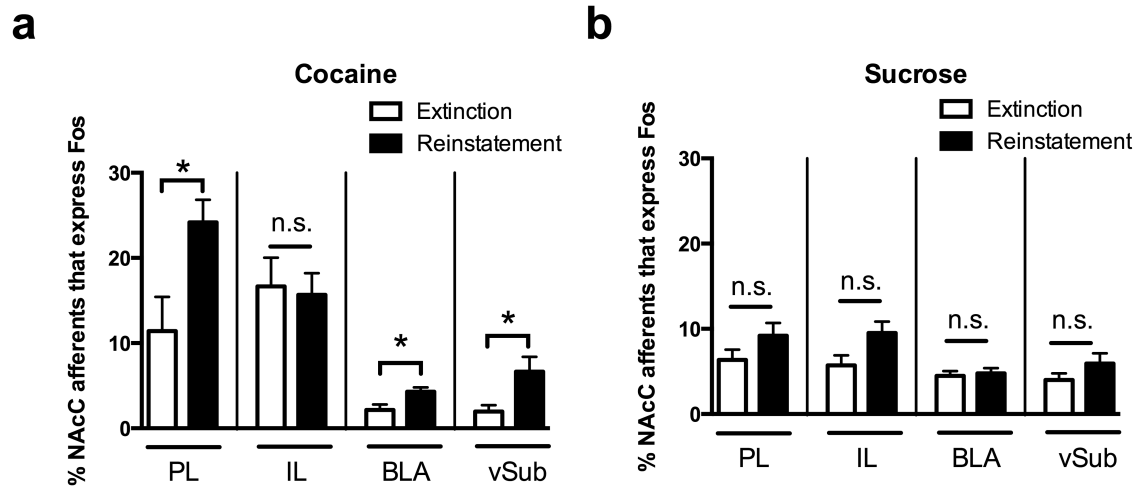

Figure 2-4. Prelimbic, basolateral amygdala, and ventral subiculum neurons that project to NAcC activate during cue-induced cocaine-, but not sucrose-seeking. (a) PL neurons retrogradely labeled from NAcC expressed a significantly greater percentage of Fos during cue-induced reinstatement of cocaine seeking (black bar, n=10) compared to extinction of cocaine seeking (white bar, n=7). There were no differences in the percentage of Fos in the IL-NAcC projection during extinction (n=7) and cue-induced reinstatement of cocaine seeking (n=10). Similar to the PL-NAcC pathway, BLA and vSub neurons that are retrogradely labeled from NAcC showed significantly greater percentages of Fos during cue-induced reinstatement of cocaine seeking (n=10) compared to extinction (n=7). (b) There were no differences in the percentage of Fos in the PL-NAcC projection during extinction (white bar, n=5) and cue-induced reinstatement of sucrose seeking (black bar, n=9). The percentages of Fos+ IL, BLA, or vSub neurons that were retrogradely labeled from NAcC also did not differ between extinction (n=5) and cue-induced reinstatement sucrose seeking (n=9). Bar graphs and error bars represent mean ± SEM; *p<0.05; n.s.=not significant.

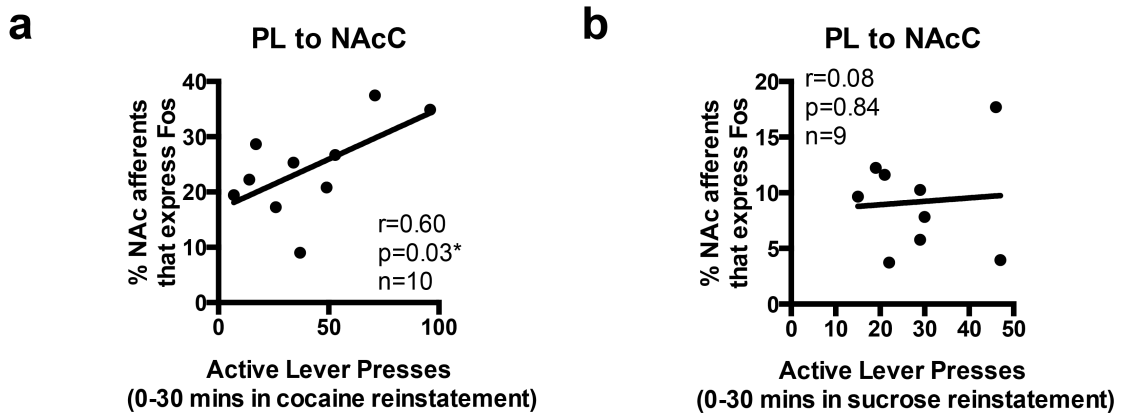

Figure 2-5. Prelimbic neurons that project to NAcC activate in proportion to cocaine seeking, but not sucrose seeking. (a) The percentages of Fos+ PL neurons retrogradely labeled from NAcC during cue-induced reinstatement of cocaine seeking correlated with the first 30 min of active lever pressing in individual rats (circles, n=10). However, Fos activation in the same circuit did not correlate with cue-induced reinstatement of sucrose seeking (panel b, n=9).

Fos expression during extinction compared to reinstatement of cocaine seeking did not differ in PL or IL projections to NAcSh (Figure 2-6a; PL: t_{15}=1.953, p=0.0697; IL: t_{15}=0.384, p=0.7062). The same result was seen with extinction and reinstatement of sucrose seeking (Figure 2-6b; PL: t_8=0.542, p=0.6026; IL: t_8=0.881, p=0.4040). There were also no correlations between Fos induction and reinstatement of cocaine- or sucrose-seeking in PL or IL projections to NAcSh (Pearson correlations; Table 2-2). These results indicate that PL projections to NAcC, but not to NAcSh, were recruited during, and in proportion to, cue-induced cocaine seeking behavior. Additionally, PL neurons that project to NAcC did not show similar results during cue-induced sucrose seeking.

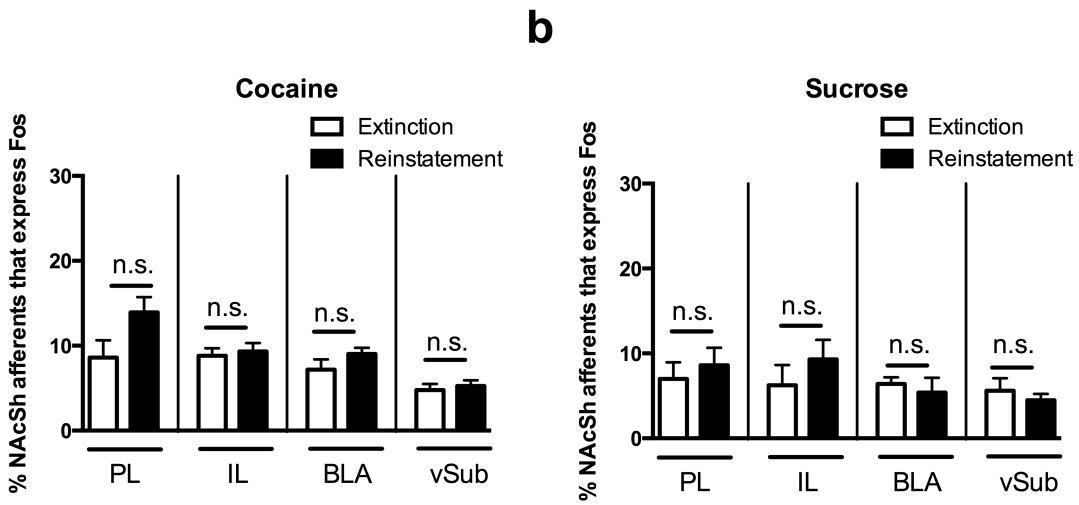

Figure 2-6. NAcSh afferents did not activate during cue-induced cocaine or sucrose seeking. (a) The percentages of Fos+ PL, IL, BLA, or vSub neurons that were retrogradely labeled from NAcSh did not differ between extinction (white bars, n=7) and cue-induced reinstatement of cocaine seeking (black bars, n=10). (b) The percentages of Fos+ PL, IL, BLA, or vSub neurons that were retrogradely labeled from NAcSh did not differ between extinction (white bars, n=4) and cue-induced reinstatement of sucrose seeking (black bars, n=6). Bar graphs and error bars represent mean ± SEM; n.s.=not significant.

Table 2-2. Correlations of Fos in NAcC and NAcSh afferent neurons with active lever responding during cue-induced reinstatement of cocaine or sucrose seeking. Correlation coefficients represent the relationships between the percentages of Fos induction in NAcC or NAcSh afferent neurons and active lever pressing during the first 30 min of cue-induced cocaine or sucrose seeking. The 0-30 min active lever responding reflects the time of peak Fos induction. *p<0.05; Pearson r correlation.

Table 2-2. Correlations between Fos+ NAcC and NAcSh afferents and reinstatement

		Reinstatement active lever presses by % Fos+ CTb cells					
		NAcC			NAcSh		
Reward	Structure	r value	n	p-value	r value	n	p-value
Cocaine	PL	0.60	10	0.03*	-0.12	10	0.73
	IL	0.28	10	0.44	0.17	10	0.65
	BLA	0.28	10	0.22	0.11	10	0.76
	vSub	0.40	10	0.25	-0.19	10	0.59
Sucrose	PL	0.08	9	0.84	0.24	6	0.65
	IL	-0.49	9	0.18	-0.48	6	0.33
	BLA	-0.28	9	0.46	0.77	6	0.07
	vSub	-0.55	9	0.12	-0.50	6	0.31

Activation of basolateral amygdala and ventral subiculum projections to nucleus accumbens core and shell during cocaine- vs. sucrose-seeking.

BLA and vSub provide substantial glutamate input to NAc (Groenewegen et al., 1987; 1999). Therefore, we examined whether BLA and vSub inputs to NAc also express Fos during conditioned cocaine seeking. We found that BLA and vSub neurons that project to NAcC expressed a greater proportion of Fos+ cells during cue-induced reinstatement of cocaine seeking relative to extinction (Figure 2-4a; BLA: t_{15}=2.652, p=0.0181; vSub: t_{15}=2.134, p=0.0498). There were no such differences between extinction and cue-induced reinstatement of sucrose seeking in NAcC-projecting BLA or vSub neurons (Figure 2-4b; BLA: t_{12}=0.307, p=0.7639; vSub: t_{12}=1.097, p=0.2942). The percentage of Fos+ BLA or vSub neurons retrogradely-labeled from NAcSh did not differ between cocaine extinction and cue-induced reinstatement of cocaine seeking (Figure 2-6a; BLA: t_{15}=0.384, p=0.7062, vSub: t_{15}=0.487, p=0.6331), or between sucrose extinction and cue-induced reinstatement of sucrose seeking (Figure 2-6b; BLA: t_{8}=0.456, p=0.6607, vSub: t_{8}=0.749, p=0.4752). In contrast to the PL-NAcC circuit, the percentages of Fos+ BLA or vSub neurons retrogradely-labeled from NAcC or NAcSh did not correlate with reinstatement of cocaine seeking (Pearson correlations, Table 2-2). Additionally, there were no correlations between Fos induction and reinstatement of sucrose seeking in BLA or vSub projections to NAcC or NAcSh (Table 2-2).

In summary, our findings showed that BLA and vSub projections to NAcC (but not to NAcSh) were activated during cue-induced cocaine-, but not sucrose-seeking. However, unlike PL projections to NAcC, this Fos activation did not correlate with the intensity of cocaine seeking behavior.

Discussion

We investigated the proportion of NAc-projecting neurons originating from PL, IL, BLA and vSub that are activated in response to cocaine cues. Our results showed that within PL, BLA, and vSub, only those cells that project to the NAcC (not the NAcSh) exhibit increased Fos protein in response to cocaine conditioned stimuli. Similar results were not seen in these circuits during cue-induced reinstatement of sucrose seeking. Furthermore, only Fos activation in NAcC-projecting PL neurons positively correlated with the magnitude of cocaine reinstatement behavior. Our results indicate distinct activation of the PL-NAcC pathway in reinstatement of drug seeking.

Activated circuits target NAcC, not NAcSh

Prelimbic, BLA, and vSub neurons that expressed Fos predominately targeted NAcC, and not NAcSh during cue-induced reinstatement of cocaine seeking. Many studies have implicated NAcC as a central region necessary for discrete, response-contingent cue-induced cocaine seeking (Fuchs et al., 2004; Bäckström and Hyytiä, 2007), whereas projections to NAcSh are important for other drugs of abuse (e.g. heroin) and reinstatement elicited by drug-associated discriminative contexts (Bossert et al., 2007; 2012; Cruz et al., 2014). For example, neural responses to cocaine conditioned cues are heightened in NAcC, but not NAcSh, following prolonged abstinence (Hollander and Carelli, 2007). Further, inactivation of NAcC blocks cue-induced cocaine seeking whereas inactivation of NAcSh has no such effect (Fuchs et al., 2004). Our current results support previous literature concerning the importance of NAcC in cocaine seeking in response to discrete cues, and further elucidate addiction-related projection targets from activated neuronal subpopulations within brain structures.

Multiple NAcC afferents express Fos during cocaine seeking to conditioned cues

Multiple glutamatergic inputs interact to modulate NAc activity (Mulder et al., 1998; Stern et al., 1998; McGinty and Grace, 2008; Calhoon and O'Donnell, 2013). For example, in anesthetized rats mPFC stimulation only induces NAc firing when combined with hippocampal activation (O'Donnell and Grace, 1995). Additionally, input from multiple glutamatergic regions (e.g. vSub and BLA, or vSub and mPFC) converge on the same neurons in NAc (French and Totterdell, 2002; 2003). These data indicate that a combination of multiple excitatory inputs may be involved in regulating NAc neural activity. Our results show that PL, BLA, and vSub neurons that activate during reinstatement of cocaine seeking all innervate NAcC. The combined activation of these glutamatergic inputs to NAcC may make drug-associated cues more salient than sucrose-associated cues.

Specific activation of PL projection to NAcC during cue-induced cocaine seeking, but not sucrose seeking

PL promotes drug seeking via its projection to NAcC (McFarland et al., 2003; Stefanik et al., 2013), but only subsets of neurons in PL are involved in drug seeking behaviors (West et al., 2014; Moorman et al., 2015). Distinct cell populations characterized by their projection targets are gradually being disentangled (Taha and Fields, 2005; Bossert et al., 2011; Cruz et al., 2013; 2014). For example, different populations of mPFC neurons project to NAc vs. VTA (Pinto and Sesack, 2000), and although cue-induced cocaine seeking activates PL neurons (Kufahl et al., 2009), this behavior does not activate PL projections to VTA (Mahler and Aston-Jones, 2012). Rather, we found that cue-induced reinstatement of cocaine-, but not sucrose-seeking activated the PL to NAcC circuit. This was similarly seen in BLA and vSub projections to NAcC, however the proportion of NAcC-projecting PL neurons that co-expressed Fos

correlated with the intensity of cocaine seeking across rats. Similar correlations were not seen in BLA or vSub projections to NAcC during cocaine seeking, or in PL projections to NAcC during sucrose seeking.

Notably, IL has also been implicated in drug abuse behaviors. IL inactivation, or inactivation of its projection to NAcSh, drives rats to reinstate extinguished cocaine-seeking (Peters et al., 2008a; 2008b). However, inactivation of IL or its projection to NAcSh also attenuates drug seeking for heroin, cocaine, and methamphetamine (Koya et al., 2009; Rocha and Kalivas, 2010; Bossert et al., 2011; 2012). Due to these functional intricacies of the IL to NAcSh circuit, it is unsurprising that our results revealed similar Fos expression between extinction of cocaine self-administration and cue-induced cocaine seeking in IL neurons that are retrogradely labeled from NAcSh.

Conclusions

Overall, these results untangle important circuits in addiction pathology by identifying neuronal subpopulations activated during cue-conditioned cocaine-seeking that target NAcC, but not NAcSh. We examined and compared multiple glutamatergic inputs to NAcC and NAcSh, and compared the proportions of neurons in these circuits that were Fos-activated during seeking for cocaine versus a natural reward (sucrose). We found that multiple, functionally distinct circuits activate and converge in NAcC during cue-induced reinstatement of cocaine seeking, but only neurons in the PL to NAcC circuit expressed Fos in a manner that correlated with active lever responding during cocaine seeking. The same circuits did not activate during cue-induced reinstatement of sucrose seeking. As such, subpopulations of PL neurons that project to NAcC are potential therapeutic targets for treating cocaine relapse.

CHAPTER 3: DORSAL HIPPOCAMPUS DRIVES CONTEXT-INDUCED REINSTATEMENT OF COCAINE SEEKING VIA INPUTS TO LATERAL SEPTUM

Introduction

Lateral septum (LS), a brain structure within the limbic system, has re-emerged in the literature as a critical node in reward-related behaviors. Intracranial self-stimulation studies in both humans and rodents first established LS as a reward-related structure (Olds and Milner, 1954; Heath, 1963). More recent studies implicate LS in addiction and relapse to numerous drugs of abuse. Rats rapidly self-administer morphine into LS (Le Merrer et al., 2007), methamphetamine seeking upregulates Fos expression (a marker of neuronal activity) in LS (Cornish et al., 2012), and pharmacological inhibition of LS blocks cocaine-conditioned place preference (Sartor and Aston-Jones, 2012).

Lateral septum is highly connected to brain regions associated with drug addiction, such as ventral tegmental area (VTA), nucleus accumbens, lateral hypothalamus, and hippocampus, among others (Risold and Swanson, 1997; Sheehan et al., 2004). Our lab revealed a circuit between dorsal hippocampal CA3 neurons to the VTA via a relay in LS (Luo et al., 2011), indicating that LS may be an important region linking memories of salient stimuli with their associated rewards.

Hippocampus is the densest afferent to LS (Risold and Swanson, 1997), but also is a heterogeneous structure. Dorsal and ventral hippocampus projections to LS differ anatomically. The CA3 region of the dorsal hippocampus selectively projects to the limbic system via the caudal/dorsal area of LS (Risold and Swanson, 1996). This contrasts with the ventral hippocampus (CA1 and CA3 regions) which projects strongly to the intermediate-ventral LS (Risold and Swanson, 1997). Dorsal hippocampus and

ventral hippocampus also have distinct functional roles in a multitude of associative learning behaviors including spatial learning, classical fear conditioning, and drug-stimulus conditioning (Moser et al., 1995; Moser and Moser, 1998; Degoulet et al., 2008; Hunsaker and Kesner, 2008; Kenney et al., 2012; Wilkinson et al., 2013). Evidence suggests dorsal hippocampus may form and integrate memories of environmental contexts, whereas ventral hippocampus may encode emotional memories associated with discrete and contextual cues (Fanselow and Dong, 2010). These distinctions may be important for how drug addicts encode stimuli associated with drug experience, such as drug-taking environments and discrete drug cues—and how these stimuli trigger relapse (or reinstatement of extinguished lever pressing in an rodent model).

Different triggers of relapse recruit distinct brain circuits. For example, inhibition of dorsal hippocampal neurons attenuates only context-, but not cue-induced reinstatement of cocaine seeking (Fuchs et al., 2005), and ventral pallidum inhibition attenuates cue-induced, but not cocaine-primed reinstatement (Mahler et al., 2014). These results signify that specific brain regions drive particular reinstatement modalities. It is important to elucidate the neural circuitry involved in relapse triggers to identify potential targets for future therapeutics. Lateral septum could be a promising target to treat addiction given its involvement in multiple drugs of abuse, but to date no studies have investigated LS afferents that drive reinstatement behavior.

To elucidate the circuitry of specific relapse triggers, we developed a self-administration paradigm to dissociate context- (Crombag and Shaham, 2002) versus discrete cue-induced (Meil and See, 1997) reinstatement within the same subjects. We combined the behavioral paradigm with anatomical, pharmacological, and chemogenetic techniques to investigate inputs to LS driving reinstatement behavior. We predicted that dorsal hippocampal inputs to LS would be necessary specifically for context-, but not

cue-induced reinstatement given hippocampal involvement in encoding and interpreting contextual representations (Jarrard, 1993).

Methods

Subjects

Male Sprague Dawley rats (n=112; arrival weight 275-325g; Charles River, Raleigh, NC) were single or double housed in a temperature- and humidity-controlled vivarium under a reverse 12:12 h light cycle with ad libitum access to food and water. The Institutional Animal Care and Use Committees at the Medical University of South Carolina and Rutgers University approved of all laboratory procedures. All procedures were conducted according to the specifications of the Guide for the Care and Use of Laboratory Animals. Rats were handled daily after a 3 d acclimation period at the facility; all experiments were performed in the dark phase.

Intravenous catheter

All surgical tools and materials were autoclaved and the surgery area prepped for proper aseptic technique prior to any surgical procedures. Rats were then anesthetized with a mixture of ketamine and xylazine (56.5 mg/kg; 8.7 mg/kg, i.p., respectively) followed by an injection of an analgesic (meloxicam, 1 mg/kg or rimadyl, 5 mg/kg, s.c.). Pre-operative procedures included shaving the designated surgical sites and disinfecting the areas with sterilized alcohol and iodine. Ophthalmic lubricant was administered to prevent the corneas from drying (artificial tears ointment; Rugby). All rats were implanted with a chronic indwelling catheter (made with a modified 22 ga cannula, Plastics One) into the right jugular vein that exited the body via a biopsy hole on the back. Surgical sites were sutured with sterilized black thread or closed with wound clips. Catheters

were flushed with cefazolin (0.1 ml; 100 mg/ml) and heparin (0.1 ml; 100 U/ml) immediately following surgery, daily beginning 3 d after surgery, and continuing throughout self-administration. Rats were monitored daily for post-operative care and allowed to recover for a minimum of 1 week after surgery prior to beginning behavioral training.

Injection of retrograde tracer in LS

Rats that were assigned to anatomical tracer experiments received a unilateral microinjection of cholera toxin *β* subunit (CTb, 40-60nl; 1% dissolved in 0.1 M phosphate buffer, Sigma) into LS (n=30). Injections were made at 0.25 mm rostral to bregma, 0.6 mm from midline, and 5.0 mm ventral to skull surface using a glass micropipette (15-20 μm diameter tip) and a pneumatic pressure source (Picospritzer; General Value, Inc.) Pressure injections were made over a 2 min period, and pipettes remained in place for 10-15 min to minimize diffusion along the pipette track.

Bilateral cannula in LS

Rats that were assigned to pharmacological inhibition experiments were implanted with an intracerebral guide composed of bilateral cannulae that were 1.2 mm apart from center to center (26 ga; 0.19 mm anterior to bregma, 0.5 mm from midline, 3.9 mm ventral to skull; n=12). The cannulae were aimed 2 mm above LS to account for injector length. The cannulae were attached to the skull using dental cement supported by four jeweler screws. Sterilized dummies (Plastics One) were inserted into the cannulae (without any projection beyond the cannulae) to reduce risk of infection. Guide cannulae and dummies were topped with an aluminum dust cap for additional protection (Plastics One).

Injection of DREADDs in hippocampus and CNO in LS

Rats that were assigned to DREADD experiments received bilateral microinjections of a lentivirus or adeno-associated virus (AAV) vector with the DREADD or GFP gene driven under a synapsin promoter. Vector injections were aimed at the dorsal CA3 or ventral CA1 regions of the hippocampus. The lentivirus DREADD construct contained the hM4Di gene fused to a hemoagglutanin (HA) tag and a GFP reporter tag (*lenti-hsyn-hM4Di-HA-EGFP*; custom packaged by University of Pennsylvania vector core). The AAV (serotype 8) DREADD construct contained the hM4Di gene fused to an mCherry tag (*AAV8-hsyn-hM4Di-mcherry*; University of North Carolina (UNC) vector core). The GFP control virus was driven by the same neuronal promoter, but lacked the hM4Di gene (*AAV8-hsyn-EGFP*; UNC vector core). This construct was used to assess any non-specific effects produced by the hM4Di gene or CNO microinjections. Dorsal hippocampal (3.2 mm posterior to bregma, 3.2 mm from midline, 3.9 mm ventral to skull) and ventral hippocampal (5.7 mm posterior to bregma, 5.3 mm from midline, 7.8 mm ventral to skull) injections were made using a glass micropipette (30-40 μm diameter tip) and a pneumatic pressure source (Picospritzer). Injection volumes ranged between 1.0 and 1.5 μl per hemisphere. Pressure injections targeting dorsal hippocampus (n=23; 14 hM4Di, 9 GFP controls) were made over a 10 min period and pipettes remained in place for 10-20 min to minimize diffusion along the pipette track. Pipettes for ventral hippocampus injections (n=19; 11 hM4Di, 8 GFP controls) remained in place only for a few minutes to increase the likelihood of diffusion up the pipette track to infect a greater number of ventral CA1 cells. For CNO administration, rats were implanted with cannula aimed above LS as described above.

Self-administration & Extinction training

A modified self-administration paradigm was developed to dissociate reinstatement to contexts versus discrete cues using a within-subjects approach (Figure 3-1a,b). All self-administration chambers were within a sound-attenuating box, controlled by Med-PC IV software (Med Associates, St. Albans, VT) and equipped with two retractable levers with cue lights above each lever and a house light on the opposite wall. Two contextually different chambers were used during self-administration training. One context was in a smaller self-administration chamber that included wallpaper with black circles, grid flooring, a lemon odor placed in a weigh boat under the active lever, and a red house light. The alternative context was a larger self-administration chamber that included wallpaper with black stripes, mesh flooring, a vanilla odor, and a white house light (Figure 3-1b). Rats were counterbalanced to learn self-administration in one of the contexts. Responses on the active lever (fixed ratio-1 schedule) resulted in cocaine infusions (0.2 mg / 50 µl infusion; cocaine HCl dissolved in 0.9% sterile saline, NIDA) paired with discrete light and tone cues (white cue light above the active lever and a 78 dB, 2.9 KHz tone) during training. After each reward a 20 s timeout occurred (signaled by turning off the house light) when additional presses did not yield rewards or cues. Presses on the inactive lever were recorded, but had no programmed consequences. After 10+ d of criterion self-administration performance (at least 10 infusions per 2 h session), rats were placed into the alternative context for extinction training. Some rats received one to four additional days of criterion self-administration training beyond the standard 10 days as not every extinction context became immediately available. During extinction training, responding on either lever did not result in discrete cue presentations or cocaine infusions. Rats received extinction training for a minimum of 7 d and until they met the criteria of <25 active lever presses for 2 or more

consecutive days. Rats in the extinction group underwent self-administration and extinction training, but were given a final extinction session upon reaching extinction criteria performance before being euthanized for Fos.

Reinstatement testing

Upon meeting criteria performance for extinction, rats were counterbalanced to receive a context- or a cue-induced reinstatement test (Figure 3-1a). For context-induced reinstatement, rats returned to their original training context, but did not get the contingent discrete light and tone cues or cocaine they received during self-administration (ABA design). For cue-induced reinstatement, rats stayed in their extinction context, but responding on the active lever resulted in the presentation of the discrete light and tone cues (but no cocaine) given during training (ABB design). This ensured that only the surrounding environment or discrete cues elicited reinstatement behavior during context- or cue-induced reinstatement tests, respectively. Between reinstatement tests, rats were placed back into their extinction environment until they re-extinguished their lever pressing behavior to <25 active lever presses for a minimum of 2 consecutive days before initiating the alternative reinstatement test type.

For experiments that required Fos analysis, rats were perfused immediately following a 2 h context reinstatement (n=8), cued reinstatement (n=6), extinction (n=5), or yoked saline context (n=5) session. For the yoked saline group (Neisewander et al., 2000), rats were placed in the same operant environment as cocaine self-administering rats, but their lever presses had no programmed outcomes. Instead, rats were paired to cocaine self-administering rats and received yoked passive infusions of saline and light/tone pairings to the same degree as their cocaine self-administering counterparts. They then were placed in the alternative environment for a minimum of 7 days, and then

re-exposed to their original context. This examined whether activation of brain regions occurred upon returning to a familiar, but neutral (non-drug) context. For all groups, Fos labeling should reflect neural activity at ~0-30 min in the test session as maximal Fos induction occurs ~90-120 min following neuronal activation (Müller et al., 1984; Young et al., 1991). Fos expression at this time point also corresponds to the period of peak active lever pressing.

For experiments that required microinjections, rats were acclimated to the microinjection procedure by gently restraining the rat and lowering the injectors into LS (33 ga, 1.2 mm center-to-center distance, protruding 2 mm below the cannula, Plastics One) without any infusions made. This occurred immediately following the last extinction session. Rats received a total of 4 reinstatements (2 context, 2 cue; alternating, randomized, and counterbalanced), such that rats either had a context, cue, context, cue reinstatement design or a cue, context, cue, context reinstatement design. Rats received a minimum of 2 extinction sessions of consecutive criteria performance (<25 active lever presses) in their original extinction context between reinstatement tests. On each reinstatement test day, rats received either vehicle or drug microinfusions immediately prior to each reinstatement test in such an order that either the first two microinjections were vehicle, followed by 2 drug microinjections, or vice versa. Microinjections of either a cocktail of the $GABA_A$ and $GABA_B$ agonists, baclofen and muscimol (B-M; 1 mM and 0.1 mM, respectively; 0.5 µl/side, Luo et al., 2011) or artificial cerebral spinal fluid (aCSF, 0.5 µl/side) were administered into LS to determine if pharmacological inhibition of LS attenuates cocaine seeking. In a separate experiment, CNO (1 mM) or aCSF (0.3 µl/side) microinjections were administered onto hM4Di- or GFP-expressing dCA3 terminals in LS to determine if specific neural circuits are necessary for reinstatement behavior. Reinstatement testing and CNO microinjections in the DREADD experiments

did not begin until 5 weeks after the initial virus injections to provide sufficient time to express the hM4Di receptors in hippocampal terminals in LS. In both experiments, the injectors remained in place for one minute after the microinjection to reduce the likelihood of diffusion up the cannula track. Rats were then immediately placed in their reinstatement chamber for testing. Two rats were excluded for not completing reinstatement testing and one rat was excluded for reinstatement lower than extinction criteria on every reinstatement test.

Locomotor testing

Rats were assessed for general locomotor activity to ensure that any pharmacological results were not due to non-specific motor effects. Open field locomotor chambers (Omnitech Electronics, Inc.) consisted of 3 sensor pairs each configured with 16 infrared light photobeams to record horizontal X- and Y-axes, and a Z-axis for rearing behavior/vertical activity. Each clear, acrylic locomotor chamber (42 cm x 42 cm x 30 cm) was either contained within an environmental control chamber (60 cm x 64 cm x 64 cm) or surrounded by a black curtain. The locomotor boxes were equipped with either Fusion or VersaMax software (Omnitech Electronics, Inc.) to record horizontal and vertical beam breaks and total distance traveled, amongst other behavioral readouts. Rats received locomotor testing upon completion of all reinstatement testing. Rats acclimated to the locomotor boxes for 2 d (2 h sessions) to reduce novelty-induced locomotion. The following day, rats were microinjected with either drug (B-M or CNO) or vehicle (aCSF) immediately prior to being placed into the locomotor chamber. Rats then received a no-injection day in the locomotor chamber, followed by a second round of testing the following day. The order in which rats received drug versus aCSF microinjections was randomized and counterbalanced.

Tissue preparation

Rats were either time-sacrificed immediately following their reinstatement test (for Fos analysis) or sacrificed after all behavioral testing was complete (microinjection experiments). For all experiments requiring immunohistochemistry, rats were deeply anesthetized with ketamine/xylazine and perfused with 0.9% saline followed by 4% paraformaldehyde. Brains were carefully removed from the skull and postfixed in 4% paraformaldehyde for 24 h, then transferred to a 20% sucrose-azide solution and stored at 4°C. Rats that received cannulae for B-M microinjections into LS only required localizing injection sites; therefore these rats were deeply anesthetized with ketamine/xylazine, rapidly decapitated, and the brain flash frozen with 2-methylbutance (Fischer Scientific) that was chilled in a -80°C freezer for at least 30 min prior. The brain was then placed in a -20°C freezer until sectioning. For sectioning, brains were frozen on dry ice, and sliced at 40 µm coronal sections on a cryostat (Leica). For Fos analysis, brain slices were sequentially placed in 6 wells so that brain sections per well were 240 µm apart. For injection site analyses, brain slices of regions with injection sites (hippocampus and/or lateral septum) were collected sequentially across 3 wells to examine the central point of the injections. Brain slices were collected in 0.1 M phosphate buffered saline with 1% sodium azide (PBS-Az).

Staining procedures & localization of injection sites

Immunohistochemistry procedures for Fos, and dual labeling of Fos and CTb, were similar to previously published methods; discussed in detail in Chapter 2. An abbreviated version is as follows: tissue was quenched with hydrogen peroxide, washed with PBS, and the blocked in a 2% normal donkey serum in PBS with Triton (PBST) for 2 h. The rabbit anti-Fos primary antibody (1:10,000; CalBioChem; PC38) mixed with the

tissue overnight. The tissue was then incubated in a biotinylated donkey anti-rabbit secondary antibody (1:500; Jackson ImmunoResearch Laboratories) for 2 h followed by incubation in an avidin-biotin complex solution (ABC, 1:500) for 1.5 h. The tissue was then reacted with 3,3'-diaminobenzidine (DAB; Sigma) to visualize antibody location. The DAB solution was mixed with Tris buffer, hydrogen peroxide, and nickel ammonium sulfate to produce a black Fos nuclear stain. For tissue that required CTb staining, the same tissue was incubated with goat anti-CTb primary antibody (1:20,000; List Biological Laboratories; #703), and then transferred to a biotinylated donkey anti-goat secondary antibody (1:500; Jackson ImmunoResearch Laboratories). This DAB reaction did not include nickel ammonium sulfate; it produced a brown cytoplasmic stain for CTb. Brain slices were mounted on glass slides, dehydrated with alcohols and xylenes, and coverslipped with DPX (Sigma). Retrograde tracer injections that were mostly in an adjacent region (e.g. medial septum) were excluded from analyses (n=1).

Immunohistochemistry procedures to localize DREADD receptors were similar to above. Brain tissue with hM4Di viral injections was incubated in mouse anti-HA (1:1000; Covance; MMS-101P) or rabbit anti-DsRed (1:500; Clontech; 632496) primary antibodies, followed by biotinylated donkey anti-mouse (1:500; Jackson ImmunoResearch Laboratories) or donkey anti-rabbit (1:500; Jackson ImmunoResearch Laboratories) secondary antibodies, respectively. Control virus brain tissue (synapsin promoter-driven GFP virus without the DREADD gene) was incubated with chicken anti-GFP primary antibody (1:2000; Abcam; ab13970) and donkey anti-chicken secondary antibody (1:500; Jackson ImmunoResearch Laboratories). DAB staining procedures were similar to the Fos stain where nickel ammonium was used to produce a black stain at the injection sites and at hM4Di- or GFP-expressing terminals. Sections were then mounted and counterstained with neutral red, dehydrated, and coverslipped. Brain slices

that were flash frozen without immunohistochemistry procedures were stained with neutral red or cresyl violet to localize injector sites. DREADD experiments were examined for hippocampal injection site and terminal expression in LS on a Leica DMRXA research microscope. Rats without expression in hippocampus (n=2) or with injection sites in the ventricle (n=11; for all experiments) were excluded from analyses.

Fos & CTb Quantification

Brain tissue of rats that required Fos analysis without CTb tracing was photographed at 10x magnification using a Leica DMRXA research microscope with OpenLab software (Improvision). Regions photographed included the dorsal hippocampus (between 2.52 mm and 3.48 mm posterior to bregma), rostral lateral septum (between 2.16 mm and 1.20 mm anterior to bregma), caudal lateral septum (between 1.08 mm and 0.12 mm anterior to bregma) and ventral hippocampus (between 5.04 mm and 6.12 mmm posterior to bregma). Three brain sections within each structure for each group (extinction, cue-induced reinstatement, and context-induced reinstatement, and yoked saline) were photographed for cell quantification. An individual blinded to groups quantified Fos cells using standardized thresholds for size and intensity in Image J (NIH).

For rats injected with CTb for retrograde tracing, LS sections were photographed using a 5x objective with Openlab software, and compared to rat atlas sections of LS (Paxinos and Watson, 2007) to localize the injection sites. Three tissue sections per rat representing rostral, intermediate, and caudal bregma levels were quantified for each LS afferent (dorsal hippocampus; ventral hippocampus; BLA) for each of the extinction, context-, and cue-induced reinstatement groups. These afferent structures were photographed with a 10x objective on a Leica microscope and stitched together

automatically using Stereoinvestigator Virtual Slice module (MBF Biosciences). Tissue sections from each rat were taken at similar rostrocaudal levels for each structure to ensure that quantified areas were similar across animals. Structure borders were defined based on rat atlas (Paxinos and Watson, 2007). The total numbers of CTb-labeled neurons (with or without Fos) within each LS afferent structure were manually counted for each of the 3 tissue sections per rat (sections were at least 240 μm apart to prevent double counting). Dorsal hippocampal neurons ipsilateral and contralateral to the CTb injections were quantified, as both projections densely innervate LS (Risold and Swanson, 1997). Ventral hippocampal and BLA neurons only ipsilateral to the CTb injections were quantified, as their contralateral projections are not dense. The percentage of CTb+ cells that expressed Fos was determined for each tissue section. Percentages of retrogradely labeled neurons that expressed Fos were analyzed to normalize for relative strength of projections and effectiveness of tracer injections. Means of these percentages were computed for each rat, and these means were averaged across behavioral groups for each brain region.

Data analysis

Self-administration and reinstatement data were analyzed with repeated measures two-way analyses of variance (ANOVAs) with Bonferroni corrections as appropriate. One-way ANOVAS with Tukey multiple comparison tests were used to analyze the average number of Fos cells or the percentages of Fos-positive LS afferents between groups. Active lever responding during reinstatement following vehicle (aCSF) or drug (B-M or CNO) microinjections was analyzed using a paired t-test for each reinstatement test. Locomotor activity across the 2 h session was analyzed using a paired t-test. Non-parametric Kruskal-Wallis tests with Dunn's post hoc analyses or

Wilcoxon matched-pairs signed rank tests were used when data were not normally distributed according to a D'Agostino-Pearson omnibus normality test. All statistics were two-tailed and alpha was set to 0.05. Data analyses were performed with Prism version 6 or SPSS 21 (IBM).

Results

Self-administration, extinction, and reinstatement

These experiments used a modified self-administration paradigm to dissociate context- and discrete cue-induced reinstatement within the same subjects (Figure 3-1a,b). Rats learned to self-administer cocaine paired with response-contingent light/tone discrete cues in one context, and then extinguished this behavior in a different context without cue presentations or cocaine infusions. Context-induced reinstatement of cocaine seeking was later assessed in their original training context without cue or cocaine presentation; cue-induced reinstatement was assessed in their extinguished context by presenting cocaine-associated discrete cues with active lever presses (but no cocaine; Figures 3-1a-c). In this paradigm, all rats showed stable self-administration and extinction training (Figure 3-1c) and a preference for the active (cocaine-associated) lever compared to the inactive lever throughout self-administration training (two-way ANOVA; $F_{1,178}$=104.1, p<0.0001). Rats extinguished lever pressing behavior over extinction days (two-way ANOVA, $F_{6,540}$=65.72, p<0.0001). We observed robust, and similar lever pressing during context- (n=67) and cue-induced (n=65) reinstatement behavior (Figure 3-1d; two-way ANOVA; no test x lever interaction: $F_{1,130}$=0.0085, p=0.9266; no main effect of test: $F_{1,130}$=0.7840, p=0.3776; main effect of lever: $F_{1,130}$=168.0, p<0.0001). Mean ± standard error of mean (SEM) active lever presses for rats randomized to the extinction (n=15) or yoked saline (n=5) groups for Fos immunohistochemistry experiments were 8.667 ± 0.9445 and 9.2 ± 2.267, respectively.

a

Self-administration (Context A or B)	Extinction (Opposite context)	Reinstatement (counterbalanced)	
		Cue-induced ↔	Context-induced
Contingent light/tone cues (CS+)	**No light/tones cues**	**Remain in extinction context with CS+ cues**	**Return to training environment without CS+ cues**

b

	Visual stimuli	Tactile stimuli	Olfactory stimuli
Context A	Shorter SA box, circles on walls, red house light	Grid floor	Lemon odor
Context B	Taller SA box, stripes on walls, white house light	Mesh floor	Vanilla odor

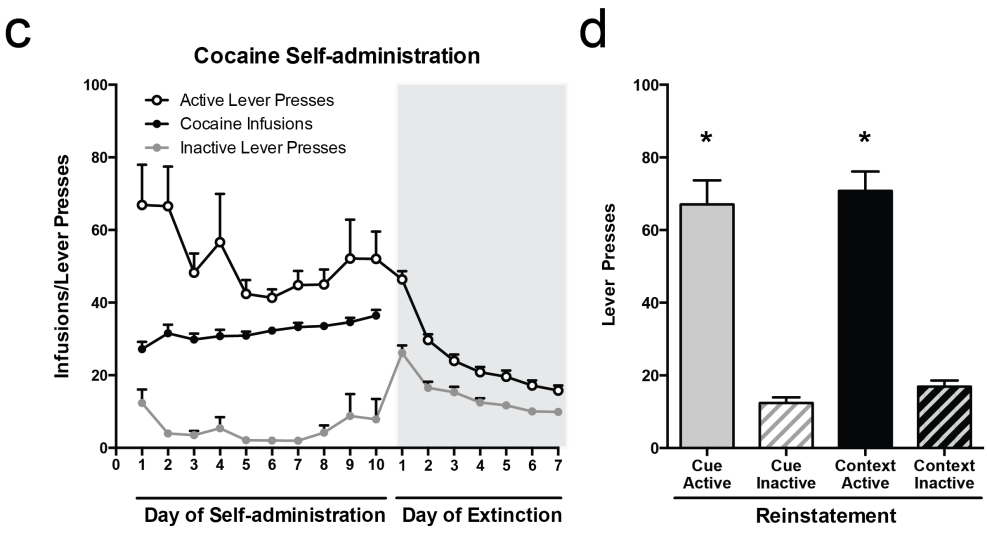

Figure 3-1. Experimental design, behavioral training, and testing. (a) Experimental design of the self-administration paradigm that provides within-subjects testing of context- and cue-induced reinstatement of drug seeking. (b) Stimulus components of each training environment. (c) Mean behavioral performance of all rats during cocaine self-administration and extinction training (n=90). Rats showed a preference for the active (drug-associated) lever compared to the inactive lever throughout self-administration training and extinguished this behavior over extinction days. (d) Mean active and inactive lever presses during 2 h cue- (n=65; gray bar) or context-induced reinstatement tests (n=67; black bar). Bar graphs represent mean ± standard error of mean (SEM).

We also confirmed that training in both contexts was equivalent for self-administration and extinction. Rats that trained in context A (n=47) versus context B (n=43) showed similar cocaine infusions (Figure 3-2a; two-way ANOVA, no context x time interaction; $F_{9,792}$=0.6775, p=0.7298), active (Figure 3-2b; $F_{9,792}$=1.057, p=0.3929), and inactive (Figure 3-2c; $F_{9,792}$=0.6592, p=0.7462) lever responding across self-administration days. Days until acquisition of self-administration also did not differ between the training contexts (unpaired t-test, t_{90}=1.650, p=0.1024). Active (context x time interaction; $F_{6,534}$=0.5174, p=0.7953) and inactive ($F_{6,534}$=0.8606, p=0.5237) lever presses during extinction training also did not differ between rats trained to self-administer in context A versus context B (Figure 3-4d).

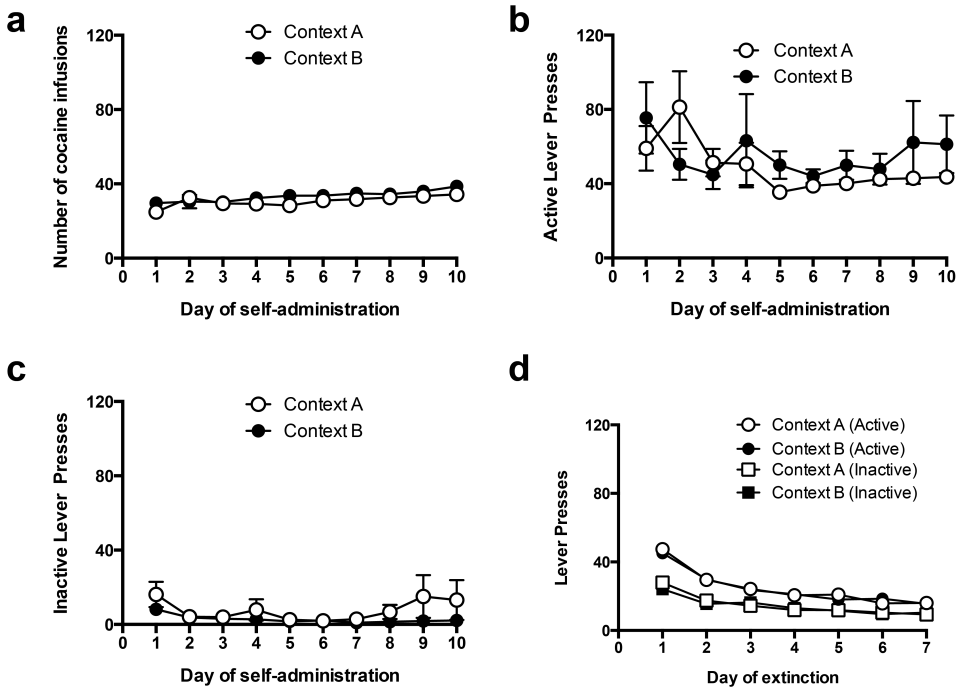

Figure 3-2. No differences between Context A and Context B during self-administration and extinction training. (a) Rats trained in context A (n=47) and those trained in context B (n=43) received a similar number of cocaine infusions across self-administration days. (b) No interactions of context x time were observed in the number of active lever presses or (c) inactive lever presses across self-administration days. (d) Active and inactive lever presses during extinction training also did not differ between rats trained to self-administer in context A versus context B. SEM indicated by error bars.

Lateral septum function during context- or cue-induced reinstatement of cocaine seeking

We first assessed a marker of neuronal activity as measured by the amount of Fos-protein induction using immunohistochemistry to determine if context- and cue-induced reinstatement differentially activated LS. Results showed rostral (Figure 3-3b; one-way ANOVA; $F_{3,20}$=5.532, p=0.0062) and caudal (Figure 3-3a,c; $F_{3,20}$=6.387, p=0.0033) LS expressed more Fos during context-, compared to cue-induced reinstatement of cocaine seeking. Caudal LS also expressed a significantly greater number of Fos cells when rats returned to a drug-associated versus yoked saline-associated contexts.

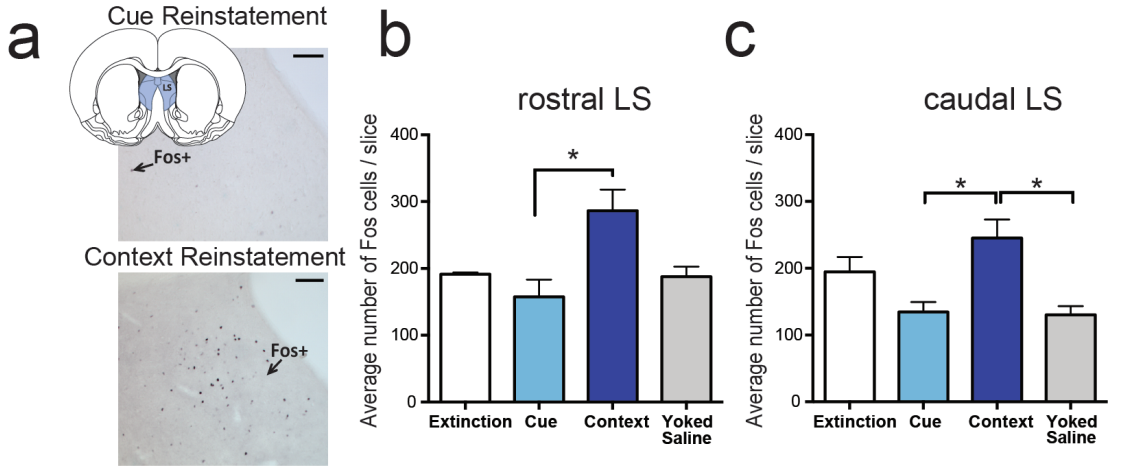

Figure 3-3. Lateral septum neurons activate during context-, but not cue-induced reinstatement of cocaine seeking. (a) Representative photomicrographs of Fos expression in LS during a cue- or context-induced reinstatement test; inset shows location of LS in a rat atlas coronal section (0.72 mm anterior to bregma; Paxinos and Watson, 2007). Scale bars represent 100 µm. Whole images adjusted for contrast and brightness in representative tissue for cue-induced reinstatement. (b) Context reinstatement (dark blue bars, n=8) induced a greater number of Fos cells compared to cue-induced reinstatement (light blue bars, n=6) in rostral LS (**p<0.01) or (c) compared to cue-induced reinstatement or yoked saline rats (gray bars, n=5) in caudal LS (**p<0.01). White bars represents the extinction group, n=5. SEM indicated by error bars.

To determine if there was a causal role of LS during context- or cue-induced reinstatement of cocaine seeking, we microinjected a cocktail of $GABA_A$ and $GABA_B$ agonists, baclofen and muscimol (B-M) into LS immediately prior to context- or cue-induced reinstatement. Figure 3-4a illustrates a representative microinjection site in LS as seen by a Nissl stain, and injection sites in LS for all rats collapsed onto one rat atlas section (0.72 mm anterior to bregma; Paxinos and Watson, 2007). Injection sites in LS ranged between 0.96 mm to 0.48 mm anterior to bregma. We found that B-M microinjections into LS attenuated both context-induced (Figure 3-4b; paired t-test, t_{11}=4.902, p=0.0005) and cue-induced (Figure 3-4c; Wilcoxon signed rank, z_{11}=-2.432, p=0.015) reinstatement compared to microinjections of artificial cerebral spinal fluid (aCSF), indicating that LS has a functional role for both reinstatement modalities.

We next assessed the same rats for general locomotor activity to determine if the behavioral effects on reinstatement reflected decreased motor activity. We found that B-M microinjections did not reduce horizontal activity (Figure 3-4d; t_{11}=1.882, p=0.0865), vertical activity (Figure 3-4e, z_{11}= -1.726, p=0.084), or total distance traveled (Figure 3-4f, t_{11}=1.005, p=0.3364) compared to microinjections of aCSF over the entire 2 h session. These results indicate that attenuated active lever responding during reinstatement following B-M injections were not the result of reduced general locomotor activity.

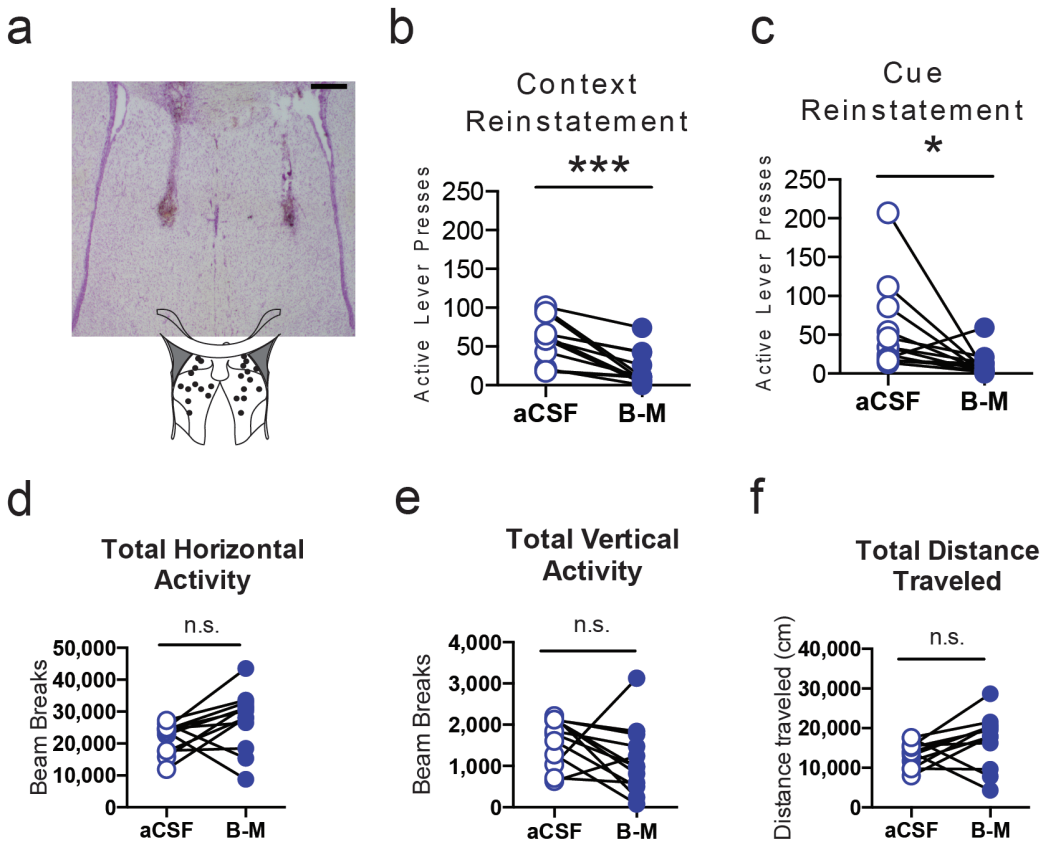

Figure 3-4. Inhibiting lateral septum blocks context- and cue-induced reinstatement of cocaine seeking. (a) Representative photomicrograph of cannulae injection sites in LS (Nissl stain, frontal section, dorsal up, midline at center). Brain atlas inset shows bilateral injection sites across all rats (black circles; n=12). Scale bar represents 200 μm. (b) Microinjections of the inhibitory agents baclofen plus muscimol (B-M, solid blue circles) into LS attenuated context-induced reinstatement (***p<0.001) and (c) cue-induced reinstatement relative to vehicle/aCSF (open blue circles) microinjections (*p<0.05). (d) Baclofen-muscimol microinjections into LS did not change total horizontal activity (t_{11}=1.882, p=0.0865) (e) total vertical activity (z_{11}=-1.726, p=0.084) or (f) total distance traveled (t_{11}=1.005, p=0.3364) compared to microinjections of aCSF over the 2 h session (n=12). n.s.=not significant.

Dorsal hippocampus inputs to lateral septum drive context-, but not cue-induced reinstatement of cocaine seeking

We next examined the possible roles of hippocampal regions in context- or cue-induced reinstatement of cocaine seeking. Fos data revealed that dorsal CA1 (dCA1;

Figure 3-5a,b; Kruskal-Wallis; $H_{3,20}$=11.71, p=0.0084), dorsal CA3 (dCA3; Figure 3-5a,c; one-way ANOVA; $F_{3,20}$=4.219, p=0.0183), dorsal dentate gyrus (dDG; Figure 3-5a,d; $F_{3,20}$=8.594, p=0.0007) expressed more Fos during context- compared to cue-induced reinstatement. Hippocampal regions also expressed more Fos-positive cells during context-induced reinstatement than during late extinction testing, albeit not significantly in dCA3. All septal (Figure 3-3) and hippocampal (Figure 3-5) regions expressed more Fos-positive cells (albeit not significantly in some cases) when rats returned to drug-associated versus yoked saline-associated context.

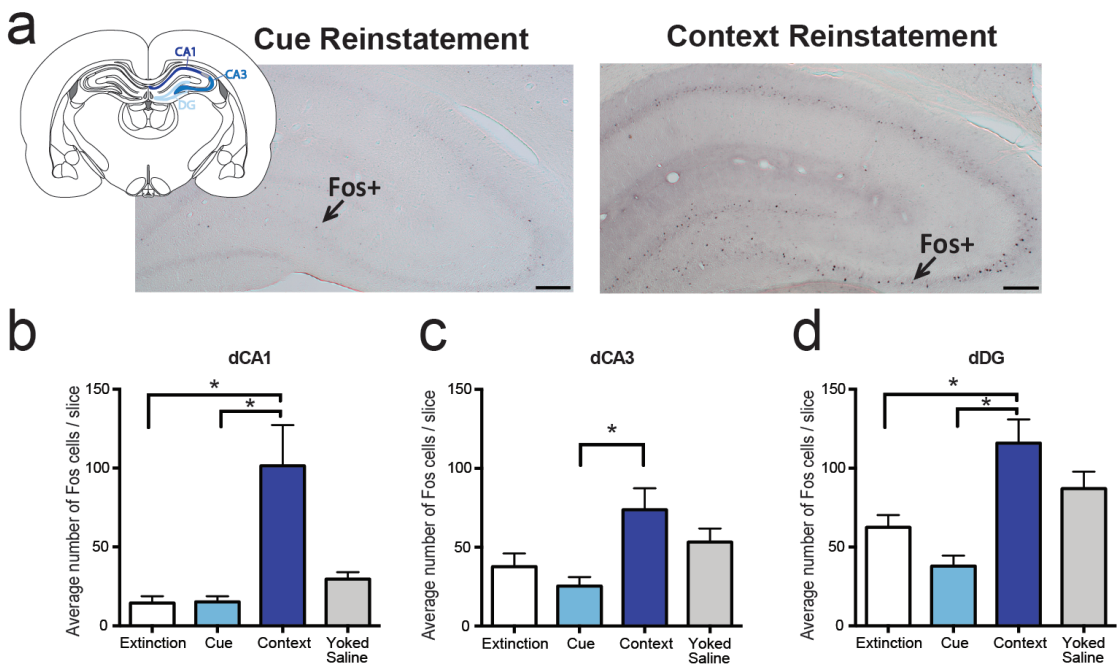

Figure 3-5. Dorsal hippocampal neurons activate during context-, but not cue-induced reinstatement of cocaine seeking. (a) Representative photomicrographs of Fos expression during cue- and context-induced reinstatement in dorsal hippocampal slices with inset of locations of dCA1, dCA3, and dentate gyrus (dDG) regions of dorsal hippocampus in a rat atlas coronal section (2.92 mm posterior to bregma; Paxinos and Watson, 2007). Scale bars represent 100 µm. Whole images adjusted for contrast and brightness in representative tissue for cue-induced reinstatement. (b) Context reinstatement (dark blue bars, n=8) induced more Fos cells than cue-induced reinstatement (light blue bars, n=6) or extinction (white bars, n=5) in dCA1 (**p<0.01) (c) than cue-induced reinstatement in dCA3 (*p<0.05), and (d) than cue-induced reinstatement and extinction in dDG (***p<0.001). Gray bars represent yoked saline group (n=5). SEM indicated by error bars.

We then questioned if neurons in dorsal hippocampus that express Fos during context-, but not cue-induced, reinstatement project to LS. We injected the retrograde tracer, CTb, unilaterally into LS (Figures 3-6a-c), and examined the percentage of hippocampal neurons retrogradely labeled from LS that expressed Fos (Figures 3-6d,e).

The center of the injection sites for LS ranged between 0.96 mm and 0.36 mm anterior to bregma. LS injection sites were aimed at the dorsal-intermediate region of LS, spreading ~ 2 mm anteroposteriorly. CTb labeling displays some anterograde properties that are visually distinct from injection sites and retrogradely-labeled neurons (which appear as individual cells). An example of the anterograde properties can be seen in the opposite hemisphere to the ipsilateral LS injection site (e.g., Figure 3-6b). This labeling most likely reflects the strong collateral projections within LS (Paxinos, 1995). With this analysis, we confirmed that CTb injection sites were strictly unilateral (Figure 3-6c).

Lateral septum retrograde tracer injections showed ipsilateral and contralateral labeling exclusively in CA3 of the dorsal hippocampus, similar to previous studies (Risold and Swanson, 1997). Both hemispheres of dCA3 projected nearly equally to one hemisphere of LS (Table 3-1).

Results from retrograde tracer experiments revealed that ipsilateral (Figure 3-6f; one-way ANOVA; $F_{2,26}=7.680$, $p=0.0024$) and contralateral (Figure 3-6g; $F_{2,26}=5.227$, $p=0.0124$) inputs from dCA3 to LS expressed more Fos during context- than cue-induced reinstatement or extinction.

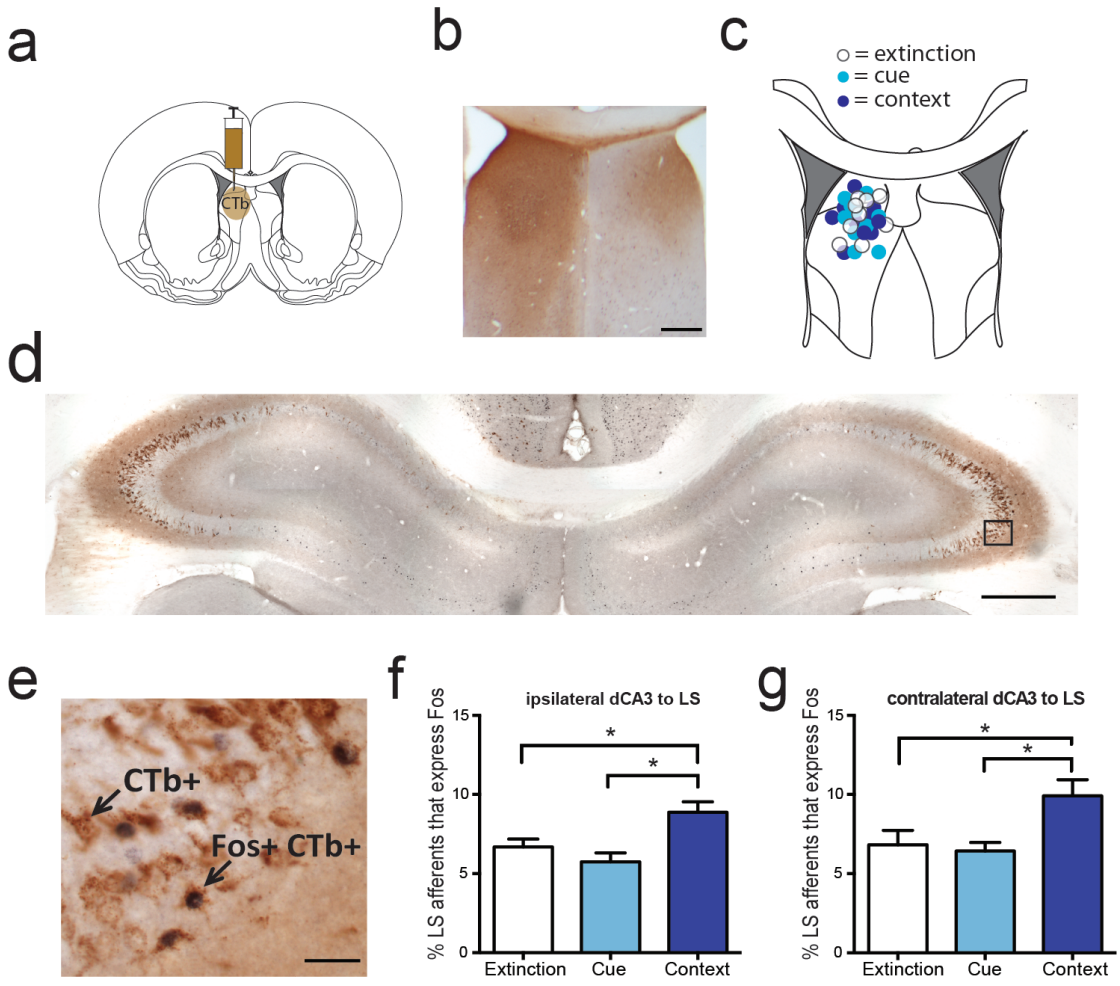

Figure 3-6. Dorsal hippocampus inputs to lateral septum activate during context-, but not cue-induced reinstatement of cocaine seeking. (a) Unilateral retrograde tracer (CTb) injection into LS. (b) Representative photomicrograph of a unilateral CTb injection into LS. Scale bar represents 200 μm. (c) The center of each CTb injection collapsed onto one atlas section zoomed into LS (0.72 mm anterior to bregma; Paxinos and Watson, 2007). White circles indicate rats assigned to the extinction group, light blue circles indicate rats assigned to the cue-induced reinstatement group, and dark blue circles represent rats assigned to the context-induced reinstatement group. (d) CTb and Fos expressed in dorsal hippocampus. Unilateral CTb injections in LS retrogradely label neurons in both hemispheres of dCA3. The black square represents the area magnified in panel e. Scale bar represents 500 μm. (e) Magnified photomicrograph of dCA3 neurons retrogradely-labeled from LS (CTb+; brown cytoplasmic stain), Fos (black nuclear stain), and CTb+ neurons co-labeled with Fos (Fos+CTb+). Scale bar represents 50 μm. (f) Ipsilateral dCA3 neurons retrogradely-labeled from LS expressed a significantly greater percentage of Fos during context- (n=10) compared to cue-induced reinstatement (n=10) or extinction (n=9) of cocaine seeking (*$p<0.01$). (g) Contralateral dCA3 neurons retrogradely-labeled from LS also expressed a significantly greater percentage of Fos during context- compared to cue-induced reinstatement or extinction of cocaine seeking (*$p<0.05$). SEM indicated by error bars.

Table 3-1. Mean ± SEM CTb-labeled cells per slice for hippocampal and BLA inputs to LS. Values represent the mean (± standard error of mean; SEM) of retrogradely labeled (CTb+) neurons in dorsal hippocampal CA3 regions (dCA3 ipsi, dCA3 contra, dCA3 both) after tracer injection into LS. The mean ± SEM values of the number of CTb cells in ventral hippocampal and BLA were quantified ipsilateral to the LS injection site based on the mainly ipsilateral nature of these projections. dCA3 ipsi, CA3 region of the dorsal hippocampus ipsilateral to the retrograde tracer injection in LS; dCA3 contra, CA3 region of the dorsal hippocampus contralateral to the retrograde tracer injection in LS; dCA3 both, the sum of both hemispheres of the CA3 region of the dorsal hippocampus; vCA1, CA1 region of the ventral hippocampus ipsilateral to the retrograde tracer injection in LS; vCA3, CA3 region of the ventral hippocampus ipsilateral to the retrograde tracer injection in LS, BLA, basolateral amygdala ipsilateral to the retrograde tracer injection in LS.

Number of CTb+ cells that project to LS

	CTb cells/slice		
Structure	Mean	±	SEM
dCA3 ipsi	279		16
dCA3 contra	277		21
dCA3 both	557		36
vCA1	932		50
vCA3	269		46
BLA	31		3

Inhibiting dorsal CA3 hippocampal inputs to lateral septum blocks context-, but not cue-induced reinstatement of cocaine seeking

We next sought to determine if hippocampal inputs to LS have a causal role in context- or cue-induced reinstatement. As dCA3 densely projects to LS, a pharmacological disconnection approach would not sufficiently inhibit all dCA3 projections to LS. To circumvent this issue, we bilaterally expressed the inhibitory hM4Di DREADD (Armbruster et al., 2007) under a synapsin promoter (*lenti-hsyn-hM4Di-HA-EGFP* or *AAV8-hsyn-hM4Di-mcherry*) in dCA3 and microinjected the DREADD agonist, clozapine-*N*-oxide (CNO) bilaterally into LS (Figures 3-7a-e).

Results showed hM4Di DREADD injections in dorsal hippocampus ranged between 2.76 mm to 3.12 mm posterior to bregma. Microinjection sites in LS (verified by Nissl stain) ranged between 1.44 mm to 0.36 mm anterior to bregma. Immunohistochemistry for the HA and mCherry tags fused to the hM4Di receptors

identified the locations of the hM4Di receptors at the injection sites and at the terminals (Figures 3-7c-e). DREADD and microinjection sites were each collapsed onto one rat atlas section at 2.92 mm posterior, and 0.72 mm anterior, to bregma, respectively (Figures 3-7b; Paxinos and Watson, 2007).

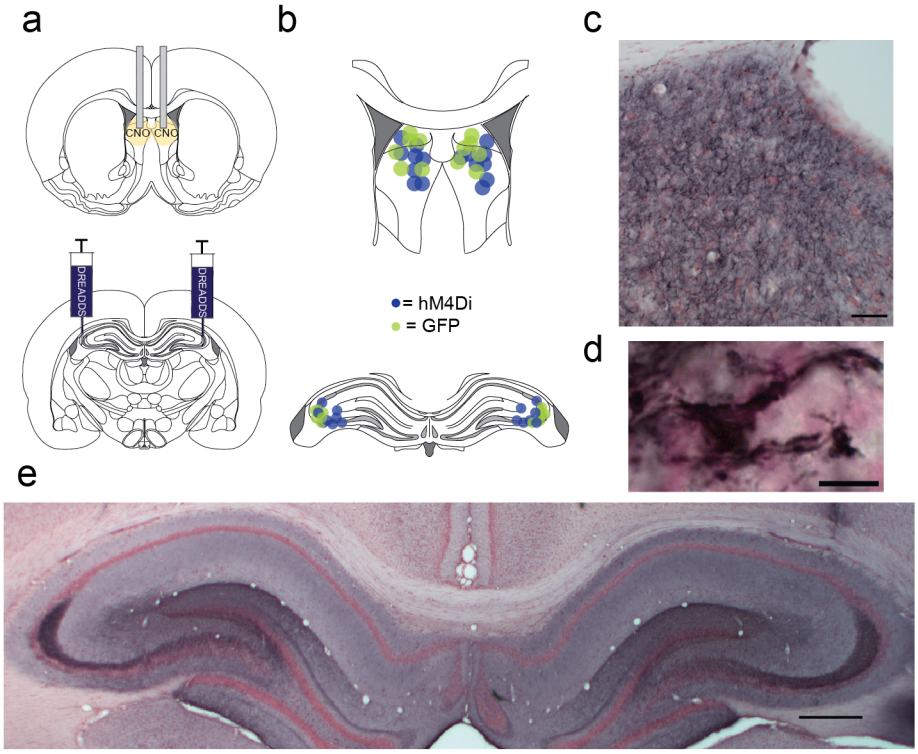

Figure 3-7. DREADD expression for injection sites in dorsal hippocampus and terminals in lateral septum. (a) Illustration of paradigm for DREADD hM4Di viral injections into dorsal hippocampus and CNO locally infused onto hM4Di-expressing dorsal hippocampal terminals in LS. (b) The schematic of LS (upper panel) shows bilateral microinjector sites collapsed onto one rat atlas section (0.72 mm anterior to bregma, Paxinos and Watson, 2007). The schematic of dorsal hippocampus (lower panel, 2.92 mm posterior to bregma, Paxinos and Watson, 2007) shows the central site of injection of the DREADD virus collapsed onto one rat atlas section. Blue circles represent the locations of injections of rats that received a viral vector expressing the gene of the hM4Di DREADD receptor ("hM4Di"). Green circles represent the locations of injections of rats that received a viral vector without hM4Di receptor gene ("GFP"). (c) Representative photomicrograph of hM4Di-expressing dCA3 terminals in LS seen with an antibody against the HA-tag fused to the hM4Di receptor (purple/black terminals) and counterstained with neutral red. Scale bar represents 100 μm. (d) Magnified photomicrograph of DREADD labeling in a dorsal CA3 hippocampal neuron within panel e. Scale bar represents 10 μm. (e) Representative photomicrograph of bilateral DREADD injection into dCA3 of hippocampus (purple/black stain). As above, an antibody against the HA-tag fused to the hM4Di receptor stained the DREADDs and tissue was counterstained with a neutral red Nissl stain. Scale bar represents 500 μm.

We found that microinjections of CNO among hM4Di-expressing dCA3 terminals in LS attenuated context- (Figure 3-8a; paired t-test, t_9=2.286, p=0.0481), but not cue-induced (Figure 3-8b; Wilcoxon signed rank, z_9=-0.296, p=0.767) reinstatement compared to aCSF microinjections, indicating the dorsal hippocampus only drives context-induced reinstatement via LS. CNO microinjections in LS did not alter reinstatement behavior in rats with hippocampal injections of a control virus lacking the hM4Di gene (*AAV8-hsyn-EGFP;* Figure 3-8a,b; paired t-test, context: t_7=0.09353, p=0.9281; cue: t_7=0.1211, p=0.9070). These results indicate that viral injections or CNO microinjections did not produce non-specific effects.

We also assessed general locomotor activity to determine if effects with CNO on context-induced reinstatement were due to reduced motor function. Microinjection of CNO onto hM4Di-expressing dCA3 terminals in LS did not alter horizontal activity (Figure 3-8c; paired t-test; t_8=0.2878, p=0.7808), vertical activity (Figure 3-8d; t_8=0.7761, p=0.4600), or total distance traveled (Figure 3-8e; t_8=0.4461, p=0.6674) during the 2 h session.

Overall these results indicate that dorsal CA3 hippocampal inputs to LS drive specifically context-, but not cue-induced reinstatement of cocaine seeking.

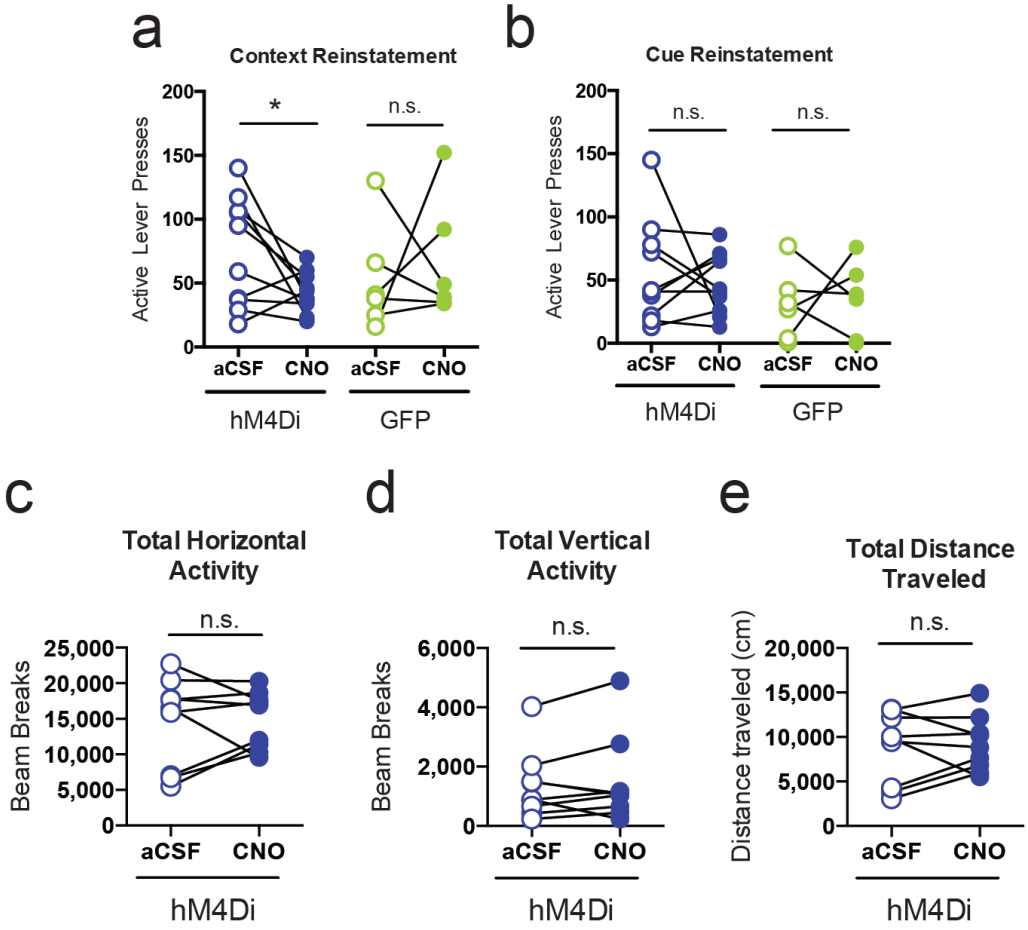

Figure 3-8. Dorsal hippocampus inputs to lateral septum drive context-, but not cue-induced reinstatement. (a) Inhibiting hM4Di-expressing dorsal hippocampal terminals in LS with local CNO attenuated active lever responding during context-induced reinstatement of cocaine seeking compared to the same rats that received aCSF microinjections (n=10, paired t-test, $p<0.05$*). There were also no differences between CNO versus aCSF microinjections in GFP control rats (n=6). (b) Inhibiting hM4Di-expressing dorsal hippocampal terminals in LS with CNO (n=10, solid blue circles) did not affect lever responding during cue-induced reinstatement of cocaine seeking compared to the same rats that received aCSF (n=10, open blue circles). There were also no effects of CNO (solid green circles) or aCSF (open green circles) on GFP control rats (n=6). (c) CNO microinjections in LS onto hM4Di-expressing dCA3 terminals did not alter total horizontal activity, (d) total vertical activity, or (e) total distance traveled compared to aCSF microinjections over the 2 h session (n=9, $p>0.05$, n.s.). n.s.= not significant.

Basolateral amygdala and ventral hippocampal inputs to lateral septum

We found that B-M microinjections in LS attenuated both context- and cue-induced reinstatement, but CNO microinjections onto hM4Di-expressing dCA3 terminals

in LS only attenuated context-induced reinstatement. These data imply functional specificity of inputs to LS. We next examined other afferents to LS that may be involved in cue-induced reinstatement behavior. Basolateral amygdala (BLA) seemed a likely target because of its involvement in encoding conditioned cues (Maren and Fanselow, 1996; Meil and See, 1997; McLaughlin and See, 2003; Stefanik and Kalivas, 2013) despite it being a sparse projection to LS (Risold and Swanson, 1997). However, the percentages of Fos+ BLA neurons retrogradely labeled from LS did not differ between context-induced reinstatement, cue-induced reinstatement, or extinction (Figure 3-9; one-way ANOVA, $F_{2,26}$=7.306, p=0.4913).

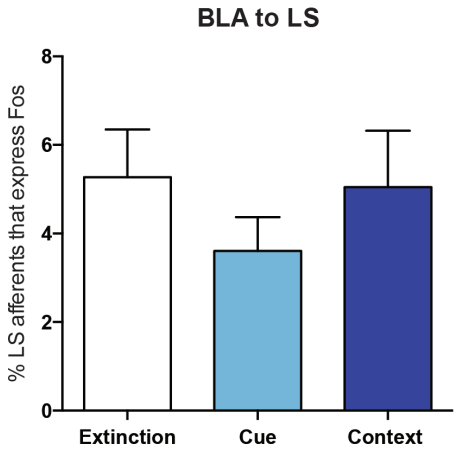

Figure 3-9. Basolateral amygdala inputs to lateral septum do not activate during reinstatement. Basolateral amygdala (BLA) neurons retrogradely labeled from LS did not express a greater percentage of Fos during cue- (n=10, light blue bars) or context-induced reinstatement (n=10, dark blue bars) compared to extinction (n=9, white bars) of cocaine seeking. SEM indicated by error bars.

We then hypothesized that ventral hippocampus inputs to LS may be involved because ventral hippocampus has a strong projection to LS (Risold and Swanson, 1997), and inhibiting ventral hippocampus blocks cue-induced reinstatement of cocaine seeking (Rogers and See, 2007). We first examined whether ventral CA1 (vCA1) and ventral CA3 (vCA3) neurons expressed Fos during context- or cue-induced reinstatement of cocaine seeking. Ventral CA1 and CA3 hippocampal cells expressed (a

trend in) elevated Fos during both context- and cue-induced reinstatement behavior, relative to extinction and yoked saline controls (Figure3-10a-c; vCA1: $F_{3,20}$=2.413, p=0.0968; vCA3: $F_{3,20}$=1.508, p=0.1082).

We then quantified and calculated the percentages of vCA1 and vCA3 neurons retrogradely labeled from LS that expressed Fos during context- or cue-induced reinstatement, or extinction. We found that ventral hippocampal CA1 inputs to LS expressed significantly greater percentages of Fos during both context- and cue-induced reinstatement compared to during extinction sessions (Figure 3-10d,e; one-way ANOVA; $F_{2,26}$=7.983, p=0.0020). Ventral hippocampal CA3 inputs to LS did not express significantly greater percentages of Fos during either reinstatement modality relative to extinction (Figure 3-10f; $F_{2,26}$=0.9968, p=0.3827). In contrast to dCA3-LS pathway (which induced Fos only during context-induced reinstatement), these results indicate that vCA1 neurons that project to LS were activated during both reinstatement modalities.

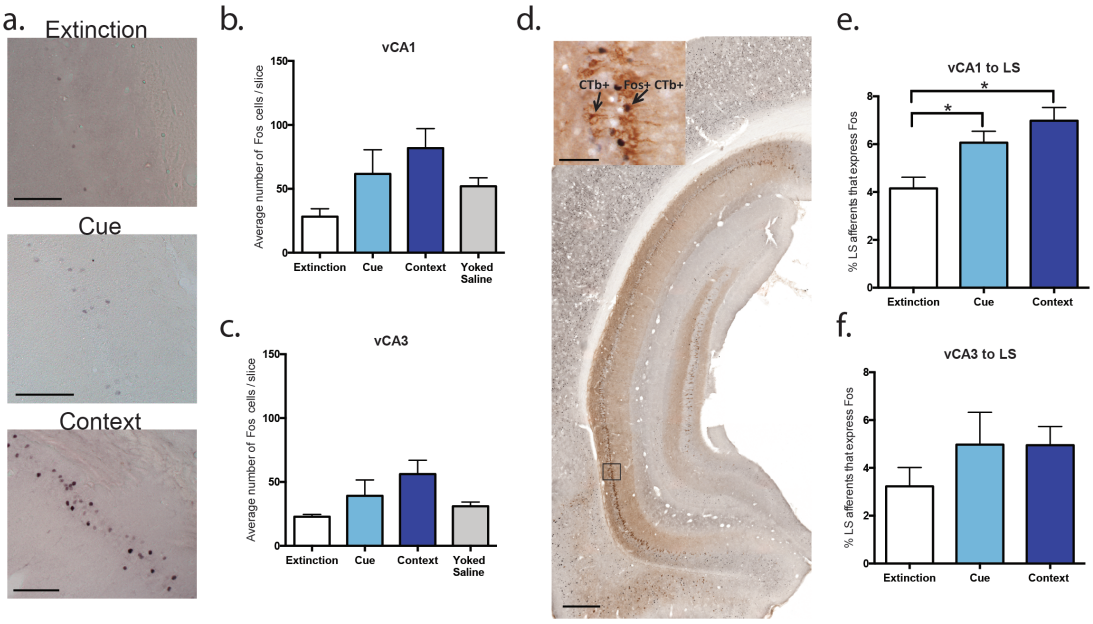

Figure 3-10. Ventral hippocampal neurons retrogradely labeled from lateral septum activate during context- and cue-induced reinstatement. (a) Representative photomicrographs of Fos labeling in CA1 region of the ventral hippocampus (vCA1) during different sessions. Rats sacrificed immediately following late extinction (upper panel) expressed a small number of Fos+ cells, whereas rats sacrificed immediately following cue- (middle panel) or context-induced (bottom panel) reinstatement expressed more Fos cells, albeit not significantly (panel b). Scale bars represent 100 μm. (b) The average number of Fos cells in vCA1 showed a trend for more Fos cells during cue- (light blue bars; n=6) and context-induced (dark blue bars; n=8) reinstatement relative to extinction (white bars; n=5) or yoked saline groups (gray bars; n=5). (c) The CA3 region of ventral hippocampus (vCA3) also showed a trend in enhanced Fos expression during context- and cue-induced reinstatement compared to extinction. (d) Representative photomicrograph of retrograde labeling in ventral hippocampus after CTb injection into LS. The black square represents the area of the magnified region of the inset. Scale bar represents 500 μm. Inset shows magnified photograph of neurons with or without Fos in vCA1 labeled by retrograde tracer from LS (CTb+; brown cytoplasmic stain), Fos+ (black nuclear stain), and CTb+ neurons co-labeled with Fos (Fos+CTb+, combined brown cytoplasmic stain with black nuclear stain). Scale bar represents 50 μm. (e) Ipsilateral vCA1 neurons retrogradely labeled from LS expressed a significantly greater percentage of Fos during both context-induced reinstatement (n=10, dark blue bars) and cue-induced reinstatement of cocaine seeking (n=10, light blue bars) compared to extinction (n=9, white bars). (f) There were no differences across all groups in the percentages of ipsilateral vCA3 neurons retrogradely labeled from LS that expressed Fos. n.s.=not significant. SEM indicated by error bars.

In the same manner that we investigated dorsal hippocampal inputs to LS, we next determined if there was a causal role for ventral hippocampal inputs to LS during context- and cue-induced reinstatement of cocaine seeking. We bilaterally expressed the inhibitory hM4Di DREADD under a synapsin promoter in ventral hippocampus and microinjected CNO in LS (Figure 3-11a-c).

Results showed that hM4Di DREADD injections in ventral hippocampus ranged between 4.92 mm to 5.76 mm posterior to bregma. Microinjection sites in LS (as verified by Nissl stain) ranged between 0.96 mm and 0.36 mm anterior to bregma. Immunohistochemistry for the mCherry tag fused to the hM4Di receptor identified the locations of the hM4Di receptors at the injection sites and at the terminals. DREADD and microinjections sites were each collapsed onto one rat atlas section at 5.04 mm posterior, and 0.72 mm anterior, to bregma, respectively (Figures 3-11a-c; Paxinos and Watson, 2007).

We found that inhibiting hM4Di-expressing ventral hippocampal terminals in LS did not block context- (Figure 3-11d; paired t-test, t_7=0.09353, p=0.9281) or cue-induced (Figure 3-11e; t_7=0.1211, p=0.9070) reinstatement compared to aCSF controls. Unsurprisingly, rats expressing the virus lacking the hM4Di gene (GFP controls) in ventral hippocampus also did not show differences between context- (Figure 3-11d; t_5=0.4589, p=0.6655) or cue-induced (Figure 3-11e; t_5=0.8815, p=0.4184) reinstatement when microinjected with CNO versus aCSF in LS. CNO microinjections in LS onto hM4Di-expressing ventral hippocampal terminals did not alter general locomotor function as measured by horizontal activity (Figure 3-11f; t_7=0.8257, p=0.4362), vertical activity (Figure 3-11g; t_7=0.3729, p=0.7202), or total distance traveled (Figure 3-11h; t_7=0.9601, p=0.3690) compared to aCSF microinjections during the 2 h session.

Overall, these results indicate that inhibiting ventral hippocampal inputs to LS are not sufficient to reduce context- or cue-induced reinstatement of cocaine seeking.

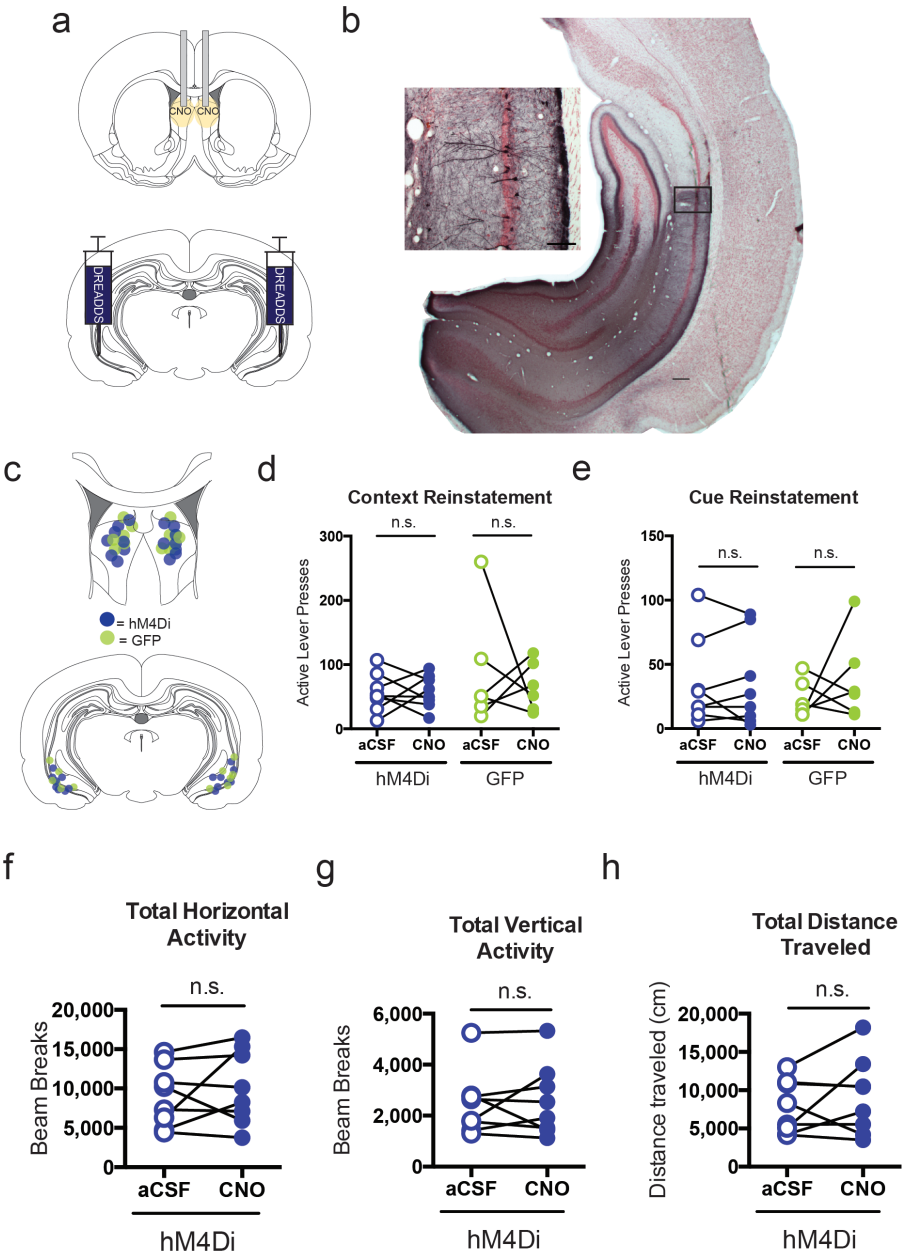

Figure 3-11. Inhibiting ventral hippocampal inputs to lateral septum does not block reinstatement. (a) Schematic of experimental design. DREADD hM4Di virus was injected bilaterally into ventral hippocampus and CNO was microinfused onto hM4Di-expressing ventral hippocampal terminals in LS. *Continued.*

(b) Representative photomicrograph of DREADD injection into ventral hippocampus as seen by a DsRed antibody against the mcherry-tag fused to the hM4Di DREADD receptor (purple/black regions) and counterstained with neutral red. Black square represents the zoomed area seen in the inset. Scale bar represents 1000 μm. Inset shows cell body and axonal labeling of ventral hippocampal neurons expressing the hM4Di receptor. Inset scale bar represents 100 μm. Whole images adjusted for brightness. (c) The magnified section of LS shows bilateral microinjector sites collapsed onto one rat atlas section (upper panel, frontal; 0.72 mm anterior to bregma, Paxinos and Watson, 2007). The section of ventral hippocampus shows the central site of injection of the DREADD and control viruses collapsed onto one rat atlas section (lower panel, 5.04 mm posterior to bregma). Blue circles represent injection sites of rats that received a vector expressing the hM4Di DREADD receptor gene ("hM4Di"). Green circles represent the injection sites of rats that received the same vector but contained GFP instead of the hM4Di receptor gene. (d) Microinjection of CNO among hM4Di-expressing ventral hippocampal terminals in LS (n=8, solid blue circles) did not affect active lever responding during context-induced reinstatement of cocaine seeking compared to the same rats that received aCSF microinjections (open blue circles, paired t-test, $p>0.05$, n.s.). CNO or aCSF injections into LS did not induce any non-specific effects in non-DREADD (GFP) rats (n=6, green circles). (e) Microinjection of CNO among hM4Di-expressing ventral hippocampal terminals in LS had no effect on active lever responding during cue-induced reinstatement of cocaine seeking compared to the same rats that received aCSF microinjections ($p>0.05$, n.s.). There were also no effects of CNO versus aCSF microinjections in GFP control rats. (f) CNO microinjections in LS onto hM4Di-expressing dCA3 terminals did not alter total horizontal activity (g) total vertical activity or (h) total distance traveled (n=8, $p>0.05$, n.s.). n.s.= not significant.

Discussion

This study adapted a within-subjects assessment to investigate both the involvement of LS in reinstatement to contextual vs. discrete drug cues, and hippocampal regions that drive context- vs. cue-induced reinstatement of cocaine seeking via LS. Our data revealed that dorsal hippocampal neurons that project to LS were activated specifically during context-, but not cue-induced reinstatement. In support of these results, chemogenetic-mediated inhibition of the dorsal hippocampal-LS pathway attenuated context-, but not cue-induced reinstatement. Interestingly, we found that pharmacological inhibition of LS reduced both context- and cue-induced reinstatement of cocaine seeking. In an effort to examine other LS afferents, we found that ventral hippocampal, but not BLA, inputs to LS were activated during both context- and cue-induced reinstatement; however, chemogenetic-mediated inhibition of the

ventral hippocampal-LS circuit did not block either reinstatement modality. These results indicate a functional role of LS in multiple reinstatement modalities, and that dorsal hippocampus drives context-induced reinstatement via inputs to LS.

Our data support a handful of studies that implicate LS in drug addiction and relapse to numerous drugs of abuse including cocaine (Zahm et al., 2010), morphine (Le Merrer et al., 2007), and methamphetamine (Cornish et al., 2012). Previous findings in our lab demonstrated that inhibition of LS, and of lateral hypothalamus orexin afferents from LS block the preference for the cocaine-paired context in conditioned place preference (Sartor and Aston-Jones, 2012). In addition, the LS-VTA circuit activates during cue-induced reinstatement (Mahler and Aston-Jones, 2012), and a disconnection approach of the LS-VTA pathway blocks context-induced reinstatement (Luo et al., 2011). Here, we showed for the first time that bilateral inhibition of LS blocks both context- and cue-induced reinstatement of cocaine seeking, indicating LS is an important node in stimulus-induced relapse behavior.

Interestingly, although LS had a causal role in both context- and cue-induced reinstatement, cue-induced reinstatement did not induce Fos in LS. This may be due to insufficient synaptic stimulation during cue-induced reinstatement that fails to meet a threshold to produce Fos within the nucleus of LS neurons. Fos is minimally expressed under basal conditions or under conditions of 'usual' intensity from afferent inputs, and its induction depends in part on the temporal patterns of action potential firing (Kovács, 1998). Thus, it may be that during cue-induced reinstatement, the 'usual' intensity or temporal patterns generated by afferent inputs to LS neurons still support a functional role, despite not inducing Fos.

Nonetheless, our Fos data supported several previous findings indicating that returning to a drug-associated context enhances Fos expression in dorsal hippocampal

and LS neurons (Brown et al., 1992; Neisewander et al., 2000; Trouche et al., 2016), but with a few exceptions (Neisewander et al., 2000; Kufahl et al., 2009). Differences in Fos expression in LS and hippocampus from other studies may be attributed to our unique behavioral paradigm, the time at which Fos was collected in the session, extinction versus abstinence training, and/or the methods of Fos quantification.

Our data also support studies that indicate dorsal and ventral hippocampus as anatomically and functionally distinct brain structures (Swanson and Cowan, 1977; Moser and Moser, 1998; Fanselow and Dong, 2010). For example, lesions of dorsal, but not ventral, hippocampus block spatial learning in both watermaze and radial arm maze tasks (Moser et al., 1995; Pothuizen et al., 2004). In addition, dorsal hippocampus lesions attenuate freezing behavior in a contextual, but not tone fear-conditioning paradigm (Kim and Fanselow, 1992), whereas inhibition or lesions of ventral hippocampus block freezing responses to tones and contexts associated with fear (Maren and Holt, 2004; Hunsaker and Kesner, 2008). Reinstatement behavior reveals similar functional dissociations. Inhibition of the dorsal hippocampus attenuates context-, but not cue-induced reinstatement of cocaine seeking (Fuchs et al., 2005), whereas inhibition of ventral hippocampus attenuates both context- (Lasseter et al., 2010) and cue-induced cocaine seeking (Rogers and See, 2007). At the circuit level, we found that chemogenetic-mediated inhibition of dorsal hippocampal inputs to LS attenuated context-, but not cue-induced reinstatement, whereas inhibition of ventral hippocampal-LS pathway did not block either reinstatement modality. Thus, our data also revealed dissociations between the functions of dorsal and ventral hippocampus via their inputs to LS.

We found it interesting that although inhibition of ventral hippocampus to LS pathway did not reduce reinstatement behavior, vCA1 inputs to LS expressed Fos during

both context- and cue-induced reinstatement relative to extinguished cocaine seeking. Possible explanations may be that the DREADD virus did not infect a sufficient number of ventral hippocampal neurons (given the size of the structure) or CNO did not effectively inhibit enough terminals in LS. However, our results more likely indicate that other areas, aside from ventral hippocampus, may integrate in LS to drive reinstatement behavior. Some of these inputs may be from canonical addiction-related regions such as mPFC or VTA, which are important for cue-induced reinstatement (McLaughlin and See, 2003; Di Ciano and Everitt, 2004b), and project to LS (Sheehan et al., 2004). The BLA-LS circuit also may be functionally important during context- or cue-induced reinstatement, despite not inducing more Fos relative to extinguished cocaine seeking. To our knowledge, these experiments are the first to investigate brain regions that drive drug relapse via LS. Thus, an immense number of questions remain unanswered about LS. Future studies will need to seek the input to LS driving cue-induced reinstatement behavior.

Moreover, our results revealed that specific brain circuits are involved in particular triggers of relapse. These data may provide a circuitry-based explanation for the concept of goal-trackers and sign-trackers (Silva et al., 1992; Flagel et al., 2010), and for why individual rats are sensitive to specific relapse triggers (Robinson et al., 2014; Saunders et al., 2014). Preferences of a specific relapse trigger may be related to how the dorsal hippocampus and its projections to LS encode the relative saliency of the drug-associated contextual versus discrete cues. Similarly, but from a clinical perspective, these data may provide a circuitry-based explanation for why addicts frequently relapse upon returning home despite successfully extinguishing psychological and physiological responses to drug-conditioned cues in the clinic (Childress et al., 1993; Price et. al, 2010).

In conclusion, we adapted a within-subjects assessment of context- vs. cue-induced reinstatement, useful to characterize circuits involved in triggers for specific aspects of stimulus-induced drug relapse. Our studies revealed a dorsal, but not ventral, hippocampal-LS circuit that drives context, but not cue-induced reinstatement behavior. These findings support other results indicating separate behavioral functions for dorsal vs. ventral hippocampus. Our data also lend support to a growing number of studies that implicate LS in drug addiction. Collectively, our results identified a neural circuit that provokes drug seeking triggered by drug-associated contexts, thus identifying a novel target for treating drug addiction.

CHAPTER 4: CONCLUSIONS & FUTURE DIRECTIONS

CONCLUSIONS

Decades of research have demonstrated that addiction is a complex and intricate pathological disease. Our overall understanding of addiction as a disease has continued to progress as scientists unravel the neurocircuitry of addiction pathology, but much more still needs to be understood to ultimately treat addiction. One of the biggest hurdles in treating addiction is the complex neurocircuitry that mediates relapse. As such, this dissertation focused on providing insight into the neurocircuits mediating stimulus-induced reinstatement of cocaine seeking.

In Chapter 2, we investigated NAcC and NAcSh afferents that induced Fos during cue-induced reinstatement of cocaine and sucrose seeking. We found that PL, BLA, and vSub neurons that project to NAcC, but not NAcSh, exhibited Fos induction during cue-induced reinstatement compared to extinguished cocaine seeking behavior. Similar results were not seen during cue-induced reinstatement of sucrose seeking. In contrast to BLA and vSub projections to NAcC, only the PL-NAcC pathway positively correlated with the degree of cue-induced cocaine seeking behavior. These results support NAcC as an important structure in cue-induced reinstatement of cocaine seeking and indicate a particular recruitment of PL inputs to NAcC during cue-induced reinstatement of cocaine seeking. These results were the first to show that multiple excitatory regions that project to NAcC, but not to NAcSh, induce Fos during cue-induced reinstatement of cocaine seeking; also that these same circuits do not activate during cue-induced reinstatement of sucrose seeking. These results provide insight into neurocircuits mediating conditioned cues associated with drug versus natural rewards.

In Chapter 3, we investigated LS, and LS afferents during context- and cue-induced reinstatement of cocaine seeking. We found that dorsal hippocampus, LS, and dorsal hippocampus inputs to LS induced more Fos during context- than cue-induced reinstatement of cocaine seeking. In support of these data, dorsal hippocampal neurons that project to LS also had a causal role in context-, but not cue-induced reinstatement. Lateral septum inhibition revealed a functional role in both context- and cue-induced reinstatement. We also explored ventral hippocampal inputs to LS and found this circuit was Fos-activated during context- and cue-induced reinstatement of cocaine seeking, but did not appear to play a functional role in either reinstatement modality. These results were the first to elucidate brain regions that drive relapse via LS and revealed a specific circuit mediating context-, but not cue-induced reinstatement of cocaine seeking.

In total, NAcC and LS are both critical nodes in cocaine seeking behavior elicited by drug-associated stimuli and certain afferents to these regions are important for stimulus-induced reinstatement. These results provide greater insight into the neurocircuits that mediate relapse to contextual and discrete drug cues. Figure 4-1 reflects a schematic of the neurocircuitry examined in this dissertation, including PL, IL, BLA, and (ventral) hippocampal projections to NAcC or NAcSh (Chapter 2), as well as, BLA and (dorsal + ventral) hippocampal projections to LS (Chapter 3).

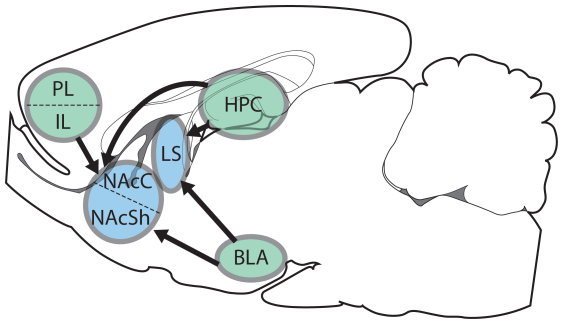

Figure 4-1. Schematic of inputs to NAc and LS examined during stimulus-induced reinstatement of cocaine seeking. PL, prelimbic; IL, infralimbic; NAcC, nucleus accumbens core; NAcSh, nucleus accumbens shell; LS, lateral septum; HPC, hippocampus; BLA, basolateral amygdala. Brain section is 1.13mm lateral from midline (Paxinos and Watson, 2007). Arrows represent projections from various brain structures.

Mechanism of cue-induced relapse behavior via canonical accumbens addiction circuitry

The mPFC, BLA, and vSub of the hippocampus are all sources of glutamate release into NAc. Glutamate induces firing of NAc neurons, which leads to activation of motor output systems (Haber, 2016) that initiate drug-seeking behaviors. This is portrayed in the canonical addiction circuitry seen in Figure 4-2. Our data support the importance of glutamate inputs to NAc during addiction pathology as we found neurons from multiple NAc glutamatergic afferents were activated during cue-induced reinstatement of cocaine seeking. The mPFC, BLA, and vSub may carry functional distinct information about drug-associated cues that provoke craving in addicts. However, the convergence of these signals in NAcC may be what initiates drug seeking and ultimately leads to relapse (Britt et. al., 2014; Floresco, 2015). Our data also showed that only Fos in the PL-to-NAcC pathway positively correlated with the degree of cocaine-seeking behavior, indicating this circuit may be particularly important in eliciting cocaine-seeking behaviors.

Overall, our data support the importance of NAc glutamatergic afferents, reveal activation of projections to NAcC, but not to NAcSh, and show a particular importance of the PL-NAcC pathway during cue-induced cocaine (but not sucrose) seeking behavior.

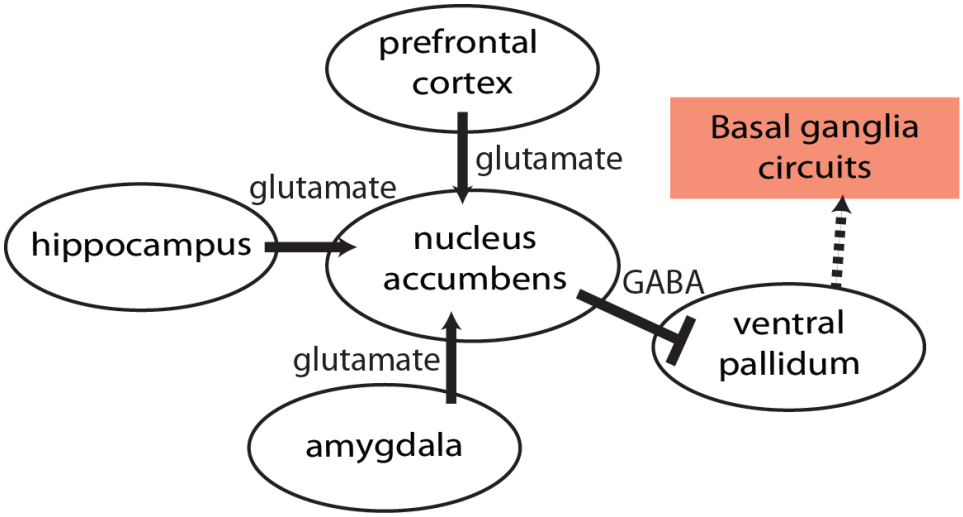

Figure 4-2. Canonical relapse circuitry involving glutamate release into nucleus accumbens. The prefrontal cortex, hippocampus, and amygdala release glutatmate into nucleus accumbens. Nucleus accumbens neurons fire and activate basal ganglia circuitry, which initiates motivationally driven movement. Diagram adapted from Kalivas and Volkow, 2005 and Floresco, 2015.

Mechanism of context- and cue-induced reinstatement of cocaine seeking via LS

Luo et al. (2011) revealed a circuit from dorsal CA3 (dCA3) to VTA via LS using the transynaptic tracer, pseudorabies virus. Furthermore, theta stimulation of dCA3 induced both excitatory and inhibitory firing patterns in VTA neurons. Inhibiting LS neurons during dCA3 theta stimulation prevented these changes in firing of VTA neurons. Pharmacological inhibition of dCA3, or of the LS-VTA pathway, attenuated context-induced reinstatement of cocaine seeking. Based on these data, it was proposed that dCA3 glutamatergic neurons excite LS GABAergic neurons, these LS neurons then inhibit GABA interneurons in VTA, resulting in disinhibition of VTA dopamine neurons, thus promoting context-driven motivational responses for reward (Figure 4-3).

We found that inhibition of LS, or of dCA3 inputs to LS, attenuated context-induced reinstatement of cocaine seeking. Our data further support this proposed model of the circuitry mediating context-reward associations. Our data are consistent with this model in which inhibition of this dCA3-LS circuit cause disinhibition of local VTA GABA

neurons, which then reduce VTA dopamine neuron firing. This would theoretically diminish context-reward associations and attenuate context-induced reinstatement, as we found.

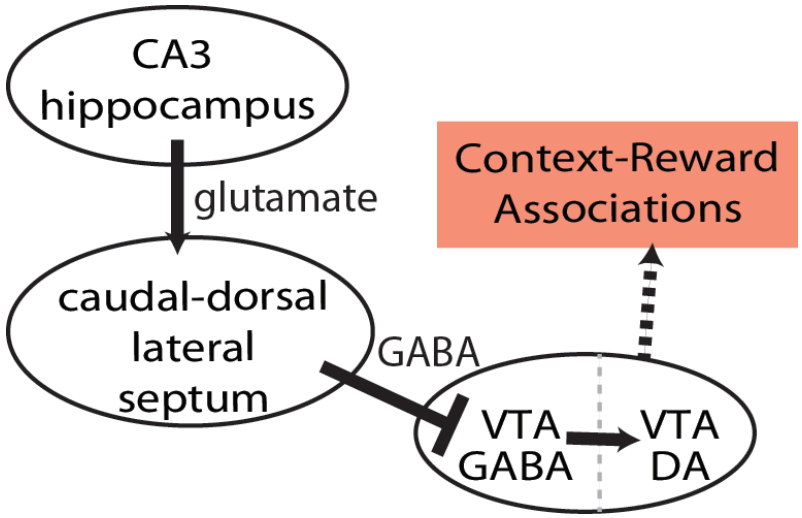

Figure 4-3. Proposed model of the formation of context and reward associations. CA3 region of the dorsal hippocampus releases glutamate on caudal-dorsal LS neurons, which release GABA to inhibit VTA interneurons causing disinhibition of VTA dopamine neurons to promote context-reward associations. VTA, ventral tegmental area; GABA, gamma-aminobutyric acid; DA, dopamine. Diagram adapted from Luo et. al., 2011.

We also found that inhibition of LS, but not inhibition of the dCA3-LS circuit, attenuated cue-induced reinstatement of cocaine seeking. These data indicate that LS has a functional role in cue-induced reinstatement of cocaine seeking, but the dorsal hippocampus is not the region that drives cue-induced reinstatement via LS. We found that ventral hippocampal, but not BLA, inputs to LS expressed more Fos during cue- and context-induced reinstatement of cocaine seeking compared to extinguished cocaine seeking behavior. However, chemogenetic-mediated inhibition of ventral hippocampal inputs to LS did not reduce cue- or context-induced reinstatement of extinguished cocaine seeking. These data likely indicate a currently unknown structure drives cue-induced reinstatement via LS. Although the mechanism in which LS induces cue-

induced reinstatement is unknown, potential mechanisms in which this may occur are suggested in the 'Future Directions' section below.

FUTURE DIRECTIONS

Are NAc afferents that induced Fos necessary for reinstatement behavior?

New technology has been created to examine if activated neuronal ensembles are functionally relevant for drug-seeking behaviors. This technology uses cFos-lacZ transgenic rats where transcription of lacZ is under the control of a cFos promoter (Cruz et al., 2013). The transcribed lacZ mRNA produces the protein beta-galactosidase. Thus, beta-galactosidase expresses only in Fos-expressing cells. The drug, Daun02, can then be injected into a region of interest to selectively inhibit Fos-activated neurons by a catalysis reaction initiated by the presence of beta-galactosidase. However, this technology has only been used in specific brain structures (Koya et al., 2009; Fanous et al., 2012; Cruz et al., 2014; Pfarr et al., 2015; Bastide et al., 2016), and not to investigate neuronal circuits. It may be interesting to use this technology to examine the functional relevance of specific neuronal ensembles in connected circuits. The Fos-lacZ transgenic rats combined with a Daun02 disconnection approach (e.g. Daun02 in one region and Daun02 in a contralateral hemisphere of a connected region) could be used to inhibit specific neuronal ensembles in connected brain regions during reinstatement behavior. If inactivation of the activated cells in both hemispheres reduced drug-seeking behavior, it would indicate that activated neuronal ensembles in the circuit are necessary (causal) for drug-seeking behavior. This would be an interesting future experiment to interpret whether the PL, BLA, and vSub projections to NAcC that induced Fos during cue-induced reinstatement of cocaine seeking were truly the neurons functionally relevant for the behavioral response.

What structure drives cue-induced reinstatement via lateral septum? Is lateral septum involved in stress-induced reinstatement of cocaine seeking?

There are a plethora of questions that may be asked regarding LS in drug addiction pathology as our scientific knowledge has only just scratched the surface. One of the more obvious questions that stems from the results is: what structure is driving cue-induced reinstatement of cocaine seeking via LS? The medial prefrontal cortex both projects to LS (Sheehan et al., 2004) and is necessary for cue-induced reinstatement of cocaine seeking (McLaughlin and See, 2003). Thus, a projection from mPFC to LS may be important for driving reinstatement to conditioned cues. LS also has reciprocal connections with VTA, bed nucleus of the stria terminalis, and hypothalamus (Sheehan et al., 2004), all which have been implicated in cocaine addiction pathology (Di Ciano and Everitt, 2004b; Aston-Jones et al., 2010; Sartor and Aston-Jones, 2012; Mahler et al., 2013). These structures may also potentially drive cue-induced reinstatement behavior via LS. Furthermore, BLA has repeatedly shown to have a functional role during cue-induced reinstatement of cocaine seeking (Meil and See, 1997; Stefanik and Kalivas, 2013). Despite the BLA-LS circuit not exhibiting enhanced Fos expression during cue-induced reinstatement behavior, BLA neurons that project to LS may still have a causal role in driving reinstatement to conditioned cues. Future studies should explore if any of these structures drive cue-induced reinstatement via LS.

It also would be interesting to explore the role of LS in stress-induced drug seeking. LS has been shown to be involved in stress and anxiety behaviors, but the literature varies regarding whether LS neurons suppress (Brady and Nauta, 1953; Sparks and LeDoux, 1995; Trent and Menard, 2010) or promote (Anthony et al., 2014) stress behaviors. Optogenetic tools used to decipher the role of LS in stress behaviors indicate activation of corticotropin-releasing factor type 2 receptors in LS promotes stress behaviors (Anthony et al., 2014). Drug-related cues evoke stress states that elicit

drug craving in cocaine-dependent individuals (Sinha et al., 1999). Also, stimulus- and stress-induced reinstatements use common corticotropin-releasing factor receptor signaling (Smith and Aston-Jones, 2011). It is plausible that LS neurons promote reinstatement to contextual and/or discrete drug cues by inducing a stress-like state. In my paradigm, inactivation of LS attenuated context- and cue-induced reinstatement of cocaine seeking. Thus, LS inhibition may suppress stress levels, which in turn attenuate reinstatement of cocaine seeking (although this may not be the case for heroin seeking; (Highfield et al., 2000); Figure 4-4). Future studies should aim to examine LS during stress-induced reinstatement of cocaine seeking to further interpret and understand LS in reinstatement behavior.

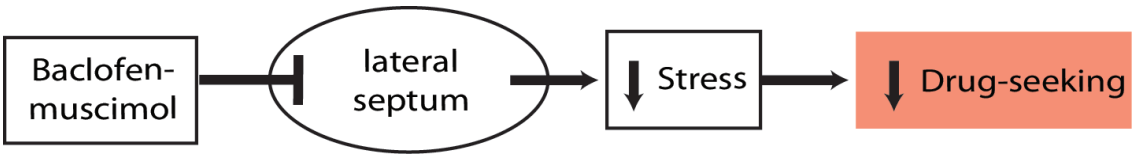

Figure 4-4. Potential model for lateral septum involvement in stress-induced reinstatement of drug-seeking. Lateral septum has recently been considered to promote stress and anxiety behaviors. Stress is known to induce drug-seeking in self-administration trained rats. Inhibiting lateral septum may reduce stress and attenuate drug-seeking behaviors.

Is there a connection between LS and NAc during cocaine seeking?

Both sets of experiments here (Chapter 2 and 3) explored the neurocircuitry mediating reinstatement of cocaine seeking behavior. However, a potentially interesting avenue to explore would be to connect accumbens and septal circuitry during reinstatement of cocaine seeking. Nucleus accumbens receives inputs from LS cells with denser inputs to NAcSh than NAcC (Brog et al., 1993; Sheehan et al., 2004). It has also been shown the pharmacological inhibition of NAcSh neurons with baclofen-muscimol potentiates reinstatement of active lever responding for cocaine (Peters et al., 2008a). Our data revealed that pharmacological inactivation of LS neurons attenuated context- and cue-induced reinstatement of cocaine seeking. It is plausible that LS GABAergic neurons may act to potentiate reinstatement of extinguished cocaine-seeking behavior via inhibition of NAcSh (Figure 4-5). Thus, when LS neurons are inactivated, it may disinhibit NAcSh neurons and attenuate cocaine-seeking behavior. In addition, it would be interesting to examine if LS neurons that project to NAcSh are activated during cue-induced reinstatement of cocaine seeking, and if that activation correlates with cocaine-seeking behavior.

To extrapolate this idea one circuit further, prior studies showed that mPFC projects to LS (Vertes, 2004; Gabbott et al., 2005). It is thus plausible that a projection from LS to NAcSh may be driven by mPFC projections to LS neurons that activate LS GABA neurons, resulting in inhibition of NAcSh neurons and potentiated cocaine-seeking behavior (Figure 4-5). This may be a mechanism by which inactivation of LS neurons attenuate context- and/or cue-induced reinstatement of cocaine seeking. In conjunction with potential functional experiments, future studies should also examine whether mPFC inputs to LS are activated during context- or cue-induced reinstatement of cocaine seeking.

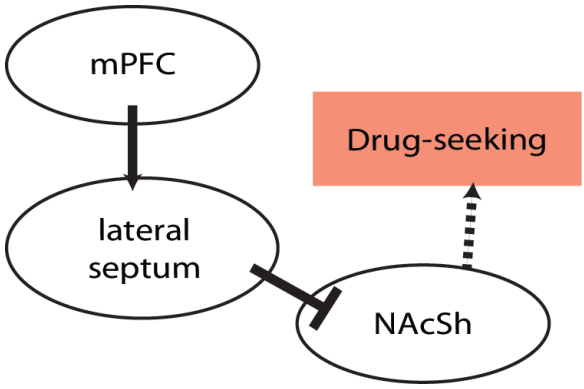

Figure 4-5. Schematic of possible link between lateral septum and nucleus accumbens driving drug-seeking. Medial prefrontal cortex (mPFC) neurons excite lateral septum (LS) GABAergic neurons that cause inhibition of nucleus accumbens shell (NAcSh) neurons, which may drive drug-seeking behaviors.

POTENTIAL THERAPEUTIC IMPLICATIONS

In Chapter 3 of this dissertation, we used DREADD technology to virally transduce a designer inhibitory hM4Di receptor into hippocampal neurons. We found that microinjections of the receptor's designer ligand onto hM4Di-expressing dCA3 terminals in LS inhibited context-induced reinstatement behavior. Although, this technology may seem far-fetched for clinical implications, it very well may be a potential therapeutic intervention in the future. Currently, viral transduction of genes (e.g. gene therapy) is being used clinically to treat a variety of rare genetic disorders with profound success (Nathwani et al., 2011; Gaudet et al., 2012; Pillay et al., 2016). The difference between current gene therapies and DREADD technology are endogenous versus exogenous genes, respectively. However, DREADDs use physiologically conserved GPCR signaling, indicating perhaps naturalistic inhibition of brain activity. For this reason, it may be within the realm of possibility that this technology could be used clinically in our lifetimes. The use of DREADD technology in the dorsal hippocampal-LS circuit could potentially be a future therapeutic intervention to reduce the likelihood of relapse in addicts.

SUMMARY

In summary, we found that multiple afferents to NAcC, but not to NAcSh, activate during cue-induced reinstatement of cocaine seeking, but only PL neurons that project to NAcC had activity that was positively correlated with cocaine seeking behavior. These results indicate heterogeneous neuronal activities of brain regions based on specific projection targets. We also found that dorsal, but not ventral, hippocampal inputs to LS drive context-, but not cue-induced reinstatement of cocaine seeking. These results indicate specific circuits are involved in different types of reinstatement modalities. Cumulative results from this dissertation emphasize the importance of elucidating the neurocircuitry of relapse behavior. Addiction is a complicated disease and understanding the neurocircuitry of addiction pathology and relapse will provide potential new therapeutic targets for treating addiction.

REFERENCES

American Psychiatric Association (2013) Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5). American Psychiatric Publishing, Arlington, VA.

Anderson SM, Schmidt HD, Pierce RC (2006) Administration of the D2 dopamine receptor antagonist sulpiride into the shell, but not the core, of the nucleus accumbens attenuates cocaine priming-induced reinstatement of drug seeking. Neuropsychopharmacology 31:1452–1461.

Anthony TE, Dee N, Bernard A, Lerchner W, Heintz N, Anderson DJ (2014) Control of stress-induced persistent anxiety by an extra-amygdala septohypothalamic circuit. Cell 156:522–536.

Armbruster BN, Li X, Pausch MH, Herlitze S, Roth BL (2007) Evolving the lock to fit the key to create a family of G protein-coupled receptors potently activated by an inert ligand. Proc Natl Acad Sci USA 104:5163–5168.

Aston-Jones G, Deisseroth K (2013) Recent advances in optogenetics and pharmacogenetics. Brain Research 1511:1–5.

Aston-Jones G, Smith RJ, Sartor GC, Moorman DE, Massi L, Tahsili-Fahadan P, Richardson KA (2010) Lateral hypothalamic orexin/hypocretin neurons: A role in reward-seeking and addiction. Brain Research 1314:74–90.

Auer J, Berent R, Eber B (2001) Cardiovascular complications of cocaine use. N Engl J Med 345:1575–authorreply1576.

Bardo MT, Bevins RA (2000) Conditioned place preference: what does it add to our preclinical understanding of drug reward? Psychopharmacology 153:31–43.

Barker JM, Corbit LH, Robinson DL, Gremel CM, Gonzales RA, Chandler LJ (2015) Corticostriatal circuitry and habitual ethanol seeking. Alcohol 49:817–824.

Bastide MF, la Crompe de B, Doudnikoff E, Fernagut P-O, Gross CE, Mallet N, Boraud T, Bézard E (2016) Inhibiting Lateral Habenula Improves L-DOPA-Induced Dyskinesia. Biol Psychiatry 79:345–353.

Baxter MG, Murray EA (2002) The amygdala and reward. Nat Rev Neurosci 3:563–573.

Bäckström P, Hyytiä P (2004) Ionotropic glutamate receptor antagonists modulate cue-induced reinstatement of ethanol-seeking behavior. Alcohol Clin Exp Res 28:558–565.

Bäckström P, Hyytiä P (2007) Involvement of AMPA/kainate, NMDA, and mGlu5 receptors in the nucleus accumbens core in cue-induced reinstatement of cocaine seeking in rats. Psychopharmacology 192:571–580.

Benwell ME, Balfour DJ (1992) The effects of acute and repeated nicotine treatment on nucleus accumbens dopamine and locomotor activity. Br J Pharmacol 105:849–856.

Bossert JM, Adhikary S, St Laurent R, Marchant NJ, Wang H-L, Morales M, Shaham Y (2016) Role of projections from ventral subiculum to nucleus accumbens shell in context-induced reinstatement of heroin seeking in rats. Psychopharmacology 233:1991–2004.

Bossert JM, Gray SM, Lu L, Shaham Y (2006) Activation of group II metabotropic glutamate receptors in the nucleus accumbens shell attenuates context-induced relapse to heroin seeking. Neuropsychopharmacology 31:2197–2209.

Bossert JM, Poles GC, Wihbey KA, Koya E, Shaham Y (2007) Differential effects of blockade of dopamine D1-family receptors in nucleus accumbens core or shell on reinstatement of heroin seeking induced by contextual and discrete cues. Journal of Neuroscience 27:12655–12663.

Bossert JM, Stern AL (2012) Role of ventral subiculum in context-induced reinstatement of heroin seeking in rats. Addict Biol.

Bossert JM, Stern AL, Theberge FRM, Cifani C, Koya E, Hope BT, Shaham Y (2011) Ventral medial prefrontal cortex neuronal ensembles mediate context-induced relapse to heroin. Nat Neurosci 14:420–422.

Bossert JM, Stern AL, Theberge FRM, Marchant NJ, Wang H-L, Morales M, Shaham Y (2012) Role of projections from ventral medial prefrontal cortex to nucleus accumbens shell in context-induced reinstatement of heroin seeking. Journal of Neuroscience 32:4982–4991.

Bouton ME, Bolles RC (1979) Role of conditioned contextual stimuli in reinstatement of extinguished fear. J Exp Psychol Anim Behav Process 5:368–378.

Boyden ES, Zhang F, Bamberg E, Nagel G, Deisseroth K (2005) Millisecond-timescale, genetically targeted optical control of neural activity. Nat Neurosci 8:1263–1268.

Brady JV, Nauta WJ (1953) Subcortical mechanisms in emotional behavior: affective changes following septal forebrain lesions in the albino rat. J Comp Physiol Psychol 46:339–346.

Britt JP, Benaliouad F, McDevitt RA, Stuber GD, Wise RA, Bonci A (2012) Synaptic and behavioral profile of multiple glutamatergic inputs to the nucleus accumbens. Neuron 76:790–803.

Brog JS, Salyapongse A, Deutch AY, Zahm DS (1993) The patterns of afferent innervation of the core and shell in the “accumbens” part of the rat ventral striatum: immunohistochemical detection of retrogradely transported fluoro-gold. J Comp Neurol 338:255–278.

Brown EE, Robertson GS, Fibiger HC (1992) Evidence for conditional neuronal activation following exposure to a cocaine-paired environment: role of forebrain limbic structures. J Neurosci 12:4112–4121.

Buffalari DM, See RE (2010) Amygdala mechanisms of Pavlovian psychostimulant conditioning and relapse. Curr Top Behav Neurosci 3:73–99.

Burattini C, Gill TM, Aicardi G, Janak PH (2006) The ethanol self-administration context as a reinstatement cue: acute effects of naltrexone. NSC 139:877–887.

Calhoon GG, O'Donnell P (2013) Closing the gate in the limbic striatum: prefrontal suppression of hippocampal and thalamic inputs. Neuron 78:181–190.

Canteras NS, Simerly RB, Swanson LW (1992) Connections of the posterior nucleus of the amygdala. J Comp Neurol 324:143–179.

Canteras NS, Simerly RB, Swanson LW (1995) Organization of projections from the medial nucleus of the amygdala: a PHAL study in the rat. J Comp Neurol 360:213–245.

Cason AM, Aston-Jones G (2013) Role of orexin/hypocretin in conditioned sucrose-seeking in rats. Psychopharmacology 226:155–165.

Cassaday HJ, Horsley RR, Norman C (2005) Electrolytic lesions to nucleus accumbens core and shell have dissociable effects on conditioning to discrete and contextual cues in aversive and appetitive procedures respectively. Behavioural Brain Research 160:222–235.

Chaudhri N, Sahuque LL, Schairer WW, Janak PH (2010) Separable roles of the nucleus accumbens core and shell in context- and cue-induced alcohol-seeking. Neuropsychopharmacology 35:783–791.

Chen S, Aston-Jones G (1995) Evidence that cholera toxin B subunit (CTb) can be avidly taken up and transported by fibers of passage. Brain Research 674:107–111.

Chen S, Aston-Jones G (1998) Axonal collateral-collateral transport of tract tracers in brain neurons: false anterograde labelling and useful tool. NSC 82:1151–1163.

Childress A, Ehrman R, McLellan AT, O'Brien C (1988) Conditioned craving and arousal in cocaine addiction: a preliminary report. NIDA Res Monogr 81:74–80.

Childress AR, Hole AV, Ehrman RN, Robbins SJ, McLellan AT, O'Brien CP (1993) Cue reactivity and cue reactivity interventions in drug dependence. NIDA Res Monogr 137:73–95.

Childress AR, Mozley PD, McElgin W, Fitzgerald J, Reivich M, O'Brien CP (1999) Limbic activation during cue-induced cocaine craving. Am J Psychiatry 156:11–18.

Ciccarone D (2011) Stimulant abuse: pharmacology, cocaine, methamphetamine, treatment, attempts at pharmacotherapy. Prim Care 38:41–58–v–vi.

Cornish JL, Hunt GE, Robins L, McGregor IS (2012) Regional c-Fos and FosB/ΔFosB expression associated with chronic methamphetamine self-administration and methamphetamine-seeking behavior in rats. Neuroscience 206:100–114.

Crandall CG, Vongpatanasin W, Victor RG (2002) Mechanism of cocaine-induced hyperthermia in humans. Ann Intern Med 136:785–791.

Crombag HS, Bossert JM, Koya E, Shaham Y (2008) Review. Context-induced relapse to drug seeking: a review. Philos Trans R Soc Lond, B, Biol Sci 363:3233–3243.

Crombag HS, Shaham Y (2002) Renewal of drug seeking by contextual cues after prolonged extinction in rats. Behav Neurosci 116:169.

Cruz FC, Babin KR, Leao RM, Goldart EM, Bossert JM, Shaham Y, Hope BT (2014) Role of nucleus accumbens shell neuronal ensembles in context-induced reinstatement of cocaine-seeking. Journal of Neuroscience 34:7437–7446.

Cruz FC, Koya E, Guez-Barber DH, Bossert JM, Lupica CR, Shaham Y, Hope BT (2013) New technologies for examining the role of neuronal ensembles in drug addiction and fear. Nat Rev Neurosci 14:743–754.

Cunningham CL, Noble D (1992) Conditioned activation induced by ethanol: role in sensitization and conditioned place preference. Pharmacol Biochem Behav 43:307–313.

Damasio H, Grabowski T, Frank R, Galaburda AM, Damasio AR (1994) The return of Phineas Gage: clues about the brain from the skull of a famous patient. Science 264:1102–1105.

Degoulet M, Rouillon C, Rostain J-C, David HN, Abraini JH (2008) Modulation by the dorsal, but not the ventral, hippocampus of the expression of behavioural sensitization to amphetamine. Int J Neuropsychopharmacol 11:497–508.

Deisseroth K (2011) Optogenetics. Nat Methods 8:26–29.

Delgado JM, Mark V, Sweet W, Ervin F, Weiss G, Bach-Y-Rita G, Hagiwara R (1968) Intracerebral radio stimulation and recording in completely free patients. J Nerv Ment Dis 147:329–340.

Di Ciano P, Everitt BJ (2004a) Direct interactions between the basolateral amygdala and nucleus accumbens core underlie cocaine-seeking behavior by rats. Journal of Neuroscience 24:7167–7173.

Di Ciano P, Everitt BJ (2004b) Contribution of the ventral tegmental area to cocaine-seeking maintained by a drug-paired conditioned stimulus in rats. Eur J Neurosci 19:1661–1667.

Di Ciano P, Robbins TW, Everitt BJ (2008) Differential effects of nucleus accumbens core, shell, or dorsal striatal inactivations on the persistence, reacquisition, or reinstatement of responding for a drug-paired conditioned reinforcer. Neuropsychopharmacology 33:1413–1425.

Diergaarde L, de Vries W, Raasø H, Schoffelmeer ANM, De Vries TJ (2008) Contextual renewal of nicotine seeking in rats and its suppression by the cannabinoid-1 receptor antagonist Rimonabant (SR141716A). Neuropharmacology 55:712–716.

Epstein DH, Preston KL, Stewart J, Shaham Y (2006) Toward a model of drug relapse: an assessment of the validity of the reinstatement procedure. Psychopharmacology 189:1–16.

Erb S, Petrovic A, Yi D, Kayyali H (2006) Central injections of CRF reinstate cocaine seeking in rats after postinjection delays of up to 3 h: an influence of time and environmental context. Psychopharmacology 187:112–120.

Everitt BJ, Parkinson JA, Olmstead MC, Arroyo M, Robledo P, Robbins TW (1999) Associative processes in addiction and reward. The role of amygdala-ventral striatal subsystems. Annals of the New York Academy of Sciences 877:412–438.

Fanous S, Goldart EM, Theberge FRM, Bossert JM, Shaham Y, Hope BT (2012) Role of orbitofrontal cortex neuronal ensembles in the expression of incubation of heroin craving. Journal of Neuroscience 32:11600–11609.

Fanselow MS, Dong H-W (2010) Are the dorsal and ventral hippocampus functionally distinct structures? Neuron 65:7–19.

Feltenstein MW, See RE (2006) Potentiation of cue-induced reinstatement of cocaine-seeking in rats by the anxiogenic drug yohimbine. Behavioural Brain Research 174:1–8.

Fischer KD, Houston ACW, Rebec GV (2013) Role of the major glutamate transporter GLT1 in nucleus accumbens core versus shell in cue-induced cocaine-seeking behavior. Journal of Neuroscience 33:9319–9327.

Flagel SB, Robinson TE, Clark JJ, Clinton SM, Watson SJ, Seeman P, Phillips PEM, Akil H (2010) An animal model of genetic vulnerability to behavioral disinhibition and responsiveness to reward-related cues: implications for addiction. Neuropsychopharmacology 35:388–400.

Floresco SB (2015) The nucleus accumbens: an interface between cognition, emotion, and action. Annu Rev Psychol 66:25–52.

Fortress AM, Hamlett ED, Vazey EM, Aston-Jones G, Cass WA, Boger HA, Granholm A-CE (2015) Designer receptors enhance memory in a mouse model of Down syndrome. Journal of Neuroscience 35:1343–1353.

French SJ, Totterdell S (2002) Hippocampal and prefrontal cortical inputs monosynaptically converge with individual projection neurons of the nucleus accumbens. J Comp Neurol 446:151–165.

French SJ, Totterdell S (2003) Individual nucleus accumbens-projection neurons receive both basolateral amygdala and ventral subicular afferents in rats. NSC 119:19–31.

Fuchs RA, Evans KA, Ledford CC, Parker MP, Case JM, Mehta RH, See RE (2005) The Role of the Dorsomedial Prefrontal Cortex, Basolateral Amygdala, and Dorsal Hippocampus in Contextual Reinstatement of Cocaine Seeking in Rats. Neuropsychopharmacology 30:296–309.

Fuchs RA, Evans KA, Parker MC, See RE (2004) Differential involvement of the core and shell subregions of the nucleus accumbens in conditioned cue-induced reinstatement of cocaine seeking in rats. Psychopharmacology 176:459–465.

Fuchs RA, Ramirez DR, Bell GH (2008) Nucleus accumbens shell and core involvement in drug context-induced reinstatement of cocaine seeking in rats. Psychopharmacology 200:545–556.

Fudala PJ, Teoh KW, Iwamoto ET (1985) Pharmacologic characterization of nicotine-induced conditioned place preference. Pharmacol Biochem Behav 22:237–241.

Gabbott PLA, Warner TA, Jays PRL, Salway P, Busby SJ (2005) Prefrontal cortex in the rat: Projections to subcortical autonomic, motor, and limbic centers. J Comp Neurol 492:145–177.

Gaffan D, Murray EA, Fabre-Thorpe M (1993) Interaction of the amygdala with the frontal lobe in reward memory. Eur J Neurosci 5:968–975.

Gaudet D, Méthot J, Kastelein J (2012) Gene therapy for lipoprotein lipase deficiency. Curr Opin Lipidol 23:310–320.

Gerfen CR, Sawchenko PE (1984) An anterograde neuroanatomical tracing method that shows the detailed morphology of neurons, their axons and terminals: immunohistochemical localization of an axonally transported plant lectin, Phaseolus vulgaris leucoagglutinin (PHA-L). Brain Research 290:219–238.

Gipson CD, Kupchik YM, Shen H, Reissner KJ, Thomas CA, Kalivas PW (2013a) Relapse induced by cues predicting cocaine depends on rapid, transient synaptic potentiation. Neuron 77:867–872.

Gipson CD, Reissner KJ, Kupchik YM, Smith ACW, Stankeviciute N, Hensley-Simon ME, Kalivas PW (2013b) Reinstatement of nicotine seeking is mediated by glutamatergic plasticity. Proc Natl Acad Sci USA 110:9124–9129.

Gloor P, Olivier A, Quesney LF, Andermann F, Horowitz S (1982) The role of the limbic system in experiential phenomena of temporal lobe epilepsy. Ann Neurol 12:129–144.

Goto Y, Grace AA (2008) Limbic and cortical information processing in the nucleus accumbens. Trends in Neurosciences 31:552–558.

Grasing K, He S, Li N (2005) Selegiline modifies the extinction of responding following morphine self-administration, but does not alter cue-induced reinstatement, reacquisition of morphine reinforcement, or precipitated withdrawal. Pharmacol Res 51:69–78.

Groenewegen HJ, Vermeulen-Van der Zee E, Kortschot te A, Witter MP (1987) Organization of the projections from the subiculum to the ventral striatum in the rat. A study using anterograde transport of Phaseolus vulgaris leucoagglutinin. NSC 23:103–120.

Groenewegen HJ, Wright CI, Beijer AV, Voorn P (1999) Convergence and segregation of ventral striatal inputs and outputs. Annals of the New York Academy of Sciences 877:49–63.

Haber SN (2016) Corticostriatal circuitry. Dialogues Clin Neurosci 18:7–21.

Hamlin A, Newby J, McNally G (2007) The neural correlates and role of D1 dopamine receptors in renewal of extinguished alcohol-seeking. NSC 146:525–536.

Hand TH, Stinus L, Le Moal M (1989) Differential mechanisms in the acquisition and expression of heroin-induced place preference. Psychopharmacology 98:61–67.

Harris GC, Aston-Jones G (2003) Critical role for ventral tegmental glutamate in preference for a cocaine-conditioned environment. Neuropsychopharmacology 28:73–76.

Harris GC, Wimmer M, Aston-Jones G (2005) A role for lateral hypothalamic orexin neurons in reward seeking. Nature 437:556–559.

Heath RG (1963) Electrical self-stimulation of the brain in man. Am J Psychiatry 120:571–577.

Highfield D, Clements A, Shalev U, McDonald R, Featherstone R, Stewart J, Shaham Y (2000) Involvement of the medial septum in stress-induced relapse to heroin seeking in rats. Eur J Neurosci 12:1705–1713.

Holland PC, Bouton ME (1999) Hippocampus and context in classical conditioning. Curr Opin Neurobiol 9:195–202.

Hollander JA, Carelli RM (2007) Cocaine-associated stimuli increase cocaine seeking and activate accumbens core neurons after abstinence. Journal of Neuroscience 27:3535–3539.

Hollander JE, Hoffman RS, Burstein JL, Shih RD, Thode HC (1995) Cocaine-associated myocardial infarction. Mortality and complications. Cocaine-Associated Myocardial Infarction Study Group. Arch Intern Med 155:1081–1086.

Hunsaker MR, Kesner RP (2008) Dissociations across the dorsal-ventral axis of CA3 and CA1 for encoding and retrieval of contextual and auditory-cued fear. Neurobiol Learn Mem 89:61–69.

Hyman SE, Malenka RC, Nestler EJ (2006) Neural mechanisms of addiction: the role of reward-related learning and memory. Annu Rev Neurosci 29:565–598.

Ito R, Dalley JW, Howes SR, Robbins TW, Everitt BJ (2000) Dissociation in conditioned dopamine release in the nucleus accumbens core and shell in response to cocaine cues and during cocaine-seeking behavior in rats. Journal of Neuroscience 20:7489–7495.

Ito R, Hayen A (2011) Opposing roles of nucleus accumbens core and shell dopamine in the modulation of limbic information processing. Journal of Neuroscience 31:6001–6007.

Ito R, Robbins TW, Everitt BJ (2004) Differential control over cocaine-seeking behavior by nucleus accumbens core and shell. Nat Neurosci 7:389–397.

Jaffe JH, Cascella NG, Kumor KM, Sherer MA (1989) Cocaine-induced cocaine craving. Psychopharmacology 97:59–64.

Jarrard LE (1993) On the role of the hippocampus in learning and memory in the rat. Behav Neural Biol 60:9–26.

Jongen-Rêlo AL, Kaufmann S, Feldon J (2003) A differential involvement of the shell and core subterritories of the nucleus accumbens of rats in memory processes. Behav Neurosci 117:150–168.

Joyce EM, Iversen SD (1979) The effect of morphine applied locally to mesencephalic dopamine cell bodies on spontaneous motor activity in the rat. Neurosci Lett 14:207–212.

Kalivas PW (2009) The glutamate homeostasis hypothesis of addiction. Nat Rev Neurosci 10:561–572.

Kalivas PW, McFarland K (2003) Brain circuitry and the reinstatement of cocaine-seeking behavior. Psychopharmacology 168:44–56.

Kalivas PW, Volkow N, Seamans J (2005) Unmanageable motivation in addiction: a pathology in prefrontal-accumbens glutamate transmission. Neuron 45:647–650.

Kalivas PW, Volkow ND (2005) The neural basis of addiction: a pathology of motivation and choice. Am J Psychiatry 162:1403–1413.

Kane MJ, Engle RW (2002) The role of prefrontal cortex in working-memory capacity, executive attention, and general fluid intelligence: an individual-differences perspective. Psychon Bull Rev 9:637–671.

Kätzel D, Nicholson E, Schorge S, Walker MC, Kullmann DM (2014) Chemical-genetic attenuation of focal neocortical seizures. Nat Commun 5:3847.

Kearns DN, Weiss SJ (2007) Contextual renewal of cocaine seeking in rats and its attenuation by the conditioned effects of an alternative reinforcer. Drug Alcohol Depend 90:193–202.

Kenney JW, Raybuck JD, Gould TJ (2012) Nicotinic receptors in the dorsal and ventral hippocampus differentially modulate contextual fear conditioning. Hippocampus 22:1681–1690.

Khantzian EJ (1985) The self-medication hypothesis of addictive disorders: focus on heroin and cocaine dependence. Am J Psychiatry 142:1259–1264.

Kim JJ, Fanselow MS (1992) Modality-specific retrograde amnesia of fear. Science 256:675–677.

Koob GF, Volkow ND (2010) Neurocircuitry of addiction. Neuropsychopharmacology 35:217–238.

Koob GF, Wall TL, Bloom FE (1989) Nucleus accumbens as a substrate for the aversive stimulus effects of opiate withdrawal. Psychopharmacology 98:530–534.

Koseki T, Mouri A, Suzuki S, Nakajima A, Mamiya T, Yan Y, Nabeshima T (2014) Galantamine attenuates reinstatement of cue-induced methamphetamine-seeking behavior in mice. Addict Biol 19:1–4.

Kosten TR, Rounsaville BJ, Kleber HD (1986) A 2.5-year follow-up of depression, life crises, and treatment effects on abstinence among opioid addicts. Arch Gen Psychiatry 43:733–738.

Kovács KJ (1998) c-Fos as a transcription factor: a stressful (re)view from a functional map. Neurochem Int 33:287–297.

Koya E, Uejima JL, Wihbey KA, Bossert JM, Hope BT, Shaham Y (2009) Role of ventral medial prefrontal cortex in incubation of cocaine craving. Neuropharmacology 56 Suppl 1:177–185.

Kufahl PR, Zavala AR, Singh A, Thiel KJ, Dickey ED, Joyce JN, Neisewander JL (2009) c-Fos expression associated with reinstatement of cocaine-seeking behavior by response-contingent conditioned cues. Synapse 63:823–835.

Kumaresan V, Yuan M, Yee J, Famous KR, Anderson SM, Schmidt HD, Pierce RC (2009) Metabotropic glutamate receptor 5 (mGluR5) antagonists attenuate cocaine priming- and cue-induced reinstatement of cocaine seeking. Behavioural Brain Research 202:238–244.

Lai M, Chen W, Zhu H, Zhou X, Liu H, Zhang F, Zhou W (2013) Low dose risperidone attenuates cue-induced but not heroin-induced reinstatement of heroin seeking in an animal model of relapse. Int J Neuropsychopharmacol 16:1569–1575.

LaLumiere RT, Kalivas PW (2008) Glutamate release in the nucleus accumbens core is necessary for heroin seeking. Journal of Neuroscience 28:3170–3177.

LaLumiere RT, Smith KC, Kalivas PW (2012) Neural circuit competition in cocaine-seeking: roles of the infralimbic cortex and nucleus accumbens shell. Eur J Neurosci 35:614–622.

Lasseter HC, Xie X, Ramirez DR, Fuchs RA (2010) Sub-region specific contribution of the ventral hippocampus to drug context-induced reinstatement of cocaine-seeking behavior in rats. Neuroscience 171:830–839.

Le Merrer J, Gavello-Baudy S, Galey D, Cazala P (2007) Morphine self-administration into the lateral septum depends on dopaminergic mechanisms: Evidence from pharmacology and Fos neuroimaging. Behavioural Brain Research 180:203–217.

Liechti ME, Lhuillier L, Kaupmann K, Markou A (2007) Metabotropic glutamate 2/3 receptors in the ventral tegmental area and the nucleus accumbens shell are involved in behaviors relating to nicotine dependence. Journal of Neuroscience 27:9077–9085.

Luo AH, Tahsili-Fahadan P, Wise RA, Lupica CR, Aston-Jones G (2011) Linking context with reward: a functional circuit from hippocampal CA3 to ventral tegmental area. Science 333:353–357.

Mahler SV, Aston-Jones GS (2012) Fos activation of selective afferents to ventral tegmental area during cue-induced reinstatement of cocaine seeking in rats. Journal of Neuroscience 32:13309–13326.

Mahler SV, Smith RJ, Aston-Jones G (2013) Interactions between VTA orexin and glutamate in cue-induced reinstatement of cocaine seeking in rats. Psychopharmacology 226:687–698.

Mahler SV, Vazey EM, Beckley JT, Keistler CR, McGlinchey EM, Kaufling J, Wilson SP, Deisseroth K, Woodward JJ, Aston-Jones G (2014) Designer receptors show role for ventral pallidum input to ventral tegmental area in cocaine seeking. Nat Neurosci.

Mantsch JR, Baker DA, Funk D, Lê AD, Shaham Y (2016) Stress-Induced Reinstatement of Drug Seeking: 20 Years of Progress. Neuropsychopharmacology 41:335–356.

Marchant NJ, Campbell EJ, Whitaker LR, Harvey BK, Kaganovsky K, Adhikary S, Hope BT, Heins RC, Prisinzano TE, Vardy E, Bonci A, Bossert JM, Shaham Y (2016) Role of Ventral Subiculum in Context-Induced Relapse to Alcohol Seeking after Punishment-Imposed Abstinence. Journal of Neuroscience 36:3281–3294.

Marchant NJ, Kaganovsky K, Shaham Y, Bossert JM (2015) Role of corticostriatal circuits in context-induced reinstatement of drug seeking. Brain Research 1628:219–232.

Maren S, Fanselow MS (1996) The amygdala and fear conditioning: has the nut been cracked? Neuron 16:237–240.

Maren S, Holt WG (2004) Hippocampus and Pavlovian fear conditioning in rats: muscimol infusions into the ventral, but not dorsal, hippocampus impair the acquisition of conditional freezing to an auditory conditional stimulus. Behav Neurosci 118:97–110.

Martín-García E, Courtin J, Renault P, Fiancette J-F, Wurtz H, Simonnet A, Levet F, Herry C, Deroche-Gamonet V (2014) Frequency of cocaine self-administration influences drug seeking in the rat: optogenetic evidence for a role of the prelimbic cortex. Neuropsychopharmacology 39:2317–2330.

Martinez D, Narendran R, Foltin RW, Slifstein M, Hwang D-R, Broft A, Huang Y, Cooper TB, Fischman MW, Kleber HD, Laruelle M (2007) Amphetamine-induced dopamine release: markedly blunted in cocaine dependence and predictive of the choice to self-administer cocaine. Am J Psychiatry 164:622–629.

McDonald AJ (1998) Cortical pathways to the mammalian amygdala. Prog Neurobiol 55:257–332.

McFarland K, Kalivas PW (2001) The circuitry mediating cocaine-induced reinstatement of drug-seeking behavior. Journal of Neuroscience 21:8655–8663.

McFarland K, Lapish CC, Kalivas PW (2003) Prefrontal glutamate release into the core of the nucleus accumbens mediates cocaine-induced reinstatement of drug-seeking behavior. Journal of Neuroscience 23:3531–3537.

McGinty VB, Grace AA (2008) Selective activation of medial prefrontal-to-accumbens projection neurons by amygdala stimulation and Pavlovian conditioned stimuli. Cerebral Cortex 18:1961–1972.

McLaughlin J, See RE (2003) Selective inactivation of the dorsomedial prefrontal cortex and the basolateral amygdala attenuates conditioned-cued reinstatement of extinguished cocaine-seeking behavior in rats. Psychopharmacology 168:57–65.

Meil WM, See RE (1997) Lesions of the basolateral amygdala abolish the ability of drug associated cues to reinstate responding during withdrawal from self-administered cocaine. Behavioural Brain Research 87:139–148.

Meredith GE (1999) The synaptic framework for chemical signaling in nucleus accumbens. Annals of the New York Academy of Sciences 877:140–156.

Meyers RA, Zavala AR, Speer CM, Neisewander JL (2006) Dorsal hippocampus inhibition disrupts acquisition and expression, but not consolidation, of cocaine conditioned place preference. Behav Neurosci 120:401–412.

Mogenson GJ, Jones DL, Yim CY (1980) From motivation to action: functional interface between the limbic system and the motor system. Prog Neurobiol 14:69–97.

Moorman DE, Aston-Jones G (2015) Prefrontal neurons encode context-based response execution and inhibition in reward seeking and extinction. Proc Natl Acad Sci USA 112:9472–9477.

Moorman DE, James MH, Kilroy EA, Aston-Jones G (2016) Orexin/hypocretin neuron activation is correlated with alcohol seeking and preference in a topographically specific manner. Eur J Neurosci 43:710–720.

Moorman DE, James MH, McGlinchey EM, Aston-Jones G (2015) Differential roles of medial prefrontal subregions in the regulation of drug seeking. Brain Research 1628:130–146.

Morris RG, Garrud P, Rawlins JN, O'Keefe J (1982) Place navigation impaired in rats with hippocampal lesions. Nature 297:681–683.

Morrison J, Thornton V, Ranaldi R (2011) Chronic intermittent heroin produces locomotor sensitization and long-lasting enhancement of conditioned reinforcement. Pharmacol Biochem Behav 99:475–479.

Moser MB, Moser EI (1998) Functional differentiation in the hippocampus. Hippocampus 8:608–619.

Moser MB, Moser EI, Forrest E, Andersen P, Morris RG (1995) Spatial learning with a minislab in the dorsal hippocampus. Proc Natl Acad Sci USA 92:9697–9701.

Mucha RF, van der Kooy D, O'Shaughnessy M, Bucenieks P (1982) Drug reinforcement studied by the use of place conditioning in rat. Brain Research 243:91–105.

Mulder AB, Hodenpijl MG, Lopes da Silva FH (1998) Electrophysiology of the hippocampal and amygdaloid projections to the nucleus accumbens of the rat: convergence, segregation, and interaction of inputs. J Neurosci 18:5095–5102.

Müller R, Bravo R, Burckhardt J, Curran T (1984) Induction of c-fos gene and protein by growth factors precedes activation of c-myc. Nature 312:716–720.

Nathwani AC et al. (2011) Adenovirus-associated virus vector-mediated gene transfer in hemophilia B. N Engl J Med 365:2357–2365.

National Institute on Drug Abuse, National Institutes of Health, U.S. Department of Health and Human Services. (2012) Drug Facts: Understanding Drug Abuse and Addiction, Bethesda MD.

National Institute on Drug Abuse, National Institutes of Health, U.S. Department of Health and Human Services. (2015) Drug Facts: Nationwide Trends: National Survey on Drug Use and Health, Bethesda MD.

National Institute on Drug Abuse, National Institutes of Health, U.S. Department of Health and Human Services. (2016). Drug Facts: Cocaine, Bethesda MD.

National Research Council (201a) Guide for the Care and Use of Laboratory Animals: eighth edition. Washtington, DC: National Academies Press.

Neisewander JL, Baker DA, Fuchs RA, Tran-Nguyen LT, Palmer A, Marshall JF (2000) Fos protein expression and cocaine-seeking behavior in rats after exposure to a cocaine self-administration environment. Journal of Neuroscience 20:798–805.

Nelson RJ, Trainor BC (2007) Neural mechanisms of aggression. Nat Rev Neurosci 8:536–546.

O'Donnell P, Grace AA (1993) Physiological and morphological properties of accumbens core and shell neurons recorded in vitro. Synapse 13:135–160.

O'Donnell P, Grace AA (1995) Synaptic interactions among excitatory afferents to nucleus accumbens neurons: hippocampal gating of prefrontal cortical input. J Neurosci 15:3622–3639.

Olds J, Milner P (1954) Positive reinforcement produced by electrical stimulation of septal area and other regions of rat brain. J Comp Physiol Psychol 47:419–427.

Owesson-White CA, Ariansen J, Stuber GD, Cleaveland NA, Cheer JF, Mark Wightman R, Carelli RM (2009) Neural encoding of cocaine-seeking behavior is coincident with phasic dopamine release in the accumbens core and shell. European Journal of Neuroscience 30:1117–1127.

Oztas E (2003) Neuronal tracing. Neuroanatomy. 2:2-5.

O'Brien CP (2005) Anticraving medications for relapse prevention: a possible new class of psychoactive medications. Am J Psychiatry 162:1423–1431.

Parkinson JA, Olmstead MC, Burns LH, Robbins TW, Everitt BJ (1999) Dissociation in effects of lesions of the nucleus accumbens core and shell on appetitive pavlovian approach behavior and the potentiation of conditioned reinforcement and locomotor activity by D-amphetamine. J Neurosci 19:2401–2411.

Parsegian A, See RE (2014) Dysregulation of dopamine and glutamate release in the prefrontal cortex and nucleus accumbens following methamphetamine self-administration and during reinstatement in rats. Neuropsychopharmacology 39:811–822.

Paulson PE, Camp DM, Robinson TE (1991) Time course of transient behavioral depression and persistent behavioral sensitization in relation to regional brain monoamine concentrations during amphetamine withdrawal in rats. Psychopharmacology 103:480–492.

Paxinos G (1995) The Rat Nervous System. Academic Press.

Paxinos G, Watson C (2007) The Rat Brain in Stereotaxic Coordinates. Academic Press.

Pennartz CM, Groenewegen HJ, Lopes da Silva FH (1994) The nucleus accumbens as a complex of functionally distinct neuronal ensembles: an integration of behavioural, electrophysiological and anatomical data. Prog Neurobiol 42:719–761.

Peñagarikano O, Lázaro MT, Lu X-H, Gordon A, Dong H, Lam HA, Peles E, Maidment NT, Murphy NP, Yang XW, Golshani P, Geschwind DH (2015) Exogenous and evoked oxytocin restores social behavior in the Cntnap2 mouse model of autism. Sci Transl Med 7:271ra8.

Peters J, Kalivas PW, Quirk GJ (2009) Extinction circuits for fear and addiction overlap in prefrontal cortex. Learn Mem 16:279–288.

Peters J, LaLumiere RT, Kalivas PW (2008a) Infralimbic prefrontal cortex is responsible for inhibiting cocaine seeking in extinguished rats. Journal of Neuroscience 28:6046–6053.

Peters J, Vallone J, Laurendi K, Kalivas PW (2008b) Opposing roles for the ventral prefrontal cortex and the basolateral amygdala on the spontaneous recovery of cocaine-seeking in rats. Psychopharmacology 197:319–326.

Pfarr S, Meinhardt MW, Klee ML, Hansson AC, Vengeliene V, Schönig K, Bartsch D, Hope BT, Spanagel R, Sommer WH (2015) Losing Control: Excessive Alcohol

Seeking after Selective Inactivation of Cue-Responsive Neurons in the Infralimbic Cortex. Journal of Neuroscience 35:10750–10761.

Pillay S, Meyer NL, Puschnik AS, Davulcu O, Diep J, Ishikawa Y, Jae LT, Wosen JE, Nagamine CM, Chapman MS, Carette JE (2016) An essential receptor for adeno-associated virus infection. Nature 530:108–112.

Pina MM, Young EA, Ryabinin AE, Cunningham CL (2015) The bed nucleus of the stria terminalis regulates ethanol-seeking behavior in mice. Neuropharmacology 99:627–638.

Pinto A, Sesack SR (2000) Limited collateralization of neurons in the rat prefrontal cortex that project to the nucleus accumbens. NSC 97:635–642.

Post RM, Weiss SR, Fontana D, Pert A (1992) Conditioned sensitization to the psychomotor stimulant cocaine. Annals of the New York Academy of Sciences 654:386–399.

Pothuizen HHJ, Zhang W-N, Jongen-Rêlo AL, Feldon J, Yee BK (2004) Dissociation of function between the dorsal and the ventral hippocampus in spatial learning abilities of the rat: a within-subject, within-task comparison of reference and working spatial memory. Eur J Neurosci 19:705–712.

Price KL, Saladin ME, Baker NL, Tolliver BK, DeSantis SM, McRae-Clark AL, Brady KT (2010) Extinction of drug cue reactivity in methamphetamine-dependent individuals. Behav Res Ther 48:860–865.

Reid LD, Hunter GA, Beaman CM, Hubbell CL (1985) Toward understanding ethanol's capacity to be reinforcing: a conditioned place preference following injections of ethanol. Pharmacol Biochem Behav 22:483–487.

Risold PY, Swanson LW (1996) Structural evidence for functional domains in the rat hippocampus. Science 272:1484–1486.

Risold PY, Swanson LW (1997) Connections of the rat lateral septal complex. Brain Res Brain Res Rev 24:115–195.

Ritz MC, Cone EJ, Kuhar MJ (1990) Cocaine inhibition of ligand binding at dopamine, norepinephrine and serotonin transporters: a structure-activity study. Life Sci 46:635–645.

Robinson TE, Becker JB (1986) Enduring changes in brain and behavior produced by chronic amphetamine administration: a review and evaluation of animal models of amphetamine psychosis. Brain Research 396:157–198.

Robinson TE, Berridge KC (1993) The neural basis of drug craving: an incentive-sensitization theory of addiction. Brain Res Brain Res Rev 18:247–291.

Robinson TE, Yager LM, Cogan ES, Saunders BT (2014) On the motivational properties of reward cues: Individual differences. Neuropharmacology 76 Pt B:450–459.

Rocha A, Kalivas PW (2010) Role of the prefrontal cortex and nucleus accumbens in reinstating methamphetamine seeking. Eur J Neurosci 31:903–909.

Rogan SC, Roth BL (2011) Remote control of neuronal signaling. Pharmacol Rev 63:291–315.

Rogers JL, See RE (2007) Selective inactivation of the ventral hippocampus attenuates cue-induced and cocaine-primed reinstatement of drug-seeking in rats. Neurobiol Learn Mem 87:688–692.

Roth BL (2016) DREADDs for Neuroscientists. Neuron 89:683–694.

Rubio FJ, Liu Q-R, Li X, Cruz FC, Leao RM, Warren BL, Kambhampati S, Babin KR, McPherson KB, Cimbro R, Bossert JM, Shaham Y, Hope BT (2015) Context-induced reinstatement of methamphetamine seeking is associated with unique molecular alterations in Fos-expressing dorsolateral striatum neurons. Journal of Neuroscience 35:5625–5639.

Sartor GC, Aston-Jones GS (2012) A septal-hypothalamic pathway drives orexin neurons, which is necessary for conditioned cocaine preference. Journal of Neuroscience 32:4623–4631.

Sasaki K, Suzuki M, Mieda M, Tsujino N, Roth B, Sakurai T (2011) Pharmacogenetic modulation of orexin neurons alters sleep/wakefulness states in mice. PLoS ONE 6:e20360.

Saunders BT, O'Donnell EG, Aurbach EL, Robinson TE (2014) A cocaine context renews drug seeking preferentially in a subset of individuals. Neuropsychopharmacology 39:2816–2823.

Schmidt HD, Anderson SM, Pierce RC (2006) Stimulation of D1-like or D2 dopamine receptors in the shell, but not the core, of the nucleus accumbens reinstates cocaine-seeking behaviour in the rat. Eur J Neurosci 23:219–228.

Scoville WB, Milner B (1957) Loss of recent memory after bilateral hippocampal lesions. J Neurol Neurosurg Psychiatr 20:11–21.

Sesack SR, Deutch AY, Roth RH, Bunney BS (1989) Topographical organization of the efferent projections of the medial prefrontal cortex in the rat: an anterograde tract-tracing study with Phaseolus vulgaris leucoagglutinin. J Comp Neurol 290:213–242.

Shaham Y, Shalev U, Lu L, De Wit H, Stewart J (2003) The reinstatement model of drug relapse: history, methodology and major findings. Psychopharmacology 168:3–20.

Shaham Y, Stewart J (1995) Stress reinstates heroin-seeking in drug-free animals: an effect mimicking heroin, not withdrawal. Psychopharmacology 119:334–341.

Shalev U, Marinelli M, Baumann MH, Piazza P-V, Shaham Y (2003) The role of corticosterone in food deprivation-induced reinstatement of cocaine seeking in the rat. Psychopharmacology 168:170–176.

Sheehan TP, Chambers RA, Russell DS (2004) Regulation of affect by the lateral septum: implications for neuropsychiatry. Brain Res Brain Res Rev 46:71–117.

Shiflett MW, Balleine BW (2010) At the limbic-motor interface: disconnection of basolateral amygdala from nucleus accumbens core and shell reveals dissociable components of incentive motivation. Eur J Neurosci 32:1735–1743.

Silva FJ, Silva KM, Pear JJ (1992) Sign- versus goal-tracking: effects of conditioned-stimulus-to-unconditioned-stimulus distance. J Exp Anal Behav 57:17–31.

Sinclair CM, Cleva RM, Hood LE, Olive MF, Gass JT (2012) mGluR5 receptors in the basolateral amygdala and nucleus accumbens regulate cue-induced reinstatement of ethanol-seeking behavior. Pharmacol Biochem Behav 101:329–335.

Sinha R, Catapano D, O'Malley S (1999) Stress-induced craving and stress response in cocaine dependent individuals. Psychopharmacology 142:343–351.

Smith KS, Bucci DJ, Luikart BW, Mahler SV (2016) DREADDS: Use and application in behavioral neuroscience. Behav Neurosci 130:137–155.

Smith RJ, Aston-Jones G (2011) α(2) Adrenergic and imidazoline receptor agonists prevent cue-induced cocaine seeking. Biol Psychiatry 70:712–719.

Smith RJ, See RE, Aston-Jones G (2009) Orexin/hypocretin signaling at the orexin 1 receptor regulates cue-elicited cocaine-seeking. Eur J Neurosci 30:493–503.

Sparks PD, LeDoux JE (1995) Septal lesions potentiate freezing behavior to contextual but not to phasic conditioned stimuli in rats. Behav Neurosci 109:184–188.

Spyraki C, Fibiger HC, Phillips AG (1982) Dopaminergic substrates of amphetamine-induced place preference conditioning. Brain Research 253:185–193.

Squire LR (1992) Memory and the hippocampus: a synthesis from findings with rats, monkeys, and humans. Psychol Rev 99:195–231.

Stefanelli T, Bertollini C, Lüscher C, Muller D, Mendez P (2016) Hippocampal Somatostatin Interneurons Control the Size of Neuronal Memory Ensembles. Neuron 89:1074–1085.

Stefanik MT, Kalivas PW (2013) Optogenetic dissection of basolateral amygdala projections during cue-induced reinstatement of cocaine seeking. Front Behav Neurosci 7:213.

Stefanik MT, Kupchik YM, Kalivas PW (2016) Optogenetic inhibition of cortical afferents in the nucleus accumbens simultaneously prevents cue-induced transient synaptic potentiation and cocaine-seeking behavior. Brain Struct Funct 221:1681–1689.

Stefanik MT, Moussawi K, Kupchik YM, Smith KC, Miller RL, Huff ML, Deisseroth K, Kalivas PW, LaLumiere RT (2013) Optogenetic inhibition of cocaine seeking in rats. Addict Biol 18:50–53.

Stern EA, Jaeger D, Wilson CJ (1998) Membrane potential synchrony of simultaneously recorded striatal spiny neurons in vivo. Nature 394:475–478.

Stewart J, Badiani A (1993) Tolerance and sensitization to the behavioral effects of drugs. Behav Pharmacol 4:289–312.

Sun W, Rebec GV (2003) Lidocaine inactivation of ventral subiculum attenuates cocaine-seeking behavior in rats. Journal of Neuroscience 23:10258–10264.

Swanson LW, Cowan WM (1977) An autoradiographic study of the organization of the efferent connections of the hippocampal formation in the rat. J Comp Neurol 172:49–84.

Sweeney P, Yang Y (2015) An excitatory ventral hippocampus to lateral septum circuit that suppresses feeding. Nat Commun.

Taha SA, Fields HL (2005) Encoding of palatability and appetitive behaviors by distinct neuronal populations in the nucleus accumbens. Journal of Neuroscience 25:1193–1202.

Thiebaut de Schotten M, Dell'Acqua F, Ratiu P, Leslie A, Howells H, Cabanis E, Iba-Zizen MT, Plaisant O, Simmons A, Dronkers NF, Corkin S, Catani M (2015) From Phineas Gage and Monsieur Leborgne to H.M.: Revisiting Disconnection Syndromes. Cerebral Cortex 25:4812–4827.

Tomita M, Katsuyama H, Watanabe Y, Shibaike Y, Yoshinari H, Tee JW, Iwachidou N, Miyamoto O (2013) c-Fos immunoreactivity of neural cells in intoxication due to high-dose methamphetamine. J Toxicol Sci 38:671–678.

Trent NL, Menard JL (2010) The ventral hippocampus and the lateral septum work in tandem to regulate rats' open-arm exploration in the elevated plus-maze. Physiology & Behavior 101:141–152.

Trouche S, Perestenko PV, van de Ven GM, Bratley CT, McNamara CG, Campo-Urriza N, Black SL, Reijmers LG, Dupret D (2016) Recoding a cocaine-place memory engram to a neutral engram in the hippocampus. Nat Neurosci.

Van Horn JD, Irimia A, Torgerson CM, Chambers MC, Kikinis R, Toga AW (2012) Mapping connectivity damage in the case of Phineas Gage.

Vardy E et al. (2015) A New DREADD Facilitates the Multiplexed Chemogenetic Interrogation of Behavior. Neuron 86:936–946.

Vazey EM, Aston-Jones G (2014) Designer receptor manipulations reveal a role of the locus coeruleus noradrenergic system in isoflurane general anesthesia. Proc Natl Acad Sci USA 111:3859–3864.

Vertes RP (2004) Differential projections of the infralimbic and prelimbic cortex in the rat. Synapse 51:32–58.

Volkow ND, Morales M (2015) The Brain on Drugs: From Reward to Addiction. Cell 162:712–725.

Volkow ND, Wang GJ, Fischman MW, Foltin R, Fowler JS, Franceschi D, Franceschi M, Logan J, Gatley SJ, Wong C, Ding YS, Hitzemann R, Pappas N (2000) Effects of route of administration on cocaine induced dopamine transporter blockade in the human brain. Life Sci 67:1507–1515.

Volkow ND, Wang GJ, Fowler JS, Logan J, Gatley SJ, Hitzemann R, Chen AD, Dewey SL, Pappas N (1997) Decreased striatal dopaminergic responsiveness in detoxified cocaine-dependent subjects. Nature 386:830–833.

Voorn P, Vanderschuren LJMJ, Groenewegen HJ, Robbins TW, Pennartz CMA (2004) Putting a spin on the dorsal-ventral divide of the striatum. Trends in Neurosciences 27:468–474.

Vorel SR, Liu X, Hayes RJ, Spector JA, Gardner EL (2001) Relapse to cocaine-seeking after hippocampal theta burst stimulation. Science 292:1175–1178.

Weiskrantz L (1956) Behavioral changes associated with ablation of the amygdaloid complex in monkeys. J Comp Physiol Psychol 49:381–391.

West EA, Saddoris MP, Kerfoot EC, Carelli RM (2014) Prelimbic and infralimbic cortical regions differentially encode cocaine-associated stimuli and cocaine-seeking before and following abstinence. Eur J Neurosci 39:1891–1902.

Wilkinson DS, Turner JR, Blendy JA, Gould TJ (2013) Genetic background influences the effects of withdrawal from chronic nicotine on learning and high-affinity nicotinic acetylcholine receptor binding in the dorsal and ventral hippocampus. Psychopharmacology 225:201–208.

Williams JC, Denison T (2013) From optogenetic technologies to neuromodulation therapies. Sci Transl Med 5:177ps6.

Wise RA (1980) Action of drugs of abuse on brain reward systems. Pharmacol Biochem Behav 13 Suppl 1:213–223.

Xie X, Lasseter HC, Ramirez DR, Ponds KL, Wells AM, Fuchs RA (2012) Subregion-specific role of glutamate receptors in the nucleus accumbens on drug context-induced reinstatement of cocaine-seeking behavior in rats. Addict Biol 17:287–299.

Young ST, Porrino LJ, Iadarola MJ (1991) Cocaine induces striatal c-fos-immunoreactive proteins via dopaminergic D1 receptors. Proc Natl Acad Sci USA 88:1291–1295.

Zahm DS (1999) Functional-anatomical implications of the nucleus accumbens core and shell subterritories. Annals of the New York Academy of Sciences 877:113–128.

Zahm DS (2000) An integrative neuroanatomical perspective on some subcortical substrates of adaptive responding with emphasis on the nucleus accumbens. Neurosci Biobehav Rev 24:85–105.

Zahm DS, Becker ML, Freiman AJ, Strauch S, DeGarmo B, Geisler S, Meredith GE, Marinelli M (2010) Fos after single and repeated self-administration of cocaine and saline in the rat: emphasis on the Basal forebrain and recalibration of expression. Neuropsychopharmacology 35:445–463.

Zahm DS, Parsley KP, Schwartz ZM, Cheng AY (2013) On lateral septum-like characteristics of outputs from the accumbal hedonic "hotspot" of Peciña and Berridge with commentary on the transitional nature of basal forebrain "boundaries". J Comp Neurol 521:50–68.

Zhou L, Pruitt C, Shin CB, Garcia AD, Zavala AR, See RE (2014) Fos expression induced by cocaine-conditioned cues in male and female rats. Brain Struct Funct 219:1831–1840.

Zhu H, Pleil KE, Urban DJ, Moy SS, Kash TL, Roth BL (2014) Chemogenetic inactivation of ventral hippocampal glutamatergic neurons disrupts consolidation of contextual fear memory. Neuropsychopharmacology 39:1880–1892.

Zhu H, Roth BL (2015) DREADD: a chemogenetic GPCR signaling platform. Int J Neuropsychopharmacol 18.