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# Influence Of Local And Circuit-Wide Modulation Of The Mesocorticolimbic Reward System On The Reinstatement Of Cocaine Seeking

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# Influence Of Local And Circuit-Wide Modulation Of The Mesocorticolimbic Reward System On The Reinstatement Of Cocaine Seeking

## **Abstract**

Cocaine abuse poses a significant public health concern both in the United States and across the globe. A critical issue with cocaine abuse is the discouragingly high rate of relapse among addicts following detoxification and abstinence. The research presented in this doctoral dissertation examines the influence of local and circuit-wide modulation of the mesocorticolimbic reward system on cocaine reinstatement, an animal model of relapse.

The data presented in the second and third chapters of this dissertation demonstrate that DBS may serve as a possible non-pharmacological therapeutic intervention in the treatment of cocaine addiction. In Chapter 2, I show that DBS of the nucleus accumbens shell attenuates the cue-induced reinstatement of cocaine seeking, expanding upon previous work demonstrating the efficacy of accumbal shell DBS in attenuating cocaine priming-induced reinstatement. In Chapter 3, I demonstrate that DBS of the medial prefrontal cortex (mPFC), but not the basolateral amygdala (BLA) or the ventral hippocampus (vHipp) selectively attenuates the reinstatement of cocaine seeking. Moreover, this effect is constrained to the infralimbic subregion of the mPFC as DBS in the prelimbic or anterior cingulate cortices has no effect on cocaine reinstatement. Further, my results also suggest that infralimbic mPFC DBS attenuates cocaine reinstatement by disrupting glutamatergic transmission to the nucleus accumbens.

The data presented in Chapter 4 of this dissertation support a substantial body of evidence demonstrating that increased transmission through GluA1-containing AMPA receptors (AMPA) in the nucleus accumbens shell promotes cocaine reinstatement. These data reveal the novel role of the protein, AKAP150, in the reinstatement of cocaine seeking. My findings indicate that AKAP150 promotes cocaine reinstatement by facilitating D1-like dopamine receptor (D1DR)-induced, PKA-mediated phosphorylation of GluA1-containing AMPARs. Collectively, these findings suggest that AKAP150 may serve as a biochemical bridge linking the dopamine and glutamate systems in the nucleus accumbens during cocaine reinstatement.

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REWARD SYSTEM ON THE REINSTATEMENT OF COCAINE SEEKING

Leonardo Antonio Guercio

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*To my grandmothers -- Erminia LoBiondo and Marie Andricola.*

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*“When you eat the fruits of success, give thanks to those who planted the tree.”*

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To all my friends, thank you for all the love, kindness, and support you have shown me over the years. One of my greatest joys in life is having been in the company of such warm and wonderful people. Your friendship makes music sound fuller, wine taste sweeter, and laughter ring louder. I am grateful every day to be blessed with such good friends.

Finally, I thank my family most of all, both those that are still with here and those that have passed on. I know without a doubt that I would not be the man that I am today without all your love, compassion, and generosity. You are the greatest blessing in my life. I must especially thank my parents, Leonard and Lisa, because you gave me the greatest gift of all – you always believed in me. You sacrificed so much for me to succeed and you did so without hesitation or complaint. I hope I have made you proud.

Thanks, and thanks, and ever thanks.

## **ABSTRACT**

### INFLUENCE OF LOCAL AND CIRCUIT-WIDE MODULATION OF THE MESOCORTICOLIMBIC REWARD SYSTEM ON THE REINSTATEMENT OF COCAINE SEEKING

Leonardo Antonio Guercio

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Heath D. Schmidt, Ph.D.

Cocaine abuse poses a significant public health concern both in the United States and across the globe. A critical issue with cocaine abuse is the discouragingly high rate of relapse among addicts following detoxification and abstinence. The research presented in this doctoral dissertation examines the influence of local and circuit-wide modulation of the mesocorticolimbic reward system on cocaine reinstatement, an animal model of relapse.

The data presented in the second and third chapters of this dissertation demonstrate that DBS may serve as a possible non-pharmacological therapeutic intervention in the treatment of cocaine addiction. In Chapter 2, I show that DBS of the nucleus accumbens shell attenuates the cue-induced reinstatement of cocaine seeking, expanding upon previous work demonstrating the efficacy of accumbal shell DBS in attenuating cocaine priming-induced reinstatement. In Chapter 3, I demonstrate that DBS of the medial prefrontal cortex (mPFC), but not the basolateral amygdala (BLA) or the ventral hippocampus (vHipp) selectively attenuates the reinstatement of cocaine seeking. Moreover, this effect is constrained to the infralimbic subregion of the mPFC as DBS in



the prelimbic or anterior cingulate cortices has no effect on cocaine reinstatement. Further, my results also suggest that infralimbic mPFC DBS attenuates cocaine reinstatement by disrupting glutamatergic transmission to the nucleus accumbens.

The data presented in Chapter 4 of this dissertation support a substantial body of evidence demonstrating that increased transmission through GluA1-containing AMPA receptors (AMPA) in the nucleus accumbens shell promotes cocaine reinstatement. These data reveal the novel role of the protein, AKAP150, in the reinstatement of cocaine seeking. My findings indicate that AKAP150 promotes cocaine reinstatement by facilitating D1-like dopamine receptor (D1DR)-induced, PKA-mediated phosphorylation of GluA1-containing AMPARs. Collectively, these findings suggest that AKAP150 may serve as a biochemical bridge linking the dopamine and glutamate systems in the nucleus accumbens during cocaine reinstatement.

In sum, the findings presented herein expand our understanding of the neurobiological mechanisms underlying cocaine seeking and identify both a non-pharmacological application, deep brain stimulation (DBS), and a novel biochemical target, AKAP150, for potential therapeutic interventions in cocaine addiction and craving.

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## Chapter 1

### COCAINE ADDICTION AND RELAPSE: A GENERAL INTRODUCTION

Leonardo Antonio Guercio

“... I sneered at the poor mortals condemned to live in this valley of tears while I, carried on the wings of two leaves of coca, went flying through the spaces of 77,438 words, each more splendid than the one before... An hour later, I was sufficiently calm to write these words in a steady hand: God is unjust because he made man incapable of sustaining the effect of coca all life long. I would rather have a life span of ten years with coca than one of 10,000,000,000,000,000,000,000 centuries without coca.”

- Paolo Mantegazza, *Sulle Virtù Igieniche e Medicinali della Coca e sugli Alimenti Nervosi in Generale (1859)*

#### ***Cocaine: A Brief History***

Cocaine is derived from the leaves of the coca plant (*Erythroxylon coca*), a bushlike flower that is indigenous to the Andean mountain range of northwestern South America, particularly Colombia, Peru, and Bolivia. For over 4,000 years, the coca leaf has been chewed or brewed in tea in order to relieve fatigue from working in high altitudes (Karch, 2005). The coca plant was first introduced into Europe in the 16<sup>th</sup> century, following the Spanish invasion of Peru and the Incan empire, led by Francisco Pizarro in 1532 (MacQuarrie, 2008). However, cocaine did not become popular in Europe until the mid-19<sup>th</sup> century, when it was isolated from the coca plant.

In 1860, German chemist Albert Niemann, recognizing the powerful stimulant properties of the coca plant, extracted the pure chemical, cocaine hydrochloride, as part of his

dissertation work. In the 1880s, Karl Koller, an Austrian ophthalmologist, discovered the anesthetic properties of cocaine, which led to its use in eye surgery, and later dentistry (Karch, 2005). In 1884, the eminent Austrian psychiatrist Sigmund Freud published a book entitled *Uber Coca* ("On Coca"), in which he touted the many benefits of cocaine, including its use as an aphrodisiac, mental stimulant, digestive aid, antidepressant, among others. Freud was so enamored with the drug, he erroneously believed that chronic cocaine use was not detrimental to the body and that cocaine could also serve as a treatment for alcohol and morphine addiction, which ultimately led to the death of his best friend for whom he prescribed cocaine to cure his morphine addiction (Karch, 2005).

In addition to being used for medical purposes, cocaine was also being used recreationally. In the late 19<sup>th</sup> century, Parke, Davis & Company sold a medicinal kit that included 300 mg of powdered cocaine, dissolving solution, and a syringe for intravenous injection (Karch, 2005). Also at this time, Vin Mariani, a Bordeaux wine infused with cocaine became wildly popular, bolstered by resounding endorsements from Queen Victoria of England, Thomas Edison, Ulysses S. Grant, and many others. Pope Leo XIII even awarded the wine with a Vatican gold medal, in addition to appearing on posters endorsing the wine (Inciardi, 1992). The success of Vin Mariani ultimately led American pharmacist John Pemberton to develop a non-alcoholic form of the beverage, since the state of Georgia had strict prohibition laws at the time. His formula consisted of approximately 2.5 mg of cocaine (from coca leaves) per 100 mL of caffeinated fluid (from kola nuts), and he called it Coca-Cola (Spillane, 2000). Even today, the Coca-Cola recipe still contains coca leaves for flavor, albeit without cocaine (Karch, 2005). By the early 20<sup>th</sup> century, increased recreational use of cocaine and improved knowledge of its addictive properties caused a backlash against drug use, ultimately

leading to the passage of the Harrison Narcotics Act of 1914, which criminalized the use of cocaine (Flynn, 1993). The counterculture movement of the 1960s and 1970s led to a re-emergence of cocaine use due to a more relaxed attitude towards drugs (Miller, 2014). Cocaine use further skyrocketed in the 1980s with the advent of crack cocaine, a freebase form of cocaine that can be smoked and thereby directly inhaled into the lungs (Karch, 2005). Though the market for illegal cocaine grew most dramatically in the 1980s, it remains a global crisis with approximately 850 tons of cocaine produced in 2014 (Crime, 2016).

### ***Cocaine Addiction: A Global Public Health Concern***

Drug addiction is a major public health concern in both the United States and worldwide. It is estimated that the total costs of substance abuse including productivity, health, and crime-related costs, exceed \$600 billion annually in the United States alone (National Drug Intelligence Center, 2011). Cocaine is the fourth most commonly abused illegal drug in the world, with the United States as the global leader in cocaine demand (Crime, 2016). Approximately 1.5 million Americans aged 12 or older are regular users of cocaine, comprising about 0.5% of the US population (Sarra L Hedden, 2015). Cocaine use is also responsible for over 500,000 emergency room visits annually (National Institute on Drug Abuse, 2016). Thus, cocaine addiction is a prohibitively expensive public health epidemic that affects millions of Americans.

### ***Physiological Effects of Cocaine Use***

Cocaine is a biogenic amine transporter inhibitor, exerting its effects on the dopamine, serotonin, and norepinephrine systems (Ritz et al., 1990). Cocaine can also block voltage-gated sodium channels, making it an effective local anesthetic (O'Leary and

Hancox, 2010). The acute effects of cocaine include euphoria, elation, increased energy, increased mental alertness, decreased appetite, increased sensitivity to sights, sounds, and touch, blood vessels constriction, dilated pupils, increased body temperature, and increased heart rate and blood pressure (Karch, 2005). Chronic cocaine use can lead to addiction, increased irritability, restlessness, panic attacks, paranoia, psychosis, auditory hallucinations, heart attacks, increased risk for stroke and seizures, gastrointestinal tears or ulcerations, septal necrosis, loss of sense of smell, lung damage (typically from crack cocaine inhalation), sleep disturbances, and malnourishment (National Institute on Drug Abuse, 2016). Chronic cocaine users typically develop increased tolerance to the drug, which can lead to compulsive drug-seeking behavior and worsening withdrawal symptoms (Wolf, 2010).

### ***Drug Abuse and the Cycle of Addiction***

Substance use disorders can range from mild to severe and is determined by the number of diagnostic criteria met by the individual. A diagnosis of substance use disorder is based on evidence of impaired control, social impairment, risky use, and pharmacological criteria. The most commonly abused substances are alcohol, tobacco, cannabis, stimulants, opioids, and hallucinogens. Cocaine is the most widely abused psychostimulant (Sarra L Hedden, 2015).

Following the initial use of cocaine, drug use can quickly escalate in certain individuals. These individuals subsequently enter a cycle of chronic drug taking followed by periods of abstinence and subsequent relapse into chronic drug taking. In fact, one of the major problems facing cocaine addicts is the discouragingly high rate of relapse, even after prolonged abstinence (Carroll, 1994; O'Brien, 1997). In fact, rates of relapse increase as

time goes on — a phenomenon called incubation of craving (Gawin and Kleber, 1986). Since drug craving increases over time, long-term abstinence is an extraordinarily difficult accomplishment for addicts. However, despite many years of preclinical and clinical research focused on understanding the underlying neurobiological and neurochemical basis of addiction, there are no FDA-approved pharmacotherapeutic interventions for the treatment of cocaine abuse and relapse.

### ***Modeling Cocaine Taking and Relapse: Self-Administration and Reinstatement***

Cocaine craving and relapse in abstinent addicts can be precipitated by 3 major factors: stress, environmental stimuli previously associated with drug taking, or re-exposure to the drug itself (Wit and Stewart, 1981; Jaffe et al., 1989; O'Brien et al., 1992; Sinha et al., 1999). In order to gain a better understanding into the molecular and physiological underpinnings of cocaine addiction and relapse, researchers utilize an animal model of addiction, specifically the self-administration/extinction/reinstatement paradigm (Shalev et al., 2002; Shaham and Hope, 2005; Bossert et al., 2013). This involves training an animal to self-administer cocaine via operant conditioning. After a period of self-administration (typically several hours per day for 14-21 days), the cocaine solution is removed and replaced with saline, which extinguishes cocaine taking. Following extinction of drug taking, exposure to a stressor, re-exposure to cocaine-associated cues, or a non-contingent priming injection of cocaine reinstate drug-seeking behavior (Shalev et al., 2002). This model is invaluable for assessing the neurobiological underpinnings of drug addiction and craving-induced relapse of cocaine-seeking behavior.

### ***Neuronal Circuitry Underlying Cocaine Reinstatement***



Though cocaine can inhibit transporter activity of the dopamine, serotonin, and norepinephrine systems (Ritz et al., 1990), several studies have shown that dopamine is the critical biogenic amine underlying the reinstatement of cocaine seeking. Administration of a dopamine, but not serotonin or norepinephrine, reuptake inhibitor reinstated cocaine seeking (Schenk, 2002; Schmidt and Pierce, 2006a). Dopaminergic neurons in the ventral tegmental area (VTA) richly innervate corticolimbic nuclei, including the nucleus accumbens, medial prefrontal cortex (mPFC), amygdala, hippocampus, and ventral pallidum (Berendse et al., 1992; Brog et al., 1993; Heimer et al., 1997). Several of these corticolimbic nuclei, including the medial prefrontal cortex (mPFC), hippocampus, and amygdala send robust glutamatergic projections to the nucleus accumbens (Phillipson and Griffiths, 1985; Friedman et al., 2002). The nucleus accumbens sends efferent GABAergic projections to the VTA and ventral pallidum (Heimer et al., 1991; Groenewegen et al., 1999), which in turn, send efferent GABAergic projections to the mediodorsal thalamus (Groenewegen, 2003). The mediodorsal thalamus sends glutamatergic projections to the mPFC, effectively closing this circuit (see Figure 1.1).

The nucleus accumbens can be further divided into two functionally segregated subregions, the medial shell and the lateral core (Meredith et al., 1992; Groenewegen et al., 1999). The nucleus accumbens shell, considered part of the limbic system, has been implicated primarily in the reinforcing and rewarding properties of drugs of abuse (Di Chiara and Imperato, 1988; Pontieri et al., 1995; William A Carlezon and Wise, 1996). The nucleus accumbens core, considered an extension of the basal ganglia, contributes to drug-associated, cue-induced drug seeking (Di Ciano and Everitt, 2004; Fuchs et al., 2004; Ito et al., 2004). Therefore, the nucleus accumbens serves to integrate the

motivational information from the limbic system with the basal ganglia to facilitate an appropriate behavioral response.

### ***The Role of the Nucleus Accumbens in Cocaine Seeking***

#### *Nucleus Accumbens and Priming-Induced Reinstatement of Cocaine Seeking*

It is now clear that increased dopamine transmission in the nucleus accumbens promotes the reinstatement of cocaine seeking. Thus, intra-nucleus accumbens infusions of dopamine (Cornish and Kalivas, 2000) promoted the reinstatement of cocaine seeking in rats that previously self-administered cocaine. Co-administration of the nonselective dopamine receptor antagonist, fluphenazine, blocked the reinstatement of cocaine seeking precipitated by intra-accumbal infusions of dopamine (Cornish and Kalivas, 2000). Dopamine transmission is mediated by a family of G-protein coupled receptors, with 5 subtypes (D1-D5). These receptors subtypes can be further categorized as D1-like (D1 and D5) or D2-like (D2, D3, D4) based on their sequence homology and pharmacology (MISSALE et al., 1998; Beaulieu and Gainetdinov, 2011). There is an extensive literature indicating that dopaminergic transmission through D1-like and D2-like dopamine receptors is critical for the reinstatement of cocaine seeking in the nucleus accumbens (Bossert et al., 2005; Schmidt and Pierce, 2006b).

Systemic administration of D1-like or D2-like dopamine receptor antagonists blocked the reinstatement of cocaine seeking (Self et al., 1996; Khroyan et al., 2000; Vorel et al., 2002). Peripheral injections of D2-like dopamine receptor agonists promoted cocaine priming-induced reinstatement (Self et al., 1996; Khroyan et al., 2000; De Vries et al., 2002). However, systemically administered D1-like dopamine receptor agonists failed to promote, and actually attenuated cocaine reinstatement (Self et al., 1996; Khroyan et al.,

2000; Self et al., 2000), while intra-accumbal shell administration of D1-like dopamine receptor agonists promoted the reinstatement of cocaine-seeking behavior (Bachtell et al., 2005; Schmidt et al., 2006; Anderson et al., 2008). These results suggested that systemic administration of D1-like dopamine receptor agonists activate D1-like dopamine receptors in other brain regions, which counteract the reinstatement of cocaine seeking promoted by activation of D1-like dopamine receptors in the nucleus accumbens. It should be noted, however, that dopaminergic transmission in the core and shell subregions of the nucleus accumbens have differential effects on cocaine seeking. Administration of D1-like or D2-like dopamine receptor antagonists in the accumbens shell, but not the core, blocked priming-induced reinstatement of cocaine seeking (Anderson et al., 2003; 2005; Bachtell et al., 2005). Consistent with these findings, intra-accumbal shell, but not core, administration of D1-like and D2-like dopamine receptor agonists promoted the reinstatement of cocaine seeking (Schmidt and Pierce, 2006b). Collectively, these findings offer evidence that D1-like and D2-like dopamine receptors play a critical role in cocaine reinstatement and that D1-like dopamine receptors in other nuclei besides the nucleus accumbens shell may have differential effects on the reinstatement of cocaine seeking.

Although cocaine increases the extracellular concentration of dopamine, there is overwhelming evidence that chronic cocaine exposure also affects glutamatergic transmission, particularly in the nucleus accumbens, which can have profound effects on neuronal function and alter the behavioral effects of cocaine (Schmidt and Pierce, 2010). While cocaine has no direct action on glutamatergic neurons or glutamate levels, withdrawal from repeated exposure to cocaine reduced basal extracellular levels of

glutamate in the nucleus accumbens (Pierce et al., 1996), an effect due to decreased activity of the cysteine-glutamate antiporter (Baker et al., 2003a).

Cocaine priming-induced reinstatement is associated with increased extracellular levels of glutamate in the nucleus accumbens (Cornish et al., 1999; Cornish and Kalivas, 2000; Park et al., 2002; McFarland et al., 2003). In fact, systemic injections of N-acetyl cysteine, a pro-drug that increases the activity of the cysteine-glutamate antiporter, attenuated cocaine priming-induced reinstatement. Glutamate binds to N-methyl-D-aspartate (NMDA),  $\alpha$ -amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA), and metabotropic glutamate (mGluR) receptors, all of which play a role in cocaine reinstatement (Schmidt and Pierce, 2010).

Intra-accumbal administration of AMPA or an AMPA receptor agonist reinstated cocaine seeking, whereas intra-accumbal administration of AMPA receptor antagonists attenuated cocaine priming-induced reinstatement (Cornish et al., 1999; Cornish and Kalivas, 2000; Famous et al., 2008; Ping et al., 2008). These effects were observed in both core and shell subregions of the nucleus accumbens. AMPA receptors are heteromeric, ligand-gated ion channels expressed throughout the brain that are composed of 4 subunits: GluA1-GluA4, and are also permeable to  $\text{Ca}^{2+}$ ,  $\text{Na}^+$ , and  $\text{K}^+$ . AMPA receptors have a unique feature where conversion of a glutamine (Q) residue to an arginine (R) on the GluA2 subunit renders GluA2-containing AMPA receptors impermeable to calcium (Hume et al., 1991; Rueter et al., 1995). Since most GluA2 subunits are edited in this manner, GluA2-containing AMPA receptors are considered calcium-impermeable (Tanaka et al., 2000).

Cocaine reinstatement is associated with the phosphorylation and trafficking of GluA1 and GluA2-containing AMPA receptors. Consistent with these results, suppression of GluA1 transcription in the accumbens blocked the reinstatement of cocaine seeking induced by a priming injection of cocaine (Ping et al., 2008). Cocaine reinstatement is associated with increased phosphorylation and surface expression of GluA1-containing AMPA receptors and of GluA1 in the accumbens shell, but not the core (Anderson et al., 2008). Consistent with this, preventing the transport of GluA1-containing AMPA receptors to the cell surface in the nucleus accumbens shell attenuated priming-induced reinstatement of cocaine seeking (Anderson et al., 2008). Additionally, extended withdrawal from cocaine self-administration increased surface expression of GluA1-containing, but not GluA2-containing, AMPA receptors in the nucleus accumbens (Conrad et al., 2008). Cocaine reinstatement is also associated with increased phosphorylation and decreased surface expression of GluA2-containing AMPA receptors in the accumbens shell, but not the core (Famous et al., 2008). Disruption of trafficking of GluA2-containing AMPA receptors attenuated cocaine priming-induced reinstatement (Famous et al., 2008). Similarly, more recent studies have shown that withdrawal from cocaine self-administration and incubation of cocaine craving is associated with increased insertion of GluA1-containing, GluA2-lacking, calcium-permeable AMPA receptors (CP-AMPA) in the nucleus accumbens (Mameli et al., 2009; Ferrario et al., 2010; McCutcheon et al., 2011b). Taken together, these findings suggest that cocaine reinstatement is associated with increases in accumbal glutamatergic transmission, mediated in part by the differential trafficking of GluA1 and GluA2 subunits.

#### *Nucleus Accumbens and Cue-Induced Reinstatement of Cocaine Seeking*

Relapse to drug-seeking behavior can be induced by re-exposure to environmental cues and contexts previously associated with drug taking (O'Brien et al., 1992). This can be modeled experimentally in three major ways: context-induced reinstatement (Crombag and Shaham, 2002), discrete cue-induced reinstatement (Meil and See, 1996), and discriminative cue-induced reinstatement (Weiss et al., 2000). Behavioral findings from all three models will be outlined below, however, we will primarily focus on discrete cue-induced reinstatement.

As in priming-induced reinstatement, re-exposure to cocaine-associated stimuli resulted in increased glutamate levels in the nucleus accumbens (Hotsenpiller and Giorgetti, 2001). Systemic or intra-accumbal core administration of baclofen and muscimol attenuated cue-induced reinstatement of cocaine-seeking behavior (Di Ciano and Everitt, 2004; Fuchs et al., 2004). Additionally, excitotoxic lesions of the nucleus accumbens core, but not the shell, attenuated cocaine seeking induced by discrete cues (Ito et al., 2004). Consistent with these findings, systemic and intra-accumbal core administration of an AMPA receptor antagonist, or intra-accumbal core administration of an NMDA receptor antagonist, attenuated cue-induced reinstatement (Di Ciano, 2001; Bäckström and Hyytiä, 2006; 2007; Zavala et al., 2008) – however, these treatments had no effect when injected into the accumbens shell. Systemic administration of ceftriaxone, an antibiotic that enhances glutamate reuptake in synapses, attenuated cocaine cue-induced reinstatement (Sari et al., 2009; Sondheimer and Knackstedt, 2011; Fischer et al., 2013). Consistent with this, ceftriaxone-mediated attenuation of cue-induced cocaine reinstatement was reversed by blockade of GLT-1, which is responsible for glutamate reuptake, in the accumbens core, but not the shell (Fischer et al., 2013).

While the role of the accumbens core is well established for cue-induced reinstatement of cocaine seeking, there is evidence that the accumbens shell also contributes to cue-induced reinstatement, particularly in context-induced reinstatement. Administration of baclofen and muscimol into the accumbens shell attenuated the context-induced reinstatement of cocaine seeking (Fuchs et al., 2008). Additionally, intra-accumbal shell administration of AMPA/kainate glutamate receptor antagonist CNQX attenuated context-induced reinstatement of cocaine seeking (Xie et al., 2012). It should be noted, however, that similar effects were observed in the accumbens core in both cases. Together, these findings suggest that the nucleus accumbens core, and to a lesser extent, the accumbens shell, is critical for cue-induced reinstatement of cocaine seeking.

### ***The Role of the mPFC in Cocaine Seeking***

The mPFC can be divided into three functional components: the anterior cingulate cortex, the prelimbic cortex, and the infralimbic cortex (Krettek and Price, 1977), all of which receive dense dopaminergic projections from the VTA (Heidbreder and Groenewegen, 2003). These regions can also be segregated into the dorsal mPFC, which includes the anterior cingulate cortex and dorsal prelimbic cortex, and the ventral mPFC, which includes the ventral prelimbic and infralimbic cortices (Graybiel et al., 1990; Steketee, 2003). Notably, these regions have differential glutamatergic projections to the nucleus accumbens. The dorsal mPFC projects mainly to the nucleus accumbens core, whereas the ventral mPFC projects primarily to the nucleus accumbens shell (Berendse et al., 1992; Wright and Groenewegen, 1995; Ding et al., 2001). While there is strong evidence for the role of the dorsal mPFC in the reinstatement of cocaine seeking, there is also evidence for the role of the ventral mPFC as well.

Infusion of dopamine or cocaine into the dorsal mPFC reinstated cocaine seeking (McFarland and Kalivas, 2001; Park et al., 2002). Consistent with this, administration of baclofen and muscimol (McFarland and Kalivas, 2001) or TTX (Capriles et al., 2003) into the prelimbic, but not infralimbic, cortex attenuated cocaine priming-induced reinstatement. Additionally, administration of nonspecific, D1-like, or D2-like dopamine antagonists into the prelimbic, but not infralimbic, cortex blocked cocaine reinstatement (McFarland and Kalivas, 2001; Park et al., 2002; Capriles et al., 2003; Sun and Rebec, 2005). These findings suggest a strong role for dopaminergic transmission in the dorsal mPFC in cocaine priming-induced reinstatement.

The glutamatergic projections from the mPFC to the nucleus accumbens play a critical role in the reinstatement of cocaine seeking (Kalivas and O'Brien, 2008; Schmidt and Pierce, 2010). As mentioned previously, cocaine priming-induced reinstatement is associated with increased glutamate release in the nucleus accumbens, an effect that was blocked by pharmacological inactivation of the dorsal mPFC (McFarland et al., 2003). Consistent with this, reinstatement of cocaine seeking induced by administration of cocaine directly into the dorsal mPFC was blocked by intra-accumbal administration of AMPA antagonists (Park et al., 2002). Additionally, repeated cocaine exposure increased the excitability of glutamatergic projection neurons in the prelimbic cortex (Hearing et al., 2013), which increased their responsiveness for cocaine (Sun and Rebec, 2006).

While there is strong evidence for the role of the dorsal mPFC in cocaine reinstatement, there is also evidence, albeit conflicting, that the ventral mPFC plays a critical role as well. In one particular study, administration of baclofen and muscimol into the infralimbic



cortex reinstated cocaine seeking while microinjections of AMPA into this region attenuated cocaine seeking (Peters et al., 2008). This is inconsistent with the finding that administration of baclofen and muscimol into either the prelimbic or infralimbic cortices attenuated cocaine priming-induced reinstatement (Vassoler et al., 2013). Additionally, recent work has demonstrated that activation of the infralimbic mPFC-accumbens shell glutamatergic pathway is critical for the reinstatement of heroin and alcohol seeking (Bossert et al., 2012; Willcocks and McNally, 2013). Further, it has been shown the infralimbic cortex is involved in the consolidation of memories for the extinction of cocaine-seeking behavior (LaLumiere et al., 2010). Collectively, these results indicate that increased dopamine transmission in the mPFC, and glutamatergic transmission from the mPFC to the nucleus accumbens are critical for the reinstatement of cocaine seeking.

### ***The Role of the Basolateral Amygdala in Cocaine Seeking***

The amygdala is another nucleus that plays a critical role in the reinstatement of cocaine-seeking behavior. Like the hippocampus and mPFC, it also receives dopaminergic projections from the VTA (Fallon et al., 1978) and sends glutamatergic projections to the nucleus accumbens (Phillipson and Griffiths, 1985). The amygdala can be divided into many subnuclei, several of which have been shown to be involved in various types of cocaine reinstatement, particularly cue-induced reinstatement of cocaine seeking (Grimm, 2000; McFarland et al., 2004; Fuchs et al., 2005; Mashhoon et al., 2009; Stefanik and Kalivas, 2013).

The basolateral amygdala (BLA) has also been implicated in priming-induced reinstatement of cocaine seeking. Immediate-early genes *arc* and *zif268* in the BLA were

upregulated following priming-induced reinstatement of cocaine seeking (Ziółkowska et al., 2011). Consistent with this, lesions of the BLA attenuated cocaine priming-induced reinstatement (Yun and Fields, 2003). However, inactivation of the BLA using lidocaine had no effect on priming-induced reinstatement of cocaine seeking (McFarland and Kalivas, 2001). Administration of NMDA into the BLA reinstated cocaine seeking (Hayes et al., 2003) and antagonism of D1-like and D2-like dopamine receptors, in the BLA attenuated cocaine priming-induced reinstatement (Alleweireldt et al., 2006; Di Ciano, 2008). Additionally, pharmacological manipulations in the BLA that promote experience-dependent plasticity enhanced extinction and attenuated cocaine seeking (Xue et al., 2014). While these results indicate that the BLA is involved in the priming-induced reinstatement of cocaine seeking, further studies are required to elucidate the precise mechanisms by which this nucleus contributes to priming-induced reinstatement.

### ***The Role of the Ventral Hippocampus in Cocaine Seeking***

The hippocampus, a critical region for memory and reward-related behaviors, can be segregated into dorsal and ventral regions (Moser and Moser, 1998). The dorsal hippocampus is critical for spatial memory (Moser et al., 1995), whereas the ventral hippocampus plays more important role in motivated behaviors (Henke, 1990). Additionally, these regions have different anatomical connections, with distinct inputs and outputs (Swanson and Cowan, 1977). The ventral hippocampus is strongly innervated with dopaminergic projections from the VTA (Gasbarri et al., 1994a; 1994b). Additionally, the ventral hippocampus is the major output region of the hippocampus (Groenewegen et al., 1987) with strong projections to the nucleus accumbens, particularly the accumbens shell (Fanselow and Dong, 2010). Stimulation of the ventral hippocampus leads to increased extracellular dopamine levels in the nucleus

accumbens, an effect that was abolished through blockade of glutamate receptors in the accumbens (Blaha et al., 1997; Taepavarapruk et al., 2000).

There is also evidence for the role of the ventral hippocampus in the reinstatement of cocaine seeking. Inactivation of the ventral hippocampus with lidocaine blocked cocaine priming-induced reinstatement (Sun and Rebec, 2003). Consistent with this, administration of baclofen and muscimol to the ventral hippocampus attenuated cocaine priming-induced reinstatement (Rogers and See, 2007). These results indicate a role for the hippocampus, particularly the ventral hippocampus, in the reinstatement of cocaine seeking.

Taken together, these findings demonstrate that cocaine reinstatement is associated with both circuit-wide changes in the mesocorticolimbic reward system, as well as cellular and molecular changes within each of these subnuclei, particularly the nucleus accumbens. Focusing on circuit-level changes and potential therapeutic value, Chapters 2 and 3 will examine how deep brain stimulation (DBS) in mesocorticolimbic nuclei can modulate cocaine seeking and which nuclei are optimal targets for clinical studies.

### ***Deep Brain Stimulation***

Deep brain stimulation (DBS) was originally developed in the 1950s in order to produce “reversible lesions” in the brain and was thought to be a potential replacement for the lobotomy in the treatment of psychiatric disorders (Lozano and Lipsman, 2013).

However, DBS did not achieve widespread acceptance since decent pharmacological treatments for psychiatric disorders were developed at that time as well. In the 1980s, it became increasingly apparent that levodopa, the standard pharmacotherapy for

Parkinson's disease, both failed to halt the progression of the disease and also led to debilitating side effects (Benabid et al., 2001) – thus, the interest in DBS as a therapeutic option re-emerged. In 1987, it was shown that DBS of the thalamus relieved tremors in Parkinson's patients (Benabid et al., 1987). A few years later, the same group showed that DBS of the subthalamic nucleus (STN) ameliorated symptoms in Parkinson's patients (Pollak et al., 1993). Due to its highly effective outcomes and minimal side effects, DBS grew in popularity throughout the 1990s and 2000s (Hariz, 2012). In 1997, the US Food and Drug administration approved DBS for the treatment of essential tremor, with approval for the treatment of Parkinson's disease and dystonia following in 2002 and 2003, respectively.

### ***DBS Mechanism of Action***

Despite years of research, the mechanism of action of DBS remains unclear. General hypotheses in the field suggest that DBS acts via 1) silencing stimulated neurons through depolarization inactivation and/or activation of GABAergic interneurons or 2) activation of associated circuit structures. The first hypothesis is supported from several studies that observed suppressed firing of neuronal populations around the stimulation electrode (Benazzouz and Hallett, 2000; Kiss et al., 2002; Meissner et al., 2005). However, there are credible findings suggesting that DBS may produce local activation (McIntyre et al., 2004; Montgomery and Gale, 2008). Several studies support the second hypothesis that DBS can produce activation of nearby axons and afferent structures (Vitek, 2002; McCracken and Grace, 2007; Johnson et al., 2008; Gradinaru et al., 2009; Vassoler et al., 2013). However, a growing body of evidence suggests that the mechanism of action of DBS is far more complex than the “local inhibition, distant excitation” hypothesis, including affecting multiple neurotransmitter systems (Barat et al.,

2012; Hess et al., 2013; Martinez et al., 2013). It is clear that more research is required to provide additional insight into the potential mechanisms of DBS and potentially improve clinical outcomes.

### ***DBS and Drug Addiction***

The success of DBS in treating movement disorders paved the way for its use as a therapeutic modality in psychiatric disorders. Indeed, DBS is being studied in a number of psychiatric conditions, including obsessive-compulsive disorder, major depression, eating disorders, Tourette's syndrome, and drug addiction (Lozano and Lipsman, 2013). There have been a number of successful case studies that have examined the effects of DBS on drug addiction. In a pilot study of DBS of the accumbens in 5 patients with severe alcohol addiction, all subjects reported complete remission of their craving for alcohol (Müller et al., 2009; 2016). Another case study showed complete remission of heroin abuse by a patient for 6 years. Remarkably, the patient refrained from drug abuse during active stimulation for the first 2.5 years and remained abstinent for 3.5 years even after the stimulation was removed (Zhou et al., 2011). DBS of the nucleus accumbens was also shown to be effective in alleviating symptoms of severe alcohol dependence in one case study (Kuhn et al., 2011) and smoking cessation and weight loss in others (Kuhn et al., 2009; Mantione et al., 2010). In all cases, DBS of the nucleus accumbens produced no unwanted side effects. The majority of the studies have targeted the nucleus accumbens shell, though there is evidence that DBS of the STN is effective in treating drug addiction as well (Rouaud et al., 2010).

Recent preclinical evidence bolsters support for accumbal shell DBS as an effective treatment for drug addiction. DBS of the nucleus accumbens prevented morphine-

conditioned place preference (Liu et al., 2008), attenuated cocaine priming-induced reinstatement of drug seeking (Vassoler et al., 2008; 2013; Hamilton et al., 2015), reduced cocaine sensitization (Creed et al., 2015), attenuated cue-induced reinstatement of drug seeking (Guo et al., 2013; Guercio et al., 2015), decreased alcohol consumption (Knapp et al., 2009; Wilden et al., 2014) and reduced methamphetamine intake (Batra et al., 2016). Taken together, these clinical and preclinical findings suggest that DBS of the accumbens shell may serve as a highly effective treatment for intractable drug addiction.

While DBS may be a potential therapeutic modality in the treatment of cocaine addiction, it does not shed light on the complicated neurobiology underlying the drug addiction and relapse. Discouragingly, despite decades of research, the core pathophysiological mechanism of drug addiction remains unknown. A greater understanding of the neurobiological circuitry and mechanisms underlying drug addiction will help lead to improved pharmacotherapies. Thus, chapter 4 of this dissertation will examine how cellular and molecular changes in the nucleus accumbens contribute to the reinstatement of cocaine seeking by examining the novel role of a protein, AKAP150.

### ***Bridging the Dopamine and Glutamate Systems in the Nucleus Accumbens in Cocaine Reinstatement***

Long-term neural adaptations in both the dopaminergic and glutamatergic systems are involved in the drug-associated learning underlying cocaine reinstatement (Jones and Bonci, 2005; Kauer and Malenka, 2007). Dopaminergic transmission from the VTA to the nucleus accumbens (Schmidt et al., 2005; Shaham and Hope, 2005), the mPFC (McFarland and Kalivas, 2001; Park et al., 2002; Capriles et al., 2003), the ventral

hippocampus (Vorel, 2001; Sun and Rebec, 2003), and BLA (See et al., 2001; Alleweireldt et al., 2006) contribute to the reinstatement of cocaine seeking. While these nuclei all send glutamatergic projections to the nucleus accumbens, certain circuits play more specific roles in various types of cocaine reinstatement (Britt et al., 2012; Bossert et al., 2013). The interaction between the dopamine and glutamate systems in the nucleus accumbens, however, is crucial for the reinstatement of cocaine seeking.

The nucleus accumbens is predominately made up of medium spiny neurons that express either D1-like dopamine receptors or D2-like dopamine receptors (Gangarossa et al., 2013). Both D1-containing and D2-containing medium spiny neurons receive glutamatergic projections from the mPFC, hippocampus, and BLA (Papp et al., 2012; MacAskill et al., 2014), but exhibit different efferent projections (Smith et al., 2013). Dopamine acts to modulate excitatory input to the accumbens from the mPFC, hippocampus, and BLA (Jentsch et al., 2000; Goto and Grace, 2008). The medium spiny neurons in the accumbens integrate information from dopaminergic and glutamatergic inputs to generate an appropriate behavioral response (Papp et al., 2012).

### ***Protein Kinase A (PKA) Signaling in the Nucleus Accumbens in Cocaine***

#### ***Reinstatement***

D1-like dopamine receptor (D1DR) signaling in the nucleus accumbens shell is critical for the reinstatement of cocaine seeking (Anderson et al., 2003; Pierce and Kumaresan, 2006; Schmidt et al., 2006; Schmidt and Pierce, 2006b). D1DRs are Gs-coupled receptors that stimulate the production of cyclic adenosine monophosphate (cAMP), which ultimately lead to the activation of PKA (MISSALE et al., 1998; Beaulieu and Gainetdinov, 2011). Chronic exposure to cocaine leads to increased D1DR signaling, as

well as increased cAMP formation and PKA activity in the nucleus accumbens (Self et al., 1995; Unterwald et al., 1996; Lu et al., 2003; Anderson and Pierce, 2005).

Consistent with these observations, inhibition of PKA in the nucleus accumbens reduces cocaine self-administration (Self et al., 1998) and decreases motivation to obtain cocaine as measured by progressive ratio responding (Lynch and Taylor, 2005). Surprisingly, intra-accumbal core administration of a PKA inhibitor, Rp-cAMP, promoted the reinstatement of cocaine seeking (Self et al., 1998). A potential explanation for this unexpected result is that Rp-cAMPs can also inhibit other cAMP-activated targets, such as exchange factors directly activated by cAMP (Epacs) (Bos, 2006). Epac activation leads to increased levels of the GTPase, Rap, which can interact with the Ras/ERK cascade to modulate ERK-dependent processes (Lin et al., 2003; Johnson-Farley et al., 2005). There is considerable evidence linking ERK activation in the accumbens core with cocaine seeking (Edwards et al., 2011; Fricks-Gleason and Marshall, 2011). In summary, D1DR signaling in the accumbens shell is critical for the reinstatement for cocaine seeking and D1DR stimulation leads to downstream PKA activation. The role of PKA in cocaine reinstatement, however, has not been thoroughly characterized.

### ***GluA1 Phosphorylation and Trafficking in the Nucleus Accumbens in Cocaine Reinstatement***

AMPARs play a major role in cocaine-induced synaptic plasticity and cocaine reinstatement, as detailed above. The carboxy-terminal region of the GluA1 subunit contains all of the known protein phosphorylation sites, including residues phosphorylated by PKA, Protein Kinase C (PKC), and Ca<sup>2+</sup>/calmodulin-dependent protein kinase II (CaMKII) (Derkach et al., 2007; Anggono and Huganir, 2012). PKC and CaMKII can phosphorylate GluA1 at several residues, particularly Serine 831 (Ser831).



Phosphorylation at Ser831 is associated with GluA1 trafficking following cocaine reinstatement (Anderson et al., 2008; Pierce and Wolf, 2013).

PKA phosphorylation of GluA1 at Ser845 leads to increased open probability of AMPARs (Banke et al., 2000). PKA phosphorylation of Ser845 also increases the surface expression of GluA1-containing AMPARs. In cultured accumbal neurons, D1DR stimulation increased both Ser845 phosphorylation and GluA1 surface expression (Chao et al., 2002a; 2002b). Consistent with these findings, cocaine reinstatement was attenuated by intra-accumbal shell administration of AAV10-GluA1-C99, which impairs the trafficking of GluA1-containing AMPA receptors to the cell surface (Anderson et al., 2008). Additionally, withdrawal from cocaine self-administration led to both increased GluA1 surface expression, Ser845 phosphorylation, and increased rectification, which suggests an increase in CP-AMPA receptors (Conrad et al., 2008; McCutcheon et al., 2011b). Furthermore, withdrawal from cocaine self-administration caused an increase in the rectification index specifically in D1- but not D2-containing medium spiny neurons in the accumbens (Pascoli et al., 2014). Together, these findings suggest that D1DR/PKA/AMPA signaling in the nucleus accumbens shell is critically involved in cocaine seeking.

### ***A-Kinase Anchoring Protein 150 (AKAP150) and Neuronal Plasticity***

A-Kinase anchoring proteins (AKAPs) are a family of proteins that contain an  $\alpha$ -helical motif that binds the N-terminus of the PKA-RII subunit. By binding PKA and anchoring it at the synapse, AKAPs facilitate second messenger signaling (Wong and Scott, 2004; Carnegie et al., 2009). While there are many different forms of AKAPs, AKAP150 is the best characterized (Wong and Scott, 2004). AKAP150 is a postsynaptic scaffolding

protein that binds numerous signaling, accessory, receptor, and ion channel proteins involved in long-term synaptic plasticity (Sanderson and Dell'Acqua, 2011). AKAP150 is expressed throughout the forebrain, with highest expression seen in the striatum, including the nucleus accumbens (Glantz et al., 1992; Ostroveanu et al., 2007). AKAP150 is anchored to the plasma membrane and the post-synaptic density (PSD) in dendritic spines via interactions with F-actin, phosphatidylinositol-4,5-bisphosphate (PIP<sub>2</sub>), and cadherin cell adhesion molecules (Dell'Acqua, 1998; Gomez et al., 2002; Gorski et al., 2005). In addition to a PKA binding domain, AKAP150 also contains a membrane-associated guanylate kinase (MAGUK) motif that promotes its interaction with AMPA and NMDA receptors via binding to scaffolding proteins, PSD-95 and SAP97 (Colledge et al., 2000; Sanderson and Dell'Acqua, 2011). Furthermore, AKAP150 enhances PKA-mediated phosphorylation of AMPARs, especially Ser845 on GluA1 subunits (Colledge et al., 2000; Tavalin et al., 2002). AKAP150 is also known to bind PKC (Klauck et al., 1996), and can interact with L-type Ca<sup>2+</sup> channels via interaction with a leucine zipper (LZ) domain (Oliveria et al., 2007). The organization of this postsynaptic assembly suggests that AKAP150 is a critical scaffolding protein that regulates activity-dependent signaling processes at synapses.

There is extensive literature suggesting the importance of AKAP150 in mediating synaptic plasticity and memory formation. Administration of St-Ht31, a cell-permeable peptide that disrupts PKA binding to AKAP (Carr et al., 1992), into the lateral amygdala impairs auditory fear memory (Moita et al., 2002). AKAP150 KO mice exhibit impaired learning of the Morris Water Maze, a spatial memory task. They also show decreased AMPA currents and impaired LTD in the hippocampus (Tunquist et al., 2008). AKAP150-D36 transgenic mice, where the final 36 amino acid residues – which includes the PKA

binding domain – are deleted, exhibit impaired LTP and LTD (Weisenhaus et al., 2010). AKAP150-anchored PKA regulates GluA1 phosphorylation and CP-AMPA synaptic incorporation in NMDA-receptor mediated LTD (Sanderson et al., 2016). In addition, sleep deprived mice exhibiting memory deficits show reduced expression of AKAP150 and reduced AMPA receptor phosphorylation (Hagewoud et al., 2009). Interestingly, many of the cellular and molecular mechanisms underlying learning and memory are also critically involved in neuronal plasticity associated with cocaine addiction (Kauer and Malenka, 2007).

Despite extensive investigation on synaptic plasticity and learning mediated via AKAP150, the role of AKAP150 in drug addiction has been minimally explored. However, recent evidence shows that AKAP signaling is involved in the reinstatement of cocaine seeking (Reissner et al., 2011). Disruption of AKAP-PKA binding in the nucleus accumbens attenuates the reinstatement of cocaine seeking and reduces the post-synaptic density (PSD) content of PKA. AKAP150 is also increased in PSD fractions of the nucleus accumbens from cocaine-treated rats (Reissner et al., 2011). Together, these findings strongly suggest that AKAP150 facilitates PKA-mediated plasticity underlying cocaine seeking.

### **Summary**

As a whole, the work encompassed in this dissertation advances both our understanding of the basic science underlying cocaine relapse as well as uncovers better therapeutic modalities for the treatment of cocaine addiction and relapse.

## Figures

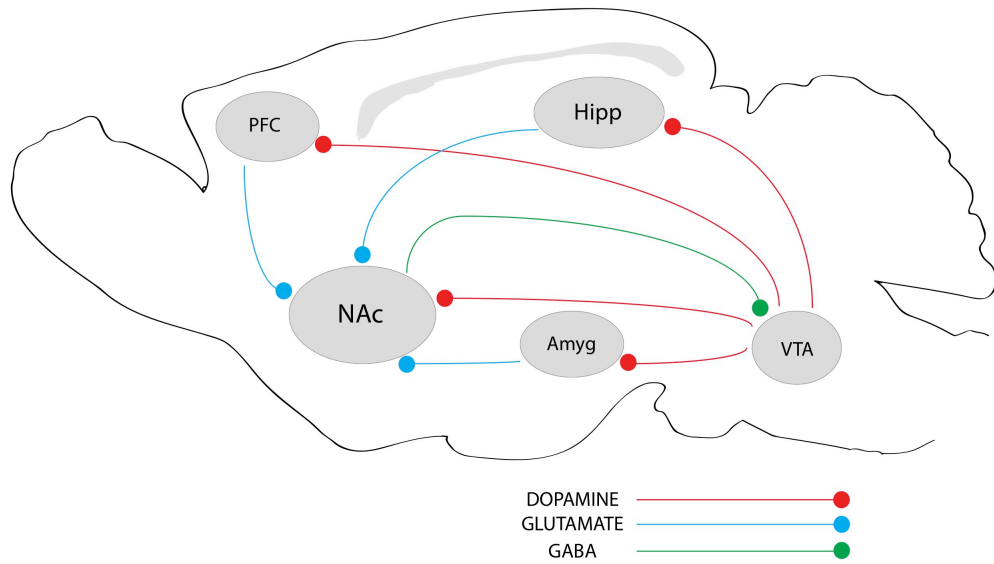


Figure 1. 1 Simplified schematic of the mesocorticolimbic reward system.

## Chapter 2

### Deep Brain Stimulation of the Nucleus Accumbens Shell Attenuates Cue-Induced Reinstatement of Both Cocaine and Sucrose Seeking in Rats

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## **Abstract**

Stimuli previously associated with drug taking can become triggers that can elicit craving and lead to relapse of drug-seeking behavior. Here, we examined the influence of deep brain stimulation (DBS) in the nucleus accumbens shell on cue-induced reinstatement of cocaine seeking, an animal model of relapse. Rats were allowed to self-administer cocaine (0.254 mg, i.v.) for 2 h daily for 21 d, with each infusion of cocaine being paired with a cue light. After 21 d of self-administration, cocaine-taking behavior was extinguished by replacing cocaine with saline in the absence of the cue light. Next, during the reinstatement phase, DBS was administered bilaterally into the nucleus accumbens shell through bipolar stainless steel electrodes immediately prior to re-exposure to cues previously associated with cocaine reinforcement. DBS continued throughout the 2 h reinstatement session. Parallel studies examined the influence of accumbens shell DBS on reinstatement induced by cues previously associated with sucrose reinforcement. Results indicated that DBS of the nucleus accumbens shell significantly attenuated cue-induced reinstatement of cocaine and sucrose seeking. Together, these results indicate that DBS of the accumbens shell disrupts cue-induced reinstatement associated with both a drug and a natural reinforcer.

## **Introduction**

Deep brain stimulation (DBS), originally developed in the 1950s, first achieved recognition in the 1980s as a potential therapeutic intervention for Parkinson's disease and other movement disorders (Lozano and Lipsman, 2013). Due to its highly effective outcomes, reversibility, and minimal side effects, DBS has grown in popularity over the past 25 years (Hariz, 2012; Lozano and Lipsman, 2013), garnering FDA approval for the treatment of several movement disorders.

The success of DBS in treating movement disorders paved the way for its use as a therapeutic modality in psychiatric disorders. Indeed, DBS is being studied in a number of psychiatric conditions, including obsessive-compulsive disorder, major depression, eating disorders, Tourette's syndrome, and drug addiction (Lozano and Lipsman, 2013; Müller et al., 2013). This is primarily due to the belief that DBS is relatively safe, free of unwanted side effects, and in some cases, may even have beneficial effects on attention, learning and memory, and executive function (Grubert et al., 2011; Bossert et al., 2013). Although DBS is highly invasive procedure with a surgical fatality rate estimated at 0.4%, the high costs associated with severe drug addiction have led many to conclude that DBS as a therapeutic intervention is a valuable avenue of research (Wit and Stewart, 1981; Müller et al., 2013).

Recent preclinical and clinical studies suggest that deep brain stimulation (DBS) of the nucleus accumbens, a limbic structure that is critically involved in the reinforcing and reinstating effects of drugs of abuse, may be a possible therapy in the treatment of drug addiction (Müller et al., 2013; Pierce and Vassoler, 2013). In a pilot study of DBS of the accumbens in 5 patients with severe alcohol addiction, all subjects reported complete

remission of their craving for alcohol (Müller et al., 2009). Another case study showed complete remission of heroin abuse by a patient for 6 years. Remarkably, the patient refrained from drug abuse during active stimulation for the first 2.5 years and remained abstinent for 3.5 years even after the stimulation was removed (Zhou et al., 2011). In all cases, DBS of the nucleus accumbens produced no unwanted side effects.

In animal models of addiction, DBS of the nucleus accumbens prevented morphine-conditioned place preference (Liu et al., 2008), attenuated cocaine priming-induced reinstatement of drug seeking (Vassoler et al., 2008; 2013), and decreased alcohol consumption (Knapp et al., 2009). However, although recent work indicates that accumbens DBS attenuated cue-induced reinstatement of heroin seeking (Guo et al., 2013), the influence of DBS on cue-induced reinstatement of cocaine seeking is unknown. Therefore, we examined the effects of DBS in the nucleus accumbens on cue-induced reinstatement of cocaine-seeking as well as sucrose-seeking behavior.



## **Materials and Methods**

*Animals and housing.* Male Sprague-Dawley rats (*Rattus norvegicus*) weighing 250-300g were ordered from Taconic Laboratories (Germantown, NY, USA). Animals were individually housed with food and water available *ad libitum*. Animals in the sucrose reinstatement study received ~25 g chow per day and had water available *ad libitum*. A 12h light/dark cycle (lights on at 7:00 am) was used and all experiments were performed during the light cycle. All experimental procedures were consistent with the ethical guidelines of the U.S. National Institutes of Health and were approved by the University of Pennsylvania Perelman School of Medicine Institutional Animal Care and Use Committee.

*Materials.* All experiments used Med-Associates (East Fairfield, VT, USA) operant chambers equipped with response levers, house light, cue light, pumps for injecting drugs intravenously, and food hoppers for dispensing sucrose pellets. Operant chambers were enclosed within ventilated, sound attenuating chambers.

*Surgery.* Prior to surgery, the rats were injected intraperitoneally with 80 mg/kg ketamine and 12 mg/kg xylazine (Sigma-Aldrich; St. Louis, MO, USA). An indwelling silastic catheter was placed into the right jugular vein (side opposite the heart) and sutured in place. The catheter was then threaded subcutaneously over the shoulder blade and was routed to a mesh backmount platform (CamCaths, UK) that was sutured below the skin between the shoulder blades. Catheters were flushed daily with 0.3 ml of an antibiotic (Timentin, 0.93 mg/ml; Henry Schein, Melville, NY, USA) dissolved in heparinized saline. Catheters were sealed with plastic obturators when not in use.

After catheter implantation, the rats were mounted in a stereotaxic apparatus (Kopf Instruments; Tujunga, CA, USA) and bipolar stainless steel electrodes (Plastics One; Roanoke, VA, USA) were implanted into the nucleus accumbens shell according to the following coordinates, relative to bregma (Paxinos and Watson, 1997): + 1.0 mm anteroposterior (A/P), +/- 3.0 mm mediolateral (M/L), - 7.3 mm dorsoventral (D/V). The stereotaxic arms were set at a 17° angle. Electrodes were cemented in place by affixing dental acrylic to three stainless steel screws fastened to the skull.

*Cocaine self-administration, extinction, and cue-induced reinstatement of drug seeking.*

Following a 7 d recovery period, the rats were placed in operant chambers and were allowed to press a lever for intravenous cocaine infusions (0.254 mg of cocaine dissolved 59 µL of saline) on a fixed ratio 1 (FR1) schedule of reinforcement. Each active lever press resulted in an infusion of cocaine and the drug-paired cue (concurrent illumination of the cue light above the active lever) for 5 s. When stable responding was achieved with the FR1 schedule (i.e., <15% variation in response rates over 3 consecutive days), they were switched to an FR5 schedule. A 20 s timeout period during which responses have no scheduled consequences followed each cocaine infusion. Active lever presses made during the time out were counted but did not result in drug delivery and inactive lever presses were of no consequence. The rats were limited to a maximum of 30 cocaine infusions per daily 2 h self-administration session.

After 21 d of cocaine self-administration, the animals underwent an extinction phase during which cocaine was replaced with saline. Additionally, presses on the active lever no longer produced presentation of the drug-paired cue light. Daily 2 h extinction sessions were conducted until responding was <15% of the response rate maintained by

cocaine self-administration. Following the extinction phase, the ability of re-exposure to the cue light to reinstate drug-seeking behavior was assessed. For the reinstatement test sessions, the FR5 schedule was used where active lever presses produced the light cue that had been presented during self-administration. However, satisfaction of the response requirements for each component resulted in a saline infusion rather than a cocaine infusion. Each reinstatement session was followed by extinction sessions until responding was again <15% of the response rate maintained by cocaine self-administration. All animals underwent 2 reinstatement sessions, counterbalanced with respect to whether stimulation was given.

*Sucrose self-administration, extinction and cue-induced reinstatement of sucrose seeking.* Rats were trained to self-administer 45 mg sucrose pellets (Research Diets; New Brunswick, NJ, USA) using the same procedures described above. After 21 days of daily 1 h food-reinforced operant sessions, rats underwent an extinction phase where responding no longer resulted in food delivery or cue light presentation. After lever pressing decreased to 15% or less of the responding maintained by contingent sucrose reinforcement, animals began reinstatement testing. Reinstatement of sucrose seeking was promoted by presentation of the cue light. For reinstatement, the FR5 schedule was used where active lever presses produced the light cue that had been presented during self-administration. However, satisfaction of the response requirements for each component did not dispense any sucrose pellets. Each 1 h reinstatement session was followed by extinction sessions until responding was again <15% of the response rate maintained by sucrose.

*Deep brain stimulation.* For DBS experiments, we used alternating current with biphasic

symmetrical pulses (60  $\mu$ s pulse width and a 160 Hz frequency) and 150  $\mu$ A of current, parameters that are consistent with previous work in this field (Chang et al., 2003; Mayberg et al., 2005; Vassoler et al., 2013). Immediately before the start of a reinstatement session, 0 or 150  $\mu$ A current was delivered continuously to the bipolar electrodes. The stimulation continued for the duration of the 2 h reinstatement session. In the 0  $\mu$ A condition, the electrodes were attached in the exact same manner as the 150  $\mu$ A condition but DBS was not administered. The 0 and 150  $\mu$ A currents were administered in a counterbalanced fashion across the multiple reinstatement test days.

*Verification of electrode placements.* After the completion of all experiments, the animals were given an overdose of pentobarbital (100 mg/kg) and perfused intracardially with 0.9% saline followed by 10% formalin. The brains were removed and coronal sections (100  $\mu$ m) were taken at the level of the nucleus accumbens with a vibratome (Technical Products International; St. Louis, MO, USA). Animals with electrode placements outside of the areas of interest, or with excessive mechanical damage, were excluded from subsequent data analysis.

*Drugs.* Cocaine hydrochloride was obtained from the National Institute on Drug Abuse (Rockville, MD, USA) and dissolved in bacteriostatic 0.9% saline.

*Statistics.* All reinstatement experiments were analyzed with two-way ANOVAs with repeated measures over reinstatement days. Pairwise analyses were made with Bonferroni post-tests ( $p < 0.05$ ).

## Results

### *DBS of the accumbens shell attenuates cue-induced reinstatement of cocaine seeking*

Following cocaine self-administration and extinction, deep brain stimulation of the nucleus accumbens shell (0 or 150  $\mu$ A) was administered throughout the 2 h cue-induced reinstatement test session. Active lever responses (mean $\pm$ SEM) during the final days of cocaine self-administration and extinction are shown in Figure 1A. Total active and inactive lever responses (mean $\pm$ SEM) from the reinstatement session are presented in Figure 1B. These data were analyzed using a two-way ANOVA (both treatment and lever were within-subject factors), which revealed significant main effects of DBS treatment ( $F_{(1,5)}=45.53$ ,  $p<0.0011$ ) and lever ( $F_{(1,5)}=29.13$ ,  $p<0.0029$ ), as well as a significant interaction between these variables ( $F_{(1,5)}=17.32$ ,  $p<0.0088$ ). Subsequent pairwise analyses indicated that the total active lever responses were significantly different between the 0 and 150  $\mu$ A treatments during cue-induced reinstatement test sessions (Bonferroni,  $p<0.05$ ). The time course data for active lever responding (Figure 1C) were analyzed with a two-way ANOVA (treatment and time were both within-subject), which revealed significant main effects of DBS treatment ( $F_{(1,5)}=45.54$ ,  $p<0.0011$ ) and time ( $F_{(11,55)}=9.474$ ,  $p<0.0001$ ) as well as a significant interaction between these variables ( $F_{(11,55)}=5.115$ ,  $p<0.0001$ ). Subsequent pairwise analyses indicated that the active lever responses between 0 and 150  $\mu$ A treatments were significantly different over the first 10 minutes of the reinstatement session (Bonferroni,  $p<0.001$ ). The electrode placements are shown in Figure 1D ( $n=6$ ). Although inactive lever responding was somewhat lower in the DBS treatment relative to control (Figure 1B), the low number of inactive responses limits the ability of this measure to accurately assess nonspecific rate suppression effects. Therefore, we also assessed the effects of DBS in the nucleus accumbens shell (0 or 150  $\mu$ A) on the reinstatement of sucrose seeking.

*DBS of the accumbens shell attenuates cue-induced reinstatement of sucrose seeking*

In order to determine if the effects of DBS in the accumbens shell were reinforcer specific, we tested the effect of DBS in the accumbens shell on sucrose cue-associated reinstatement. Active lever responses (mean±SEM) during the final days of sucrose self-administration and extinction are shown in Figure 2A. Total active and inactive lever responses (mean±SEM) from the reinstatement session during which DBS was administered to the accumbens shell during cue-induced sucrose reinstatement test sessions are shown in Figure 2B. These data were analyzed with a two-way ANOVA (treatment and lever were within-subject factors), which revealed no effect of DBS treatment ( $F_{(1,5)}=1.836$ ,  $p<0.2334$ ), a significant effect of lever ( $F_{(1,5)}=31.62$ ,  $p<0.0025$ ) and a significant interaction between these variables ( $F_{(1,5)}=9.352$ ,  $p<0.0282$ ). Subsequent pairwise analyses indicated that the total active lever responses between the 0 and 150  $\mu\text{A}$  treatments were significantly different (Bonferroni,  $p<0.05$ ). The time course data for active lever responding (Figure 2C) were analyzed with a two-way ANOVA (treatment and time were within-subject factors), which revealed no significant effect of DBS treatment ( $F_{(1,5)}=5.875$ ,  $p<0.0598$ ), but a significant main effect of time ( $F_{(11,55)}=15.73$ ,  $p<0.0001$ ) as well as a significant interaction between these variables ( $F_{(11,55)}=2.094$ ,  $p<0.0362$ ). Subsequent pairwise analyses indicated that the active lever responses between 0 and 150  $\mu\text{A}$  treatments were significantly different over the first 10 minutes of the reinstatement session (Bonferroni,  $p<0.001$ ). All data from one animal were removed because of highly unusual time course responses in the 0  $\mu\text{A}$  control condition (162 active lever presses between 40 and 60 minutes with no other presses). This is well beyond 2 SDs above the mean for this group. Electrode placements for these studies are shown in Figure 2D (n=6).

## **Discussion**

We have previously shown that DBS of the accumbens shell attenuated cocaine priming-induced reinstatement of drug seeking but had no influence on the reinstatement of sucrose seeking (Vassoler et al., 2013). The current data expand upon these findings and indicate that DBS of the nucleus accumbens shell also attenuates cue-induced reinstatement of cocaine and sucrose seeking.

The role of the accumbens core in cue-induced reinstatement of drug seeking is well established in the literature. Administration of an AMPA/kainate receptor antagonist in the nucleus accumbens core, but not the shell, reduced cue-induced cocaine-seeking behavior (Di Ciano, 2001). Additionally, excitotoxic lesions of the core, but not the shell, markedly attenuated cue-induced reinstatement of cocaine seeking (Ito et al., 2004). Indeed, the accumbens core plays a critical role in cue-induced reinstatement of other drugs of abuse as well, including heroin, amphetamine, and even natural rewards such as sucrose (Di Ciano and Everitt, 2004). While much is known about the accumbens core and its role in cue-induced reinstatement of drug seeking, the role of the accumbens shell in cue-induced reinstatement of drug seeking is much more complex.

Most studies have reported no effect or an attenuated effect in cue-induced reinstatement of drug or food seeking following pharmacological inactivation of the accumbens shell (Fuchs et al., 2004; Bossert et al., 2007; Lin and Pratt, 2014). Others have even demonstrated potentiated cue-induced reinstatement of food seeking following inactivation of the accumbens shell (Floresco et al., 2008). Our results may differ from these studies as DBS has multiple potential mechanisms of action, including, but not limited to, inactivation of target brain regions. Additionally, our findings suggest

that while accumbal shell DBS is effective at attenuating priming-induced reinstatement (Vassoler et al., 2013), it seems to be a questionable treatment for cue-induced reinstatement, as it also attenuated cue-induced reinstatement of sucrose seeking (Figure 2B). Although we show nonspecific behavioral effects of accumbal shell DBS, we do not believe that these effects are due to generalized motor inhibition, as we do not see any attenuation in inactive lever responding in either group. Additionally, we have previously shown that although DBS of the nucleus accumbens shell attenuates the priming-induced reinstatement of cocaine seeking, it has no effect on the priming-induced reinstatement of sucrose seeking (Vassoler et al., 2008). These findings lead us to believe that the nonspecific behavioral effects elicited by accumbal shell DBS are not due to generalized motor inhibition, but rather to the differences between priming-induced and cue-induced reinstatement tasks.

Our present findings show that DBS of the accumbens shell attenuates cue-induced reinstatement of cocaine and sucrose seeking. As previously stated, preclinical and clinical literature suggest that DBS of the accumbens is a promising therapeutic modality in the treatment of addiction, especially due to the lack of unwanted side effects. These findings suggest that while DBS of the nucleus accumbens shell is a promising therapeutic modality for the treatment of severe cocaine addiction, clinical trials should proceed with caution, as there may be nonselective effects of accumbal DBS.



## Figures

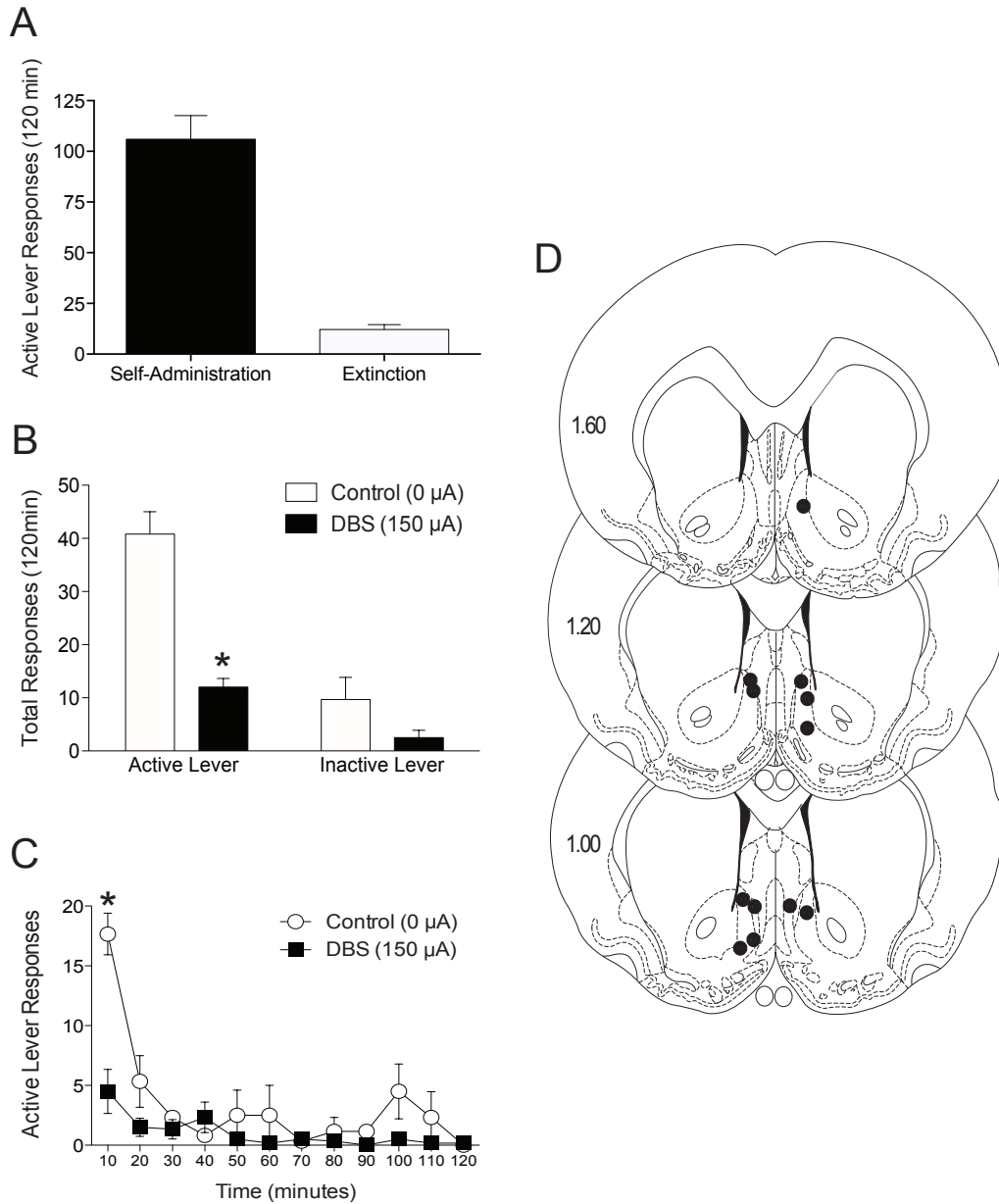


Figure 2. 1 Deep brain stimulation of the nucleus accumbens shell attenuates cue-induced reinstatement of cocaine seeking. (A) Mean ( $\pm$ SEM) active lever responses during the final days of cocaine self-administration and extinction. (B) Mean ( $\pm$ SEM) active and inactive lever responses from reinstatement sessions with 0 or 150  $\mu$ A stimulation aimed at the accumbens shell. (C) Time course of active lever responding from 0 or 150  $\mu$ A stimulation of the accumbens shell. (D) Electrode placements from the shell (dark circles). The values are in millimeters, relative to bregma. \* $p < 0.001$  0  $\mu$ A compared to 150  $\mu$ A. There were 6 animals per group.

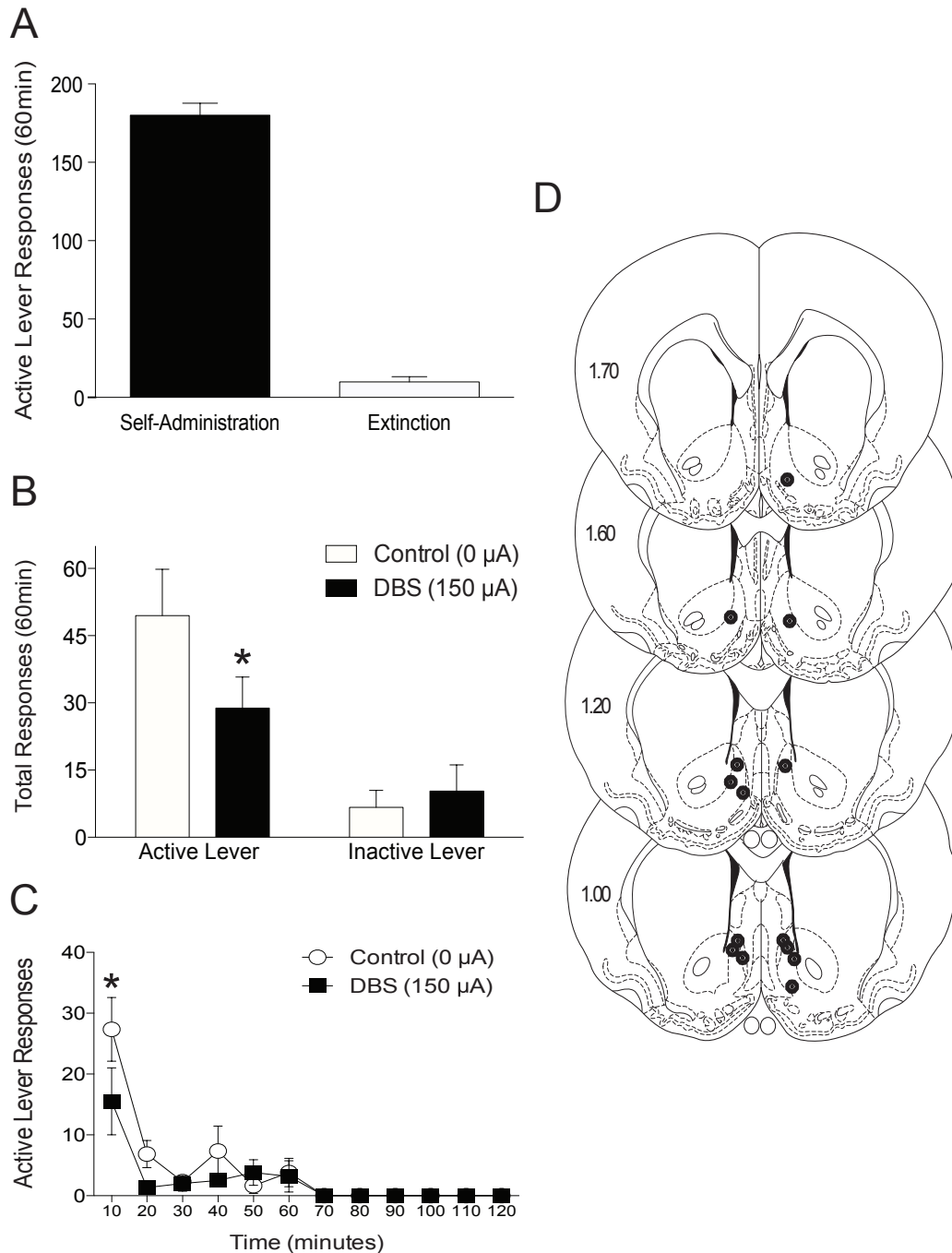


Figure 2. 2 Deep brain stimulation of the nucleus accumbens shell attenuates cue-induced reinstatement of sucrose seeking. (A) Mean ( $\pm$ SEM) active lever responses during the final days of sucrose self-administration and extinction. (B) Mean ( $\pm$ SEM) active and inactive lever responses from reinstatement sessions with 0 or 150  $\mu$ A stimulation aimed at the accumbens shell. (C) Time course of active lever responding from 0 or 150  $\mu$ A stimulation of the accumbens shell. (D) Electrode placements from the shell (dark circles). The values are in millimeters, relative to bregma. \* $p < 0.05$  0  $\mu$ A compared to 150  $\mu$ A. There were 6 animals per group.

### **Chapter 3**

Deep Brain Stimulation of the Infralimbic Medial Prefrontal Cortex Attenuates Cocaine  
Reinstatement Through Inactivation of Cortico-Striatal Projections

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## **Abstract**

Deep brain stimulation DBS is a promising therapeutic modality for the treatment of addiction. To date, most findings have examined the effects of DBS in the nucleus accumbens in drug addiction. Here, we investigate the effects of DBS in brain regions that send robust glutamatergic projections to the nucleus accumbens, specifically the medial prefrontal cortex (mPFC), basolateral amygdala (BLA), and ventral hippocampus (vHipp) on the priming-induced reinstatement of cocaine seeking, an animal model of relapse, in male Sprague Dawley rats. The current results demonstrate that DBS in the infralimbic mPFC, but not the prelimbic or anterior cingulate cortices, attenuates cocaine reinstatement. We also demonstrate that DBS of the BLA and vHipp attenuate the reinstatement of both cocaine and sucrose seeking. To examine potential circuit-wide changes, *zif268* immunohistochemistry was used to examine neuronal activity following DBS of the infralimbic mPFC. We show that infralimbic mPFC DBS is associated with decreased *zif268* immunoreactivity in the nucleus accumbens shell. Our findings suggest that infralimbic mPFC DBS attenuates cocaine reinstatement by disrupting glutamatergic transmission to the nucleus accumbens. These results support previous claims that the mPFC may be an effective target for DBS in the treatment of addiction.

## **Introduction**

Cocaine abuse is a serious public health concern both in the United States and worldwide. In the United States, cocaine is the third most commonly abused illicit drug, after marijuana and prescription opiates, with nearly 1 million regular users (SAMHSA, 2014). Despite years of preclinical and clinical research, pharmacological therapies have met with limited success and there remain no FDA-approved treatments for cocaine addiction and relapse.

Recent evidence shows that deep brain stimulation (DBS), an FDA-approved treatment for movement disorders (Lozano and Lipsman, 2013), may be a viable therapeutic option in the treatment of intractable drug addiction (Müller et al., 2013; Pierce and Vassoler, 2013). To date, the majority of these studies have focused primarily on the nucleus accumbens, a limbic structure that plays a critical role in the reinforcing properties of drugs of abuse, including cocaine. DBS of the nucleus accumbens shell, but not the core, attenuated both the priming- and cue-induced reinstatement of cocaine seeking, animal models of relapse (Vassoler et al., 2008; 2013; Guercio et al., 2015). DBS of the accumbens shell also suppressed locomotor sensitization to cocaine (Creed et al., 2015), another behavioral task that reflects aspects of plasticity related to drug craving (Robinson and Berridge, 2001; Steketee and Kalivas, 2011). However, since DBS is a highly invasive procedure, with a surgical fatality rate of approximate 0.5% (Müller et al., 2013), it is critical to determine if there are other promising target areas with high effectiveness, safety, and feasibility in treating drug addiction. The role of the nucleus accumbens in cocaine reinstatement is well known. However, other mesocorticolimbic nuclei contribute heavily to the reinstatement of cocaine seeking (Schmidt et al., 2005). In particular, the medial prefrontal cortex (mPFC), ventral

hippocampus (vHipp), and basolateral amygdala (BLA) send rich glutamatergic projections to the nucleus accumbens (Phillipson and Griffiths, 1985; Friedman et al., 2002) and are critical for the reinstatement of cocaine seeking (Grimm, 2000; McFarland and Kalivas, 2001; Sun and Rebec, 2003; Schmidt and Pierce, 2010; Lüscher and Malenka, 2011). We sought to determine whether these nuclei could also serve as target regions for DBS in the treatment of cocaine addiction.

The underlying mechanism of DBS remains unclear. We have previously shown that the attenuation of cocaine reinstatement by accumbal shell DBS is not due to inactivation of the medium spiny neurons as intra-accumbal shell infusion of GABA agonists, baclofen and muscimol, or lidocaine, did not mimic the effects seen with DBS (Vassoler et al., 2013). In fact, DBS of the accumbens shell promoted antidromic activation of GABAergic interneurons in the mPFC (Vassoler et al., 2013). This is consistent with electrophysiological evidence indicating that accumbens DBS inhibited spontaneous activity of cortico-accumbal glutamatergic projection neurons, while antidromically stimulating cortical interneurons (McCracken and Grace, 2007). Recent evidence indicated that DBS of the nucleus accumbens leads to decreased extracellular levels of glutamate and increased levels of GABA in rats that had been exposed to morphine (Yan et al., 2013). Another study showed that increases in GABA concentration mediated by accumbal DBS were attenuated by pre-treatment with memantine, an NMDA receptor antagonist (Varatharajan et al., 2015). These results suggest that DBS of glutamatergic projections to the nucleus accumbens may modulate the reinstatement of cocaine seeking.

In the current study, we investigated the effects of DBS in brain regions that send robust glutamatergic projections to the nucleus accumbens, specifically the mPFC, BLA, and vHipp, on the reinstatement of cocaine seeking. Moreover, we examined immediate-early gene activation in the nucleus accumbens to examine potential mechanisms that may contribute to the effects of DBS on cocaine reinstatement. Our results suggest that DBS attenuates cocaine reinstatement by disrupting glutamatergic transmission to the nucleus accumbens.

## **Materials and Methods**

*Animals and housing:* Male Sprague-Dawley rats (*Rattus norvegicus*) weighing 250-300 g were obtained from Taconic Laboratories (Germantown, NY). Rats were individually housed with food and water available *ad libitum*. A 12/12 hr light/dark cycle was used with the lights on at 7:00 a.m. All experimental procedures were performed during the light cycle. All experimental procedures were consistent with the ethical guidelines of the US National Institutes of Health and were approved by the Perelman School of Medicine Institutional Animal Care and Use Committee at the University of Pennsylvania.

*Materials:* All experiments used Med-Associates (East Fairfield, VT) instrumentation enclosed within ventilated, sound attenuating chambers. Each operant conditioning chamber was equipped with response levers, stimulus lights, food pellet dispensers and injection pumps for injecting drugs intravenously.

*Surgery:* Prior to surgery, rats were anesthetized with 80 mg/kg ketamine and 12 mg/kg xylazine. An indwelling silastic catheter was placed into the right jugular vein (side opposite the heart) and sutured in place. The catheter was then threaded subcutaneously over the shoulder blade and was routed to a mesh backmount platform (CamCaths, Cambridge, UK/ Strategic Applications Inc., Libertyville, IL) that was sutured below the skin between the shoulder blades. Catheters were flushed daily with 0.3 ml of an antibiotic (Timentin, 0.93 mg/ml) dissolved in heparinized saline. The catheters were sealed with plastic obturators when not in use. Following catheter implantation, the rats were mounted in a stereotaxic apparatus (Kopf Instruments, CA) and bipolar stainless steel electrodes (Plastics One, Roanoke, VA) were trimmed and implanted into to the basolateral amygdala, ventral hippocampus, infralimbic medial prefrontal cortex,



prelimbic medial prefrontal cortex, or anterior cingulate prefrontal cortex according to the following coordinates, relative to bregma (Paxinos and Watson, 1997): basolateral amygdala: -2.8 mm anteroposterior (A/P),  $\pm 5.0$  mm mediolateral (M/L), -8.5 mm dorsoventral (D/V); ventral hippocampus: -5.5 mm A/P,  $\pm 5.0$  mm M/L, -6.5 mm D/V; infralimbic prefrontal cortex: +2.5 mm A/P,  $\pm 2.0$  mm M/L, -5.39 mm D/V, 21.78° angle; prelimbic prefrontal cortex: +2.5 mm A/P,  $\pm 2.0$  mm M/L, -4.2 mm D/V, 19.5° angle; anterior cingulate prefrontal cortex: +2.5 mm A/P,  $\pm 2.0$  mm M/L, -3.0 mm D/V, 27.8° angle. Electrodes were cemented in place by affixing dental acrylic to three stainless steel screws fastened to the skull.

*Cocaine self-administration and extinction.* Following a 7 d recovery period, rats were placed in operant conditioning chambers and allowed to press a lever for intravenous cocaine infusions (0.254 mg of cocaine, 59  $\mu$ L of saline, infusion over 5 s). Rats initially were trained using a fixed ratio 1 (FR1) schedule of reinforcement. When the animals achieved stable responding with the FR1 schedule (i.e., <15% variation in total presses over 3 consecutive days), they were switched to an FR5 schedule. A 20 s timeout period during which active, drug-paired lever responses had no scheduled consequences followed each cocaine infusion. Each operant chamber was also equipped with an inactive lever. Responses made on the inactive lever had no scheduled consequences. The rats were limited to a maximum of 30 cocaine infusions per daily 2 h self-administration session. After 21 d of daily cocaine self-administration sessions, rats underwent an extinction phase during which cocaine was replaced with 0.9% bacteriostatic saline. Daily 2 h extinction sessions were conducted until active lever responding was <15% of the responses averaged over the last 3 days of cocaine self-administration.

*Sucrose self-administration and reinstatement.* Following a 7 d period for recovery from surgery, separate groups of rats were food restricted (~25 g of daily chow; Harland Teklad, Wilmington, DE) and allowed to self-administer 50 mg Noyes sucrose pellets (Research Diets; New Brunswick, NJ, USA) using the same procedures described above. After 21 d of daily 1 h sucrose-reinforced operant sessions, rats underwent an extinction phase during which active lever responding no longer resulted in sucrose delivery. Daily extinction sessions were continued until active lever responding was <15% of the responses averaged over the last 3 days of sucrose self-administration. Reinstatement of sucrose seeking was promoted by non-contingent administration of one sucrose pellet every 2 min during the first 10 min of the reinstatement test session. Each reinstatement test day was followed by extinction sessions (typically only one or two) until responding was again <15% of the responses achieved during self-administration.

*Deep brain stimulation.* In most DBS experiments (both clinical and preclinical), many parameters are fixed and uniform across studies. We used alternating current with biphasic symmetrical pulses (60  $\mu$ s pulse width and a 160 Hz frequency), parameters that are consistent with previous work from our lab and others (Chang et al., 2003; Mayberg et al., 2005; Vassoler et al., 2013). Stimulation intensities, in contrast, are often varied within and between studies, usually in the range of 50 –200  $\mu$ A (Benazzouz and Hallett, 2000; Chang et al., 2003; Mayberg et al., 2005). We previously reported that 150  $\mu$ A of current is an effective stimulation intensity in our reinstatement paradigm (Vassoler et al., 2008). Immediately before the start of a reinstatement session, 0 or 150  $\mu$ A current was delivered continuously to the bipolar electrodes. The stimulation continued

for the duration of the 2 h reinstatement session. Throughout the 0  $\mu$ A condition, the stimulation tethers were attached in the exact same manner as the 150  $\mu$ A condition. The 0 and 150  $\mu$ A currents were administered in a counterbalanced fashion across the multiple reinstatement test days.

*Immunohistochemistry.* For the IHC experiments, a separate cohort of rats were implanted with electrodes in the infralimbic mPFC and underwent cocaine self-administration and extinction as described above. Immediately prior to the reinstatement testing, rats randomly received either 0 or 150  $\mu$ A current stimulation followed a systemic priming injection of saline or cocaine (10 mg/kg, i.p.). Rats were placed immediately into the operant chambers and responding was recorded for 30 min. Rats were then returned to their home cage for 30 min, since *zif* expression is known to peak ~60 min following a stimulus (Wang, 1998). Rats were then deeply anesthetized with sodium pentobarbital (100 mg/kg) and perfused with 120 mL of ice-cold PBS followed by 60 mL of ice-cold 4% paraformaldehyde (PFA). The brains were subsequently removed and stored in 4% PFA for 24 h, at which point the brains were switched to a 30% sucrose solution in PBS for 72 h. Coronal sections (30  $\mu$ m) were taken using a vibratome in 1% Na azide in PBS and then processed for immunohistochemistry. *Zif* immunoreactivity was detected using a rabbit polyclonal antibody (SantaCruz sc-189, 1:1000). Coronal sections were mounted on electrostatic slides (both conditions, stimulated and not stimulated, were mounted on the same slide) and allowed to dry. They were then washed with PBS and blocked for 1 h in PBS with 0.1% Triton X-100 and 3% normal donkey serum. Following the blocking step, slides were incubated overnight in primary antibody, 0.1% Triton X-100, and 3% normal donkey serum at 4°C. The following day, the slides were washed in PBS and incubated for 2 h at room temperature in a fluorescent secondary antibody (AlexaFluor

488 anti-rabbit, 1:500) and 3% normal donkey serum. The slides were washed for a final time, allowed to air dry, and coverslipped using DPX mountant. Staining was visualized with a confocal microscope.

*Verification of electrode placements.* After the completion of all experiments, the animals were given an overdose of pentobarbital (100 mg/kg) and perfused intracardially with 0.9% saline followed by 10% formalin. The brains were removed and coronal sections (100  $\mu$ m) were taken with a vibratome (Technical Products International; St. Louis, MO, USA). Animals with electrode placements outside of the areas of interest, or with excessive mechanical damage, were excluded from subsequent data analysis.

*Drugs.* Cocaine hydrochloride was obtained from the National Institute on Drug Abuse (Rockville, MD, USA) and dissolved in bacteriostatic 0.9% saline.

*Statistics.* All reinstatement experiments were analyzed with two-way ANOVAs with repeated measures over reinstatement days. Pairwise analyses were made with Bonferroni post-tests ( $p < 0.05$ ).

## Results

### *Deep brain stimulation of the infralimbic, but not prelimbic or anterior cingulate, prefrontal cortex attenuates cocaine reinstatement*

The mPFC has been shown to be critically involved in the reinstatement of cocaine seeking (McFarland and Kalivas, 2001; Park et al., 2002; Stefanik et al., 2013).

However, the effect of mPFC DBS on cocaine seeking has not yet been examined. Total active and inactive lever responding (mean±SEM) from the reinstatement session during which DBS was applied to the infralimbic mPFC are presented in Figure 1A. These data were analyzed using a two-way ANOVA (both treatment and lever were within-subject factors), which revealed no effect of DBS treatment ( $F_{(1,7)} = 2.52$ ,  $p=0.16$ ), a significant main effect of lever responding ( $F_{(1,7)} = 36.91$ ,  $p<0.001$ ), and a significant interaction between these variables ( $F_{(1,7)} = 7.849$ ,  $p<0.05$ ). Subsequent pairwise analyses indicated that the total active lever responses between the 0 and 150  $\mu$ A treatment were significantly different (Bonferroni,  $p<0.05$ ).

In order to determine if the effects of DBS in the infralimbic mPFC were reinforcer-specific, we tested its effects on sucrose reinstatement. Total active and inactive lever responding (mean±SEM) from the reinstatement session during which DBS was applied to the infralimbic mPFC are presented in Figure 1B. These data were analyzed using a two-way ANOVA which revealed no effect of DBS treatment ( $F_{(1,8)} = 0.2339$ ,  $p=0.64$ ), a significant main effect of lever responding ( $F_{(1,8)} = 70.74$ ,  $p<0.0001$ ), and no interaction between these variables ( $F_{(1,8)} = 0.4386$ ,  $p=0.53$ ). The electrode placements for both cocaine and sucrose experiments are shown in Figure 1C.

Total active and inactive lever responding (mean±SEM) from the reinstatement session during which DBS was applied to the prelimbic and anterior cingulate cortices are presented in Figure 2A and 2B, respectively. These data were analyzed using separate two-way ANOVAs. The results of the prelimbic mPFC (Fig 2A) revealed no effect of DBS treatment ( $F_{(1,7)} = 1.779$ ,  $p=0.22$ ), a significant main effect of lever responding ( $F_{(1,7)} = 35.09$ ,  $p<0.001$ ), and no interaction between these variables ( $F_{(1,7)} = 0.7098$ ,  $p=0.43$ ). The results of the anterior cingulate mPFC (Fig 2B) revealed no effect of DBS treatment ( $F_{(1,6)} = 1.099$ ,  $p=0.34$ ), a significant main effect of lever responding ( $F_{(1,6)} = 34.15$ ,  $p<0.01$ ), and no interaction between these variables ( $F_{(1,6)} = 1.874$ ,  $p=0.22$ ). Subsequent pairwise analyses indicated that the total inactive lever responses between the 0 and 150  $\mu$ A treatment were significantly different (Bonferroni,  $p<0.05$ ). The electrode placements for both regions are shown in Figure 2C.

*Deep brain stimulation of the basolateral amygdala attenuates the reinstatement of both cocaine and sucrose seeking*

There is strong evidence for the BLA in the reinstatement of cocaine seeking, as lesions of the BLA attenuated cocaine priming-induced reinstatement (Yun and Fields, 2003). Additionally, optogenetic inhibition of the BLA attenuated cocaine reinstatement (Stefanik and Kalivas, 2013).

Total active and inactive lever responding (mean±SEM) from the reinstatement session during which DBS was applied to the BLA are presented in Figure 3A. These data were analyzed using a two-way ANOVA which revealed a strong trend of DBS treatment ( $F_{(1,6)} = 5.171$ ,  $p=0.06$ ), a significant main effect of lever responding ( $F_{(1,6)} = 88.07$ ,  $p<0.0001$ ), and a significant interaction between these variables ( $F_{(1,6)} = 7.76$ ,  $p<0.05$ ). Subsequent

pairwise analyses indicated that the total active lever responses between the 0 and 150  $\mu$ A treatment were significantly different (Bonferroni,  $p < 0.05$ ).

To determine if the effects of DBS in the BLA were reinforcer-specific, we tested its effects on sucrose reinstatement. Total active and inactive lever responding (mean $\pm$ SEM) from the reinstatement session during which DBS was applied to the BLA are presented in Figure 3B. These data were analyzed using a two-way ANOVA which revealed a significant main effect of DBS treatment ( $F_{(1,5)} = 10.86$ ,  $p < 0.05$ ), a significant main effect of lever responding ( $F_{(1,5)} = 28.69$ ,  $p < 0.01$ ), and a significant interaction between these variables ( $F_{(1,5)} = 26.31$ ,  $p < 0.01$ ). Subsequent pairwise analyses indicated that the total active lever responses between the 0 and 150  $\mu$ A treatment were significantly different (Bonferroni,  $p < 0.05$ ). The electrode placements are shown in Figure 3C.

*Deep brain stimulation of the ventral hippocampus attenuates the reinstatement of both cocaine and sucrose seeking*

The ventral hippocampus has been shown to be critical nucleus in promoting cocaine reinstatement. Inactivation of the ventral hippocampus with lidocaine blocked cocaine priming-induced reinstatement (Sun and Rebec, 2003). Consistent with this, administration of baclofen and muscimol to the ventral hippocampus attenuated cocaine priming-induced reinstatement (Rogers and See, 2007).

Following cocaine self-administration and extinction, DBS of the ventral hippocampus (0 or 150  $\mu$ A) was administered throughout a 2 h cocaine-primed reinstatement session. Total active and inactive lever responding (mean $\pm$ SEM) from the reinstatement session

during which DBS was applied to the ventral hippocampus are presented in Figure 4A. These data were analyzed using a two-way ANOVA which revealed a significant effect of DBS treatment ( $F_{(1,5)} = 9.453$ ,  $p < 0.05$ ), a significant main effect of lever responding ( $F_{(1,5)} = 8.092$ ,  $p < 0.05$ ), and a significant interaction between these variables ( $F_{(1,5)} = 12.83$ ,  $p < 0.05$ ). Subsequent pairwise analyses indicated that the total active lever responses between the 0 and 150  $\mu\text{A}$  treatment were significantly different (Bonferroni,  $p < 0.05$ ).

In order to determine if the effects of DBS in the ventral hippocampus were reinforcer-specific, we tested its effects on sucrose reinstatement. Total active and inactive lever responding (mean  $\pm$  SEM) from the reinstatement session during which DBS was applied to the ventral hippocampus are presented in Figure 4B. These data were analyzed using a two-way ANOVA which revealed no effect of DBS treatment ( $F_{(1,7)} = 3.507$ ,  $p = 0.1033$ ), lever responding ( $F_{(1,7)} = 23.45$ ,  $p = 0.0019$ ), and a significant interaction between these variables ( $F_{(1,7)} = 7.314$ ,  $p = 0.0304$ ). Subsequent pairwise analyses indicated that the total active lever responses between the 0 and 150  $\mu\text{A}$  treatment were significantly different (Bonferroni,  $p < 0.05$ ). The electrode placements are shown in Figure 4C.

*Deep brain stimulation of the infralimbic medial prefrontal cortex reduced neuronal activation in the nucleus accumbens shell*

It has been demonstrated that disruption of cortico-accumbal glutamatergic projections attenuates cocaine seeking (Park et al., 2002; Schmidt and Pierce, 2010). To determine if infralimbic mPFC DBS attenuated activation of glutamatergic projection neurons in the nucleus accumbens shell during cocaine reinstatement, we used immunohistochemistry to determine the levels of the immediate-early gene *zif268* following DBS. Briefly, rats



underwent cocaine self-administration and extinction as described previously.

Subsequently, rats underwent a 30 min reinstatement session and randomly received 0 or 150  $\mu$ A stimulation following a systemic priming challenge injection of either saline or cocaine (10 mg/kg, i.p.).

Total active lever responses (mean  $\pm$  SEM) from the 30 min reinstatement session are shown in Figure 5A. These data were analyzed using a two-way ANOVA (both DBS treatment and challenge were between-subject factors), which revealed significant main effects of DBS treatment ( $F_{(1,4)} = 16.32$ ,  $p < 0.05$ ), challenge injection ( $F_{(1,4)} = 33.94$ ,  $p < 0.01$ ), and a significant interaction between these variables ( $F_{(1,4)} = 15.64$ ,  $p < 0.05$ ). Post hoc analyses showed that total active lever responses were significantly different between Cocaine/Control groups and all other groups (Bonferroni,  $p < 0.05$ )

Total *zif268*-positive cells (mean  $\pm$  SEM) in the nucleus accumbens shell are quantified and shown in Figure 5B. These data were analyzed using a two-way ANOVA, which revealed significant main effects of DBS treatment ( $F_{(1,4)} = 64.57$ ,  $p < 0.01$ ), challenge injection ( $F_{(1,4)} = 62.16$ ,  $p < 0.01$ ), and a significant interaction between these variables ( $F_{(1,4)} = 64.57$ ,  $p < 0.01$ ). Post hoc analyses showed that the amount *zif268*-positive cells were significantly different between Cocaine/Control groups and all other groups (Bonferroni,  $p < 0.01$ ;  $n = 2/\text{group}$ ). Representative images of *zif268* staining are shown in Figure 5C.

## **Discussion**

The mPFC, vHipp, and BLA send rich glutamatergic projections to the nucleus accumbens (Phillipson and Griffiths, 1985; Friedman et al., 2002) and are critical for the reinstatement of cocaine seeking (Grimm, 2000; McFarland and Kalivas, 2001; Sun and Rebec, 2003; Schmidt and Pierce, 2010; Lüscher and Malenka, 2011). In this study, we examine whether these nuclei could also serve as target regions for DBS in the treatment of cocaine addiction. The present results show that DBS of the infralimbic mPFC attenuates the reinstatement of cocaine, but not sucrose, seeking. Further, we show that this effect is region-specific as DBS in the prelimbic mPFC or anterior cingulate cortex does not attenuate cocaine reinstatement. We also show that DBS of the BLA or the vHipp attenuates the reinstatement of both cocaine and sucrose seeking, illustrating possible deficits in natural reward processing and operant responding. Collectively, these results provide evidence that the infralimbic mPFC may be an effective target for DBS in the treatment of intractable drug addiction.

The mPFC can be divided into three functional subregions: the anterior cingulate cortex, the prelimbic cortex, and the infralimbic cortex (Krettek and Price, 1977). Our findings indicate that DBS of the infralimbic mPFC, but not the prelimbic or anterior cingulate cortices, attenuates cocaine reinstatement. Further, we observe an increase in inactive lever responding when DBS is applied to the anterior cingulate cortex, suggesting a generalized disruption in locomotor behavior. Since DBS of the infralimbic mPFC selectively attenuates cocaine, but not sucrose reinstatement, and we see no effect on cocaine reinstatement and generalized disruption in locomotor behavior when DBS is applied to the prelimbic and anterior cingulate cortices, this suggests that the infralimbic

mPFC is the ideal mPFC subregion to target when applying DBS for the treatment of cocaine addiction.

Our findings add to the complex literature surrounding the role of infralimbic-accumbens shell projections in the reinstatement of cocaine seeking. The mPFC subregions have differential glutamatergic projections to the nucleus accumbens. The dorsal mPFC (i.e. anterior cingulate and dorsal prelimbic cortices) projects mainly to the nucleus accumbens core, whereas the ventral mPFC (i.e. ventral prelimbic and infralimbic cortices) projects primarily to the nucleus accumbens shell (Berendse et al., 1992; Wright and Groenewegen, 1995; Ding et al., 2001). The present results are consistent with previous work showing that DBS of the accumbens shell, but not the core, attenuates the reinstatement of cocaine seeking (Vassoler et al., 2013). However, these findings appear at odds with other studies showing that administration of a cocktail of GABA receptor agonists baclofen and muscimol into the core, but not the shell, as well as all 3 subregions of the mPFC attenuates cocaine reinstatement (Vassoler et al., 2013). Moreover, administration of baclofen and muscimol into the infralimbic mPFC reinstates cocaine seeking while microinjections of AMPA into this region attenuates cocaine seeking (Peters et al., 2008). Since multiple neurotransmitter systems in the mPFC are involved in cocaine reinstatement, these conflicting findings emphasize that a single pharmacological manipulation cannot provide definitive conclusions about the role of a single brain region in cocaine reinstatement. Additionally, our results may differ from these pharmacological studies as DBS has multiple potential mechanisms including, but not limited to, inactivation of target nuclei. Though there are wide-ranging findings suggesting that the infralimbic mPFC plays a complex role in drug-seeking behavior (Moorman et al., 2015), the present results add to the growing body of literature

demonstrating a role for infralimbic mPFC-accumbens shell glutamatergic pathway in the reinstatement of drug seeking (Bossert et al., 2012; Vassoler et al., 2013; Willcocks and McNally, 2013).

In this study, we show that DBS of the BLA attenuates the reinstatement of both cocaine and sucrose seeking. The amygdala can be divided into many subnuclei, several of which play an important role in drug-seeking behavior, particularly cue-induced reinstatement of cocaine seeking (Grimm, 2000; McFarland et al., 2004; Fuchs et al., 2005; Mashhoon et al., 2009; Stefanik and Kalivas, 2013). The BLA is also implicated in drug priming-induced reinstatement of cocaine seeking. Lesions of the BLA attenuates cocaine priming-induced reinstatement (Yun and Fields, 2003). Further, antagonism of D1-like and D2-like dopamine receptors, in the BLA attenuates cocaine priming-induced reinstatement (Alleweireldt et al., 2006; Di Ciano, 2008). In addition to reducing drug-seeking behavior, our results also show that DBS of the BLA also attenuates sucrose reinstatement. This is consistent with data suggesting that the BLA plays a complex role in reward-related behaviors. Specifically, lesions of the BLA block conditioned place preference for food (Everitt et al., 1991) as well as impair approach to a conditioned stimulus predictive of sucrose reinforcement (Burns et al., 1993). Other findings show that inactivation of the BLA has no effect on, or even potentiates reinstatement of food seeking (McLaughlin and Floresco, 2007). Further, neurons in the BLA exhibit complex response properties depending on behavioral tasks and outcomes (Carelli et al., 2003; Tye et al., 2008; 2010). Since DBS of the BLA indiscriminately impairs operant responding, these findings suggest that it may not be an effective target for DBS in the treatment of cocaine addiction.

Similar to DBS in the BLA, DBS of the vHipp also attenuates both cocaine and sucrose reinstatement. The hippocampus, a critical region for memory and reward-related behaviors, can be segregated into dorsal and ventral regions (Moser and Moser, 1998). The dorsal hippocampus is critical for spatial memory (Moser et al., 1995), whereas the ventral hippocampus plays more important role in motivated behaviors (Henke, 1990). Like the mPFC and BLA, the vHipp is strongly innervated with dopaminergic projections from the VTA (Gasbarri et al., 1994a; 1994b). Additionally, the vHipp is the major output region of the hippocampus (Groenewegen et al., 1987), with strong projections to the nucleus accumbens, particularly the accumbens shell (Fanselow and Dong, 2010). There is evidence for the role of the vHipp in the reinstatement of cocaine seeking. Inactivation of the vHipp with lidocaine blocks cocaine priming-induced reinstatement (Sun and Rebec, 2003). Consistent with this, administration of baclofen and muscimol to the vHipp attenuates cocaine priming-induced reinstatement (Rogers and See, 2007). However, there is also evidence that modulation of the vHipp can affect other reward-related behaviors. Specifically, stimulation of ghrelin receptors in the vHipp increases *ad libitum* food taking and operant responding for food reward (Kanoski et al., 2013). Additionally, stimulation of glucagon-like peptide-1 (GLP-1) receptors in the vHipp attenuates both *ad libitum* food taking and operant responding for food reward (Hsu et al., 2015). These findings support the literature showing that disrupting activity in the vHipp can impair natural reward processing. For these reasons, the vHipp may not be an appropriate target for DBS in the treatment of cocaine addiction.

We also demonstrate that infralimbic mPFC DBS reduces *zif268* immunoreactivity in the nucleus accumbens shell, suggesting that the reduction in cocaine seeking is likely mediated by inactivation of glutamatergic projection neurons. This is in agreement with

our previous work showing that DBS of the accumbens shell is associated with antidromic activation of GABAergic interneurons in the infralimbic mPFC (Vassoler et al., 2013). Moreover, accumbal DBS inhibits spontaneous activity of prefrontal cortico-accumbal glutamatergic neurons while also stimulating cortical interneurons (McCracken and Grace, 2007). Taken together, these findings suggest that accumbal DBS impairs glutamatergic transmission from the mPFC. Our results expand upon this finding and suggest that infralimbic mPFC DBS attenuates glutamatergic transmission in the accumbens shell to reduce cocaine seeking. This suggests that DBS produces inactivation in the target nucleus, rather than antidromic activation of afferent structures. This is consonant with similar findings in depression showing that DBS in these regions produces similar behavioral outcomes, albeit with distinct patterns of regional activity and functional connectivity (Hamani et al., 2014).

The current findings contribute to the growing body of literature indicating that DBS may be an effective therapeutic intervention in the treatment of cocaine addiction. Moreover, these data support previous claims that the mPFC may be a potentially effective target for DBS in the treatment of addiction (Luigjes et al., 2012). Our preclinical findings strongly suggest that in addition to the nucleus accumbens shell (Vassoler et al., 2008; 2013), the infralimbic mPFC may be another effective target for DBS (present results). Our results also indicate that when considering brain regions to target for DBS as a treatment for cocaine craving, one must take into account the potential of DBS to produce adverse effects on behavior, including general motor impairments, deficits in operant learning, and altered natural reward processing. Further studies should examine in greater detail the circuit-wide influences of both infralimbic mPFC and accumbal shell DBS in cocaine reinstatement in order to optimize their therapeutic strategy.

**Figures**

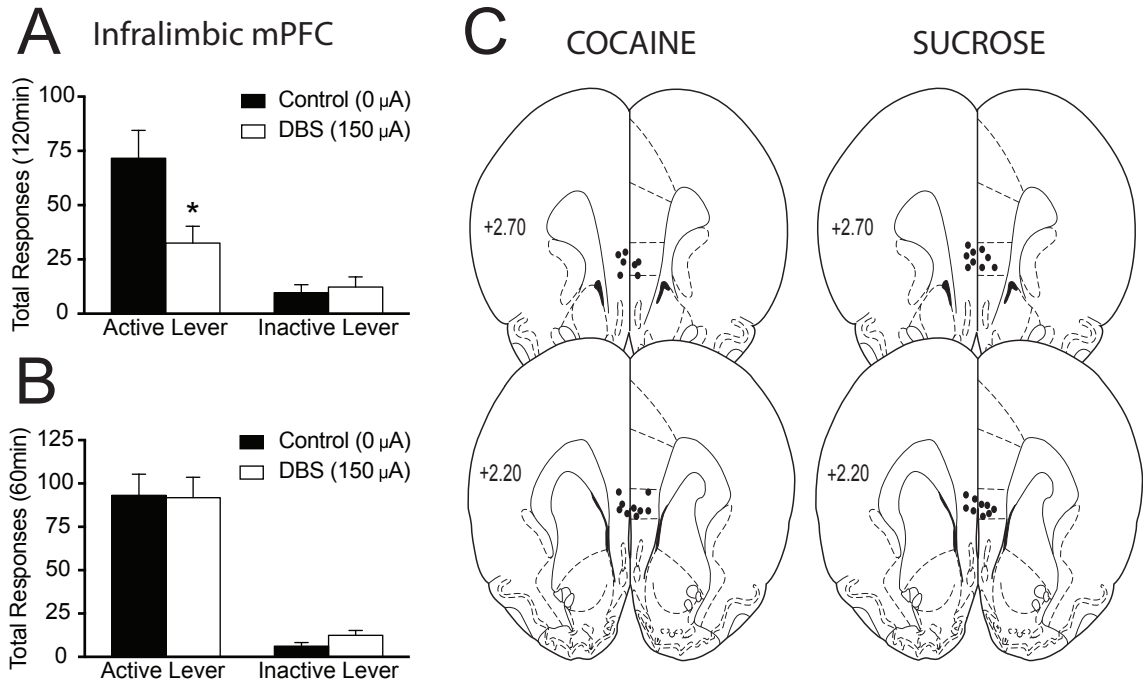


Figure 3. 1 Deep brain stimulation of the infralimbic mPFC attenuates cocaine reinstatement. (A) Mean ( $\pm$ SEM) active and inactive lever responses from cocaine reinstatement session with 0 or 150  $\mu$ A stimulation aimed at the infralimbic mPFC. (B) Mean ( $\pm$ SEM) active and inactive lever responses from sucrose reinstatement session with 0 or 150  $\mu$ A stimulation aimed at the infralimbic mPFC. (C) Electrode placements from the infralimbic mPFC (dark circles). The values are in millimeters, relative to bregma. \* $p < 0.05$  0  $\mu$ A compared to 150  $\mu$ A. There were 8-9 animals per group.

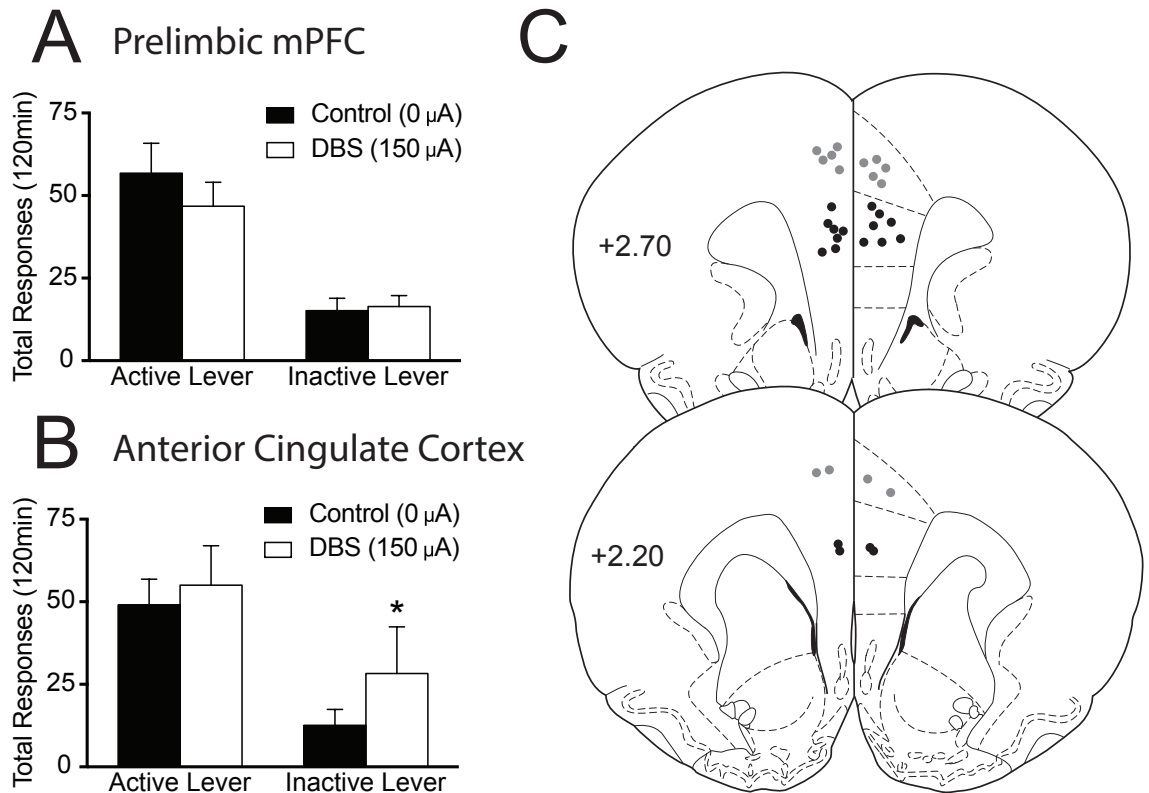


Figure 3. 2 Deep brain stimulation of the prelimbic or anterior cingulate cortices has no effect on cocaine reinstatement. (A) Mean ( $\pm$ SEM) active and inactive lever responses from cocaine reinstatement session with 0 or 150  $\mu$ A stimulation aimed at the prelimbic mPFC. (B) Mean ( $\pm$ SEM) active and inactive lever responses from cocaine reinstatement session with 0 or 150  $\mu$ A stimulation aimed at the anterior cingulate cortex. (C) Electrode placements from the prelimbic mPFC (dark circles) and anterior cingulate cortex (grey circles). The values are in millimeters, relative to bregma. \* $p < 0.05$  0  $\mu$ A compared to 150  $\mu$ A. There were 7-8 animals per group.



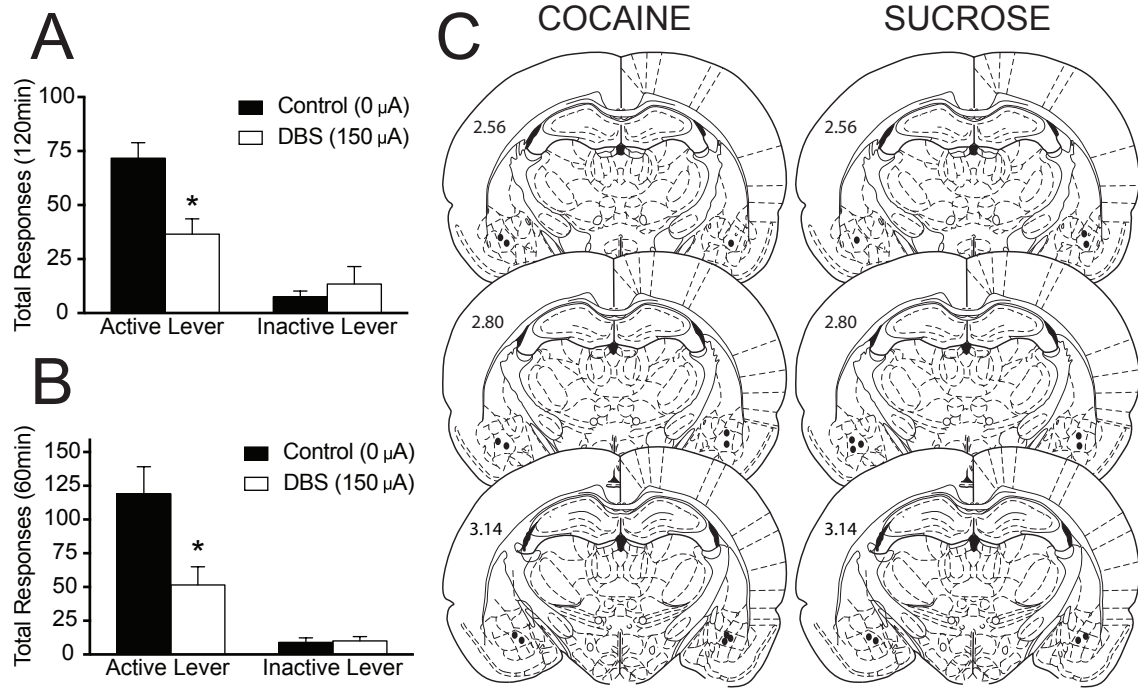


Figure 3. 3 Deep brain stimulation of the basolateral amygdala attenuates both cocaine and sucrose reinstatement. (A) Mean ( $\pm$ SEM) active and inactive lever responses from cocaine reinstatement session with 0 or 150  $\mu$ A stimulation aimed at the BLA. (B) Mean ( $\pm$ SEM) active and inactive lever responses from sucrose reinstatement session with 0 or 150  $\mu$ A stimulation aimed at the BLA. (C) Electrode placements from the BLA (dark circles). The values are in millimeters, relative to bregma. \* $p < 0.05$  0  $\mu$ A compared to 150  $\mu$ A. There were 7 animals per group.

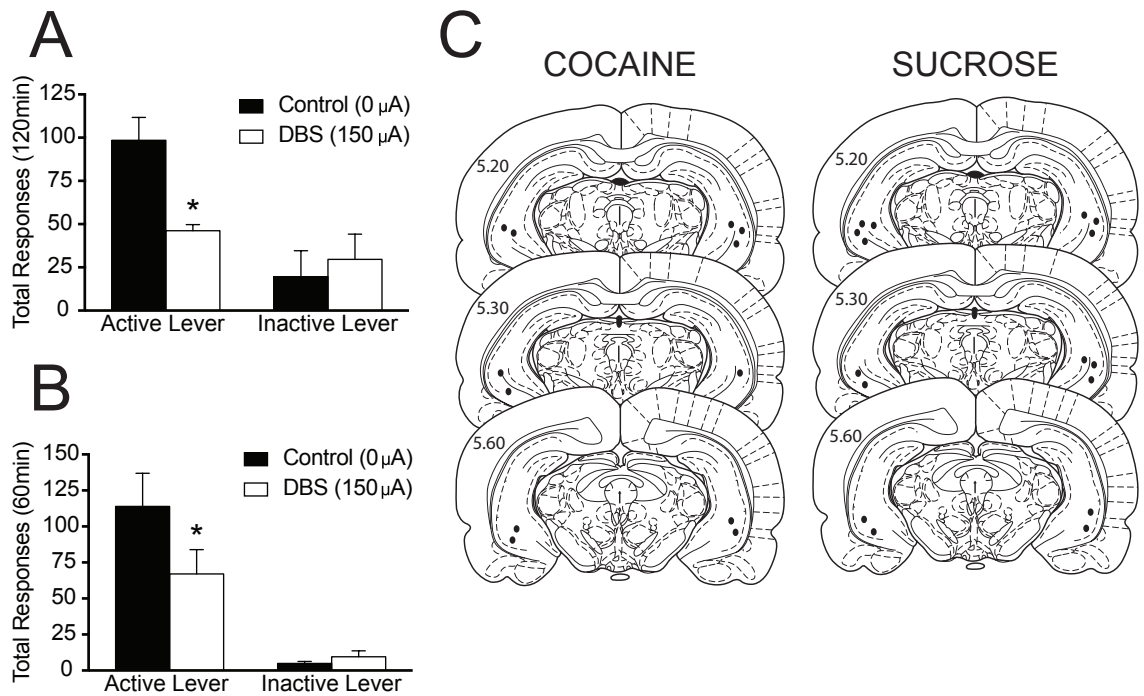


Figure 3. 4 Deep brain stimulation of the ventral hippocampus attenuates both cocaine and sucrose reinstatement. (A) Mean ( $\pm$ SEM) active and inactive lever responses from cocaine reinstatement session with 0 or 150  $\mu$ A stimulation aimed at the vHipp. (B) Mean ( $\pm$ SEM) active and inactive lever responses from sucrose reinstatement session with 0 or 150  $\mu$ A stimulation aimed at the vHipp. (C) Electrode placements from the BLA (dark circles). The values are in millimeters, relative to bregma. \* $p < 0.05$  0  $\mu$ A compared to 150  $\mu$ A. There were 6-8 animals per group.

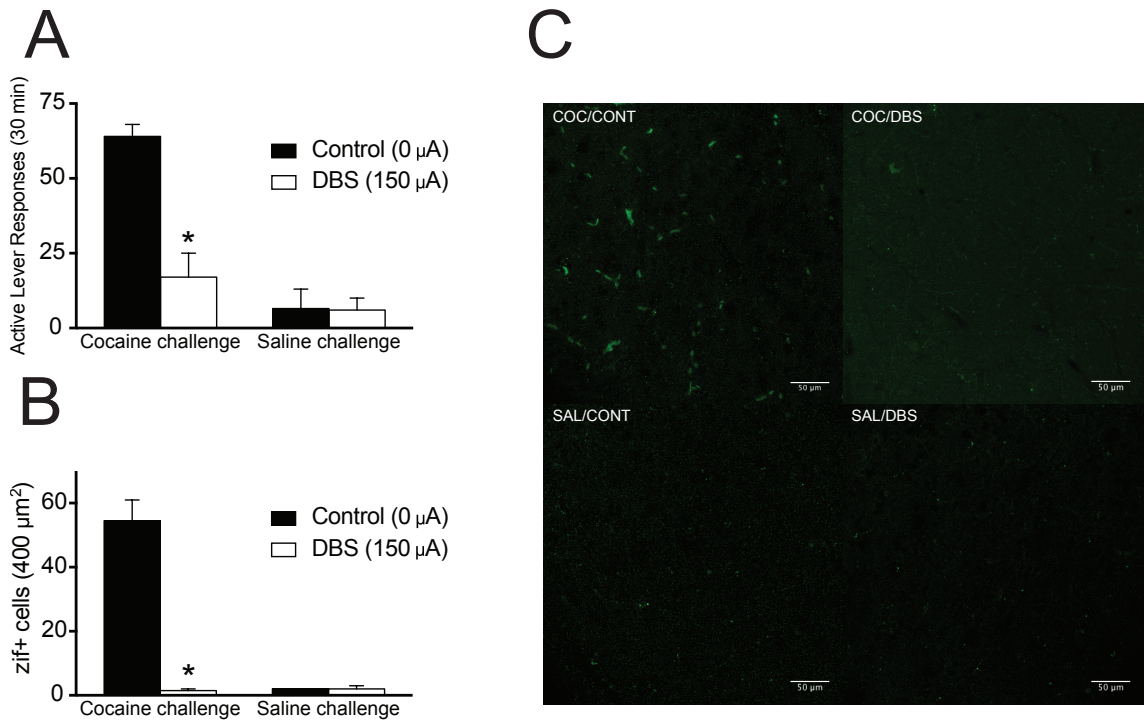


Figure 3. 5 Deep brain stimulation of the infralimbic mPFC is associated with decreased *zif268* immunoreactivity in the nucleus accumbens shell. At the reinstatement test session, all animals received either a saline or cocaine priming injection followed by either 0 or 150  $\mu$ A stimulation. (A) Mean ( $\pm$ SEM) active and inactive lever responses from 30 min reinstatement session. (B) Mean ( $\pm$ SEM) *zif268*-positive cells in the nucleus accumbens shell. (C) Representative images from all conditions. \* $p < 0.05$  0  $\mu$ A compared to 150  $\mu$ A. There were 2 animals per group.

## **Chapter 4**

AKAP150 in the nucleus accumbens shell promotes cocaine reinstatement by increasing transmission through GluA1-containing AMPA receptors

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## **Abstract**

There is a large body of evidence suggesting that many of the cellular and molecular mechanisms that underlie learning and memory are also critically involved in cocaine-induced plasticity. A-Kinase Anchoring Proteins (AKAPs) are scaffolding proteins that localize Protein Kinase A (PKA) to subcellular compartments to facilitate second messenger signaling. Though there are many different isoforms of AKAPs, AKAP150 is the most well-characterized and is highly expressed in the brain. Though the role of AKAP150 in synaptic plasticity, learning, and memory has been well established, its involvement in cocaine addiction has been minimally explored. Here, we examine the role of AKAP150 in the reinstatement of cocaine-seeking behavior, an animal model of relapse. The present results show that blockade of PKA binding to AKAPs in the nucleus accumbens shell of Sprague-Dawley rats attenuates cocaine reinstatement induced by a both priming injection of cocaine and intra-accumbal shell administration of a D1-like dopamine receptor (D1DR) agonist. Moreover, this effect seems to be dependent on the AKAP150 isoform as viral expression of a dominant negative isoform of AKAP150 lacking the PKA binding domain in the accumbens shell also attenuates cocaine reinstatement. This attenuation in cocaine reinstatement is accompanied by decreased phosphorylation of GluA1-containing AMPA receptors (AMPA receptors). Additionally, our findings also reveal decreases in AMPAR eEPSCs after disruption of PKA binding to AKAP150. Collectively, these results support the novel hypothesis that AKAP150 promotes the reinstatement of cocaine-seeking behavior by facilitating D1DR-induced, PKA-mediated phosphorylation of GluA1-containing AMPARs.

## Introduction

Neuroadaptations in both the dopamine and glutamate systems in the nucleus accumbens contribute heavily to the reinstatement of cocaine seeking, an animal model of relapse (Shaham and Hope, 2005; Kauer and Malenka, 2007; Schmidt and Pierce, 2010). It has been shown that stimulation of accumbens shell, but not core, D1-like dopamine receptors (D1DRs) promoted the reinstatement of cocaine seeking (Bachtell et al., 2005; Schmidt et al., 2006; Anderson et al., 2008). Additionally, administration of a D1DR antagonist into the accumbens shell, but not the core, attenuated cocaine reinstatement (Anderson et al., 2003; Bachtell et al., 2005). D1DR stimulation leads to increased cyclic adenosine monophosphate (cAMP) production, ultimately activating protein kinase A (PKA) (MISSALE et al., 1998; Beaulieu and Gainetdinov, 2011). Chronic exposure to cocaine leads to increased D1DR signaling, as well as increased cAMP formation and PKA activity in the nucleus accumbens (Self et al., 1995; Unterwald et al., 1996; Lu et al., 2003), which have been implicated in cocaine self-administration and reinstatement (Self et al., 1998).

One of the intracellular targets of PKA is the AMPA glutamate receptor (AMPA), which plays a major role in cocaine reinstatement (Schmidt and Pierce, 2010). PKA phosphorylation of the GluA1 subunit of AMPARs leads to increased open probability of AMPARs as well as increased surface expression of GluA1-containing AMPARs (Banke et al., 2000; Malinow, 2003). In cultured accumbal neurons, D1DR stimulation increased both GluA1 phosphorylation by PKA and GluA1 surface expression (Chao et al., 2002a; 2002b). Moreover, cocaine reinstatement was attenuated by intra-accumbal shell administration of AAV10-GluA1-C99, which impairs the trafficking of GluA1-containing AMPARs to the cell surface (Anderson et al., 2008). Thus, one of the main mechanisms

underlying cocaine priming-induced reinstatement of drug seeking is the activation of D1DRs in the accumbens shell, which results in PKA-dependent insertion of GluA1-containing AMPARs into synapses.

A-Kinase anchoring proteins (AKAPs) are a family of proteins that bind and localize PKA to distinct subcellular compartments to facilitate second messenger signaling (Wong and Scott, 2004; Carnegie et al., 2009). While there are many different forms of AKAPs, AKAP79/150 (human79/rodent150; also known as AKAP5) is the best characterized (Wong and Scott, 2004). AKAP150 is highly expressed in the brain, particularly the striatum/accumbens, and binds numerous signaling, receptor, accessory, and ion channel proteins involved in long-term synaptic plasticity (Glantz et al., 1992; Ostroveanu et al., 2007; Sanderson and Dell'Acqua, 2011). In particular, AKAP150 localizes PKA to AMPARs via interaction with the scaffolding protein SAP97, leading to enhanced phosphorylation of GluA1 (Colledge et al., 2000; Tavalin et al., 2002). This process was shown to be critical in mediating synaptic plasticity and memory formation. Thus, either total deletion or deletion of the final 36 amino acids of AKAP150, which contains the PKA binding domain, impaired operant learning, spatial memory, hippocampal LTP and LTD, and reduced AMPA currents (Tunquist et al., 2008; Weisenhaus et al., 2010). Additionally, sleep deprived mice exhibiting memory deficits showed reduced AKAP150 expression and reduced AMPAR phosphorylation (Hagewoud et al., 2009). Recent evidence also indicated that AKAP150 expression was increased in the post-synaptic density PSD fractions of the nucleus accumbens of cocaine-treated rats and disruption of AKAP-PKA binding attenuated cocaine reinstatement (Reissner et al., 2011). These findings strongly suggest a potential role for AKAP150 in mediating cocaine addiction and craving.

In the current study, we further investigated the role of AKAP150 in the reinstatement of cocaine seeking. Using biochemical and electrophysiological techniques, we examined the underlying signaling mechanisms by which AKAP150 can affect cocaine reinstatement. Our results demonstrate that AKAP150 facilitates cocaine reinstatement by promoting the D1DR-induced, PKA-mediated phosphorylation of GluA1-containing AMPARs.



## **Materials and Methods**

*Animals and housing:* Male Sprague-Dawley rats (*Rattus norvegicus*) weighing 250-300 g were obtained from Taconic Laboratories (Germantown, NY). Rats were housed individually with food and water available *ad libitum*. A 12/12 hr light/dark cycle was used with the lights on at 7:00 a.m. All experimental procedures were performed during the light cycle. All experimental procedures were consistent with the ethical guidelines of the US National Institutes of Health and were approved by the Perelman School of Medicine Institutional Animal Care and Use Committee at the University of Pennsylvania.

*Materials:* All experiments used Med-Associates (East Fairfield, VT) instrumentation enclosed within ventilated, sound attenuating chambers. Each operant conditioning chamber was equipped with response levers, food pellet dispensers and infusion pumps for injecting drugs intravenously.

*Drugs and Viruses.* Cocaine hydrochloride was obtained from the National Institute on Drug Abuse (Rockville, MD) and dissolved in bacteriostatic 0.9% saline. InCELLect AKAP inhibitor St-Ht31 and control peptide St-Ht31P were purchased from Promega (Madison, WI). D1-like dopamine receptor agonist R-(+)-6-chloro-7,8-dihydroxy-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine hydrobromide (SKF-81297) was purchased from Tocris Bioscience (Minneapolis, MN). SKF-81297 was dissolved in bacteriostatic 0.9% saline.

Herpes simplex virus (HSV) vectors were constructed and packaged at the Viral Gene Transfer Core at the McGovern Institute for Brain Research (MIT, Cambridge, MA, USA) as described previously (Neve et al., 2005). Briefly, AKAP79 $\Delta$ PKA cDNA was inserted

into the HSV amplicon HSV-PrpUC, packaged and resuspended in 10% sucrose. The average titer of the resulting HSV stocks was  $4.0 \times 10^8$  infectious units per ml.

Transgene expression was regulated by HSV IE 4/5 vectors, which produced a transient increase in transgene expression that was maximal between 3 and 4 days after infusion and dissipated completely by 6–7 days (Neve et al., 2005). All viruses were designed to co-express enhanced green fluorescent protein (eGFP) driven by a separate CMV promoter. Control viruses express a scrambled sequence plus eGFP. Control virus expressed an empty vector plus eGFP.

*Surgery:* Prior to surgery, the rats were anesthetized with 80 mg/kg ketamine and 12 mg/kg xylazine. An indwelling silastic catheter was placed into the right jugular vein (side opposite the heart) and sutured in place. The catheter was then threaded subcutaneously over the shoulder blade and was routed to a mesh backmount platform (CamCaths, Cambridge, UK/ Strategic Applications Inc., Libertyville, IL) that was sutured below the skin between the shoulder blades. Catheters were flushed daily with 0.3 ml of an antibiotic (Timentin, 0.93 mg/ml) dissolved in heparinized saline. The catheters were sealed with plastic obturators when not in use. Following catheter implantation, rats were mounted in a stereotaxic apparatus (Kopf Instruments, CA) and stainless steel guide cannula (14 mm, 24 gauge; Small Parts Inc., Roanoke, VA) were implanted dorsal to the nucleus accumbens shell according to the following coordinates, relative to bregma (Paxinos and Watson, 1997): +1.0 mm anteroposterior (A/P),  $\pm 1.0$  mm mediolateral (M/L), -5.0 mm dorsoventral (D/V). Guide cannulae were cemented in place by affixing dental acrylic to three stainless steel screws fastened to the skull. An obturator (33-gauge wire) was inserted into each guide cannula to prevent occlusion.

*Cocaine self-administration and extinction.* Following a 7 d period for recovery from surgery, rats were placed in operant conditioning chambers and allowed to press a lever for intravenous cocaine infusions (0.254 mg of cocaine, 59  $\mu$ L of saline, infusion over 5 s). Rats initially were trained using a fixed ratio 1 (FR1) schedule of reinforcement. When the animals achieved stable responding with the FR1 schedule (i.e., <15% variation in total presses over 3 consecutive days), they were switched to an FR5 schedule. A 20 s timeout period during which active, drug-paired lever responses had no scheduled consequences followed each cocaine infusion. Each operant chamber was also equipped with an inactive lever. Responses made on the inactive lever had no scheduled consequences and served as an operant control. The rats were limited to a maximum of 30 cocaine infusions per daily 2 h self-administration session. After 21 d of daily cocaine self-administration sessions, rats underwent an extinction phase during which cocaine was replaced with 0.9% bacteriostatic saline. Daily 2 h extinction sessions were conducted until active lever responding was <15% of the responses averaged over the last 3 days of cocaine self-administration.

*Sucrose self-administration and extinction.* Following a 7 d period for recovery from surgery, a separate group of rats were food restricted (~25 g of daily chow; Harland Teklad, Wilmington, DE) and allowed to self-administer 50 mg Noyes sucrose pellets (Research Diets; New Brunswick, NJ, USA) using the same procedures described above. After 21 d of daily 1 h sucrose-reinforced operant sessions, rats underwent an extinction phase where active lever responding no longer resulted in sucrose delivery. Extinction of sucrose self-administration was continued until active lever responding decreased to 15% or less of the responses averaged over the last 3 days of sucrose self-administration.

*Cocaine and Sucrose Reinstatement – St-Ht31 Microinjections.* Following the extinction phase, reinstatement was assessed. The obturators were removed from the guide cannulae and 33-gauge stainless steel microinjectors were inserted into the guide cannulae. The microinjectors extended 2 mm below the ventral end of the guide cannulae into the nucleus accumbens shell. To determine the role of AKAP signaling in cocaine reinstatement, bilateral intra-accumbal shell microinfusions of St-Ht31 or control St-Ht31P peptide (0.5  $\mu$ L, 10mM) occurred over 120 s. This dose of St-Ht31 was previously shown to modulate cocaine-seeking behavior (Reissner et al., 2011). Following microinfusions, the microinjectors remained in place for an additional 60 s to allow the solution to diffuse away from the tips of the microinjectors prior to removal. For cocaine reinstatement, 30 min following intra-accumbal shell microinjections, a systemic priming injection of cocaine (10mg/kg, i.p.) was administered immediately prior to a reinstatement test session. During reinstatement, satisfaction of the response requirements for each component resulted in a saline infusion rather than a cocaine infusion. For sucrose reinstatement, the experimenter remotely administered one sucrose pellet every 2 min for the first 10 min of the reinstatement test session. For sucrose reinstatement, active lever presses had no scheduled consequences. For both cocaine and sucrose reinstatement, each reinstatement session was followed by extinction sessions (typically only one or two) until responding was <15% of the response rate maintained by self-administration. The FR5 schedule was used throughout extinction and reinstatement. St-Ht31 or St-Ht31P were administered in the reinstatement session in a counterbalanced fashion.

In a separate experiment, cocaine reinstatement was induced by intra-accumbal shell

microinfusions of the selective D1-like dopamine receptor (D1DR) agonist SKF-81297 as described previously (Schmidt et al., 2006). To determine whether AKAPs mediate D1DR agonist-induced reinstatement of cocaine seeking, St-Ht31 or control St-Ht31P (0.5  $\mu$ L, 10mM) was microinjected into the shell 30 min before intra-accumbal shell infusions of SKF-81297 (0.5  $\mu$ L).

*Cocaine and Sucrose Reinstatement – Viral Vectors.* In these experiments, rats underwent daily extinction sessions until active lever responding was ~20% of the responses averaged over the final 3 d of self-administration. Rats then received bilateral intra-accumbal shell microinfusions of either HSV-GFP or HSV-AKAP79 $\Delta$ PKA over 10 min for a total volume of 2  $\mu$ L per hemisphere. Following the microinfusions, the microinjectors were left in place for an additional 120 s to allow the solution to diffuse away from the tips. Rats continued to undergo extinction for 3 days for peak viral expression prior to reinstatement testing. Cocaine and sucrose reinstatement were induced as described above.

*Western Blotting.* Rats underwent cocaine reinstatement with viral pretreatments as described above. Immediately prior to the reinstatement testing, animals randomly received a systemic priming injection of saline or cocaine (10 mg/kg, i.p.). Rats were placed immediately into the operant chambers following injection of saline or cocaine. Responding was recorded for 30 min, after which the pairs of rats were removed from the operant chambers and immediately decapitated. Whole brains were extracted and flash-frozen in isopentane on dry ice, then stored at -80°C. Brains were sliced on a cryostat and the nucleus accumbens shell was dissected by tissue punch (2.0 mm Harris Unicore stainless steel punchers, Ted Pella, Inc.). Tissue samples were stored at -80°C

until processing for Western blotting. Tissue was processed for Western Blotting as described previously (Anderson et al., 2008). For all samples, protein concentration was quantified using a Pierce BCA Protein Assay Kit (Thermo Fisher Scientific). Equal amounts of protein (10–20µg) were loaded and separated in 10% Tris-Glycine gels (Life Technologies, Grand Island, NY) by SDS-PAGE, then transferred to nitrocellulose membranes using the iBlot dry transfer system (Life Technologies), which were then preblocked with Tris-buffered saline containing 0.1% Tween 20 (TBST) and 5% bovine serum albumin for 1 h before overnight incubation with the following primary antibodies: pSer845-GluA1 (1:1000, Millipore #04-1073), total GluA1 (1:1000, Millipore #MAB2263), PKA-RII (1:1000, Santa Cruz #sc-909). Membranes were concurrently incubated with mouse monoclonal anti-GAPDH (1:2000, Cell Signaling #2118) as a loading control. Primary antibody incubation was followed by three washes [10 min each with rocking, room temperature (RT)] in Tris-buffered saline containing 0.1% Tween 20. Membranes were then incubated for 1 h at RT with secondary antibodies (IRDye 800 goat anti-mouse and IRDye 680 goat anti-rabbit, 1:5000) in Odyssey blocking buffer 0.05% Tween 20 (LI-COR Biosciences). Antibody/protein complexes were visualized using the Odyssey IR imaging system (Li-Cor Biosciences). For pSer845 and GluA1 analysis, gels were transferred to nitrocellulose membranes for 1 hour at 100V using SDS Running Buffer (Bio-Rad) and immunoprobed in TBST with 5% milk (Bio-Rad). HRP conjugated secondary antibody dilutions were 1:10,000 (Bio-Rad). Bands were visualized using the myECL Imager (ThermoFisher Scientific). Band intensities were quantified using either the Odyssey or mECL Imaging software. For data analysis, all bands were normalized to GAPDH and divided by the mean of the control group. The ratio of phosphorylated to native protein was then calculated.

*Electrophysiology.* A separate cohort of rats underwent cocaine reinstatement with viral pretreatments as described above. Immediately after a 30 min reinstatement session, rats were decapitated and brain slices prepared as described previously (Ortinski et al., 2013). In brief, the brain was removed and coronal slices (300  $\mu$ m) containing the nucleus accumbens were cut with a Vibratome (VT1000S, Leica Microsystems) in ice-cold dissection artificial cerebrospinal fluid solution (aCSF), in which NaCl was replaced by an equiosmolar concentration of sucrose. Dissecting aCSF consisted of 219 mM sucrose, 2.5 mM KCl, 1.2 mM  $\text{NaH}_2\text{PO}_4$ , 26 mM  $\text{NaHCO}_3$ , 10 mM glucose, 2.5 mM  $\text{MgSO}_4$ , and 1 mM  $\text{CaCl}_2$  (pH 7.2–7.4 when saturated with 95%  $\text{O}_2$ /5%  $\text{CO}_2$ ). Slices were incubated at 32–35°C for 30 min in recording aCSF and then maintained at room temperature. Recording aCSF consisted of (in mM): 219 NaCl, 2.3 KCl, 1.25 mM  $\text{NaH}_2\text{PO}_4$ , 26  $\text{NaHCO}_3$ , 10 glucose, 1  $\text{MgCl}_2$ , 2  $\text{CaCl}_2$ . Prior to experiments, slices were transferred to a recording chamber continuously perfused with oxygenated aCSF at 1.8–2 mL/min and heated to 30°C. Slices were viewed using infrared differential interference contrast optics under an upright microscope (Eclipse FN1, Nikon Instruments) with a 40X water immersion objective. Whole-cell recordings were obtained using borosilicate glass pipettes filled with intracellular solution containing: 95 mM  $\text{CsCH}_3\text{O}_3\text{S}$ , 55 mM CsCl, 0.2 mM EGTA, 10 mM HEPES, 1 mM  $\text{MgCl}_2$ , 2 mM  $\text{Na}_2\text{-ATP}$ , 0.3 mM Na-GTP, 5 mM QX-314, 0.1mM spermine, adjusted to pH 7.2–7.3 with CsOH (osmolarity 280–290 mOsm). All experiments were performed in the presence of 100  $\mu$ M picrotoxin (to block  $\text{GABA}_A$ ) and 50  $\mu$ M APV (to block NMDA). Recordings were conducted with a MultiClamp 700B, Digidata 1440A, and pClamp software (Molecular Devices) while acquiring data at 20 kHz and low pass filtered at 2 kHz. Access resistance was continuously monitored throughout the experiments and cells were discarded if the access resistance changed by >25%. To avoid recording from damaged cells, neurons

within 50  $\mu\text{m}$  of the injection cannula track were excluded from the analysis. There were no differences on any of the measures between GFP- negative cells in slices exposed to HSV-AKAP79 $\Delta$ PKA and slices exposed to HSV-GFP. Therefore, these cells were pooled for analysis.

A bipolar tungsten stimulation electrode connected to a stimulus isolator (ISO-Flex) was typically positioned dorsal to the recorded neuron to trigger evoked EPSCs (eEPSCs). Stimulation occurred at 0.1 Hz a minimal stimulation intensity required to evoke a consistent post-synaptic response was determined. Peak eEPSC amplitudes were then measured at double and triple the minimal stimulation intensity to construct the input/output curves. Average responses were then calculated based on 5–10 eEPSCs at each stimulation intensity.

*Verification of cannula placements.* After the completion of all experiments, rats were given an overdose of pentobarbital (100 mg/kg, i.p.), brains were removed and stored in 10% formalin for at least 1 week. Coronal sections (100  $\mu\text{m}$ ) were taken at the level of the nucleus accumbens with a vibratome (Technical Products International; St. Louis, MO, USA). The sections were mounted on gelatin-coated slides. Animals with cannula placements located outside of the accumbens shell, or with excessive mechanical damage, were excluded from subsequent data analysis.

*Viral expression.* To ascertain viral expression, we injected (2  $\mu\text{L}$ /hemisphere) HSV-AKAP79 $\Delta$ PKA into the nucleus accumbens shell of separate, drug-naive rats. At 3 days after injection, animals received 100 mg/kg pentobarbital (i.p.) before perfusion with 120 mL ice-cold PBS followed by 60 mL 4% PFA dissolved in ice-cold PBS. Brains were removed and placed in 4% PFA for 24 h before storage in 30% sucrose dissolved in



PBS with 1% sodium azide. Coronal sections (30  $\mu\text{m}$ ) were taken using a vibratome (Technical Products International; St. Louis, MO, USA) and mounted directly onto polarized glass slides. Dry slides were washed in 1X PBS, and then blocked for 1 h in 0.1% triton and 3% normal donkey serum in 1X PBS. We then added primary antibody (Anti-GFP, 1:500, Millipore #MAB3580; Anti-AKAP79, 1:500, Santa Cruz #sc-17772) — diluted 1:1000 in 0.1% triton + 3% donkey serum in PBS to the slides and incubated overnight at 4°C. The next day, the slides were washed in 1X PBS before incubation in secondary fluorescent antibody at room temperature for 2 h (Alexa Fluor 488, 1:500; Alexa Fluor 562, 1:500; Jackson ImmunoResearch, West Grove, PA). After 2 h, slides were washed in 1X PBS before being cover-slipped using Vectashield mounting medium (Vector Laboratories, Burlingame, CA) and imaged for GFP expression using fluorescent microscopy

*Statistics.* All reinstatement experiments were analyzed with two-way ANOVAs with repeated measures over reinstatement test days. Pairwise analyses were made with appropriate post-hoc tests ( $p < 0.05$ ).

## Results

### *AKAP signaling is required for the reinstatement of cocaine, but not sucrose seeking*

AKAP signaling in the nucleus accumbens core has been implicated in the reinstatement of cocaine seeking (Reissner et al., 2011). To confirm if these effects are also seen in the accumbens shell, we microinjected a cell-permeable inhibitory peptide (St-Ht31) that disrupts the binding of PKA to all AKAP isoforms into the nucleus accumbens shell of rats that had previously self-administered cocaine and reinstated them with an acute priming injection of cocaine (10 mg/kg, i.p.). Total active and inactive lever responses (mean±SEM) are shown in Figure 1A (n = 9). These data were analyzed using a two-way ANOVA (both treatment and lever were within-subject factors), which revealed significant main effects of treatment ( $F_{(1,8)} = 34.46$ ,  $p < 0.001$ ), lever responding ( $F_{(1,8)} = 49.62$ ,  $p < 0.001$ ), and a significant interaction between these variables ( $F_{(1,8)} = 26.05$ ,  $p < 0.001$ ). Subsequent pairwise analyses indicated that the total active lever responses between the St-Ht31 and St-Ht31P treatments were significantly different (Bonferroni,  $p < 0.0001$ ).

Previous evidence suggests that intra-accumbal infusions of St-Ht31 do not affect cocaine-induced locomotor activity (Reissner et al., 2011). However, AKAP150 mutant mice lacking the PKA binding domain exhibit, among other things, deficits in operant learning behavior (Weisenhaus et al., 2010). To determine if the effects of St-Ht31 impair operant learning generally, we tested its effects on sucrose reinstatement. Total active and inactive lever responses (mean±SEM) for rats pretreated with intra-accumbal shell microinfusions of either St-Ht31 or St-Ht31P prior to reinstatement of sucrose-seeking behavior are shown in Figure 1B (n = 8). These data were analyzed using a two-way ANOVA which revealed no effect of treatment ( $F_{(1,7)} = 1.726$ ,  $p = 0.23$ ), a significant effect

of lever responding ( $F_{(1,7)} = 50.74$ ,  $p < 0.001$ ), and no interaction between these variables ( $F_{(1,7)} = 1.472$ ,  $p = 0.26$ ). The cannula placements are shown in Figure 1C.

*AKAP signaling is required for D1DR agonist-induced reinstatement of cocaine seeking*

Stimulation of D1DRs in the accumbens shell promotes the reinstatement of cocaine seeking (Bachtell et al., 2005; Schmidt et al., 2006), an effect likely due to PKA activation (Self et al., 1998). To determine if AKAPs are required for D1DR-stimulated reinstatement of cocaine seeking, rats were pretreated with microinfusions of either St-Ht31 or St-Ht31P into the nucleus accumbens shell prior to intra-accumbal shell injections of SKF-81297. Total active and inactive lever responses (mean $\pm$ SEM) are shown in Figure 2A ( $n = 5$ ). These data were analyzed using a two-way ANOVA which revealed significant effects of treatment ( $F_{(1,4)} = 31.4$ ,  $p < 0.01$ ), lever responding ( $F_{(1,4)} = 13.48$ ,  $p < 0.05$ ), and a significant interaction between these variables ( $F_{(1,4)} = 33.57$ ,  $p < 0.01$ ). Subsequent pairwise analyses indicated that the total active lever responses between the St-Ht31 and St-Ht31P treatments were significantly different (Bonferroni,  $p < 0.01$ ). Cannula placements are shown in Figure 2B.

*Expression of HSV-AKAP79 $\Delta$ PKA in the accumbens shell attenuates the reinstatement of cocaine, but not sucrose seeking*

Although there are over 50 different isoforms of AKAPs, AKAP150 is perhaps the best characterized (Wong and Scott, 2004; Sanderson and Dell'Acqua, 2011). AKAP150 is highly expressed in the striatum and plays a critical role in learning behaviors (Ostroveanu et al., 2007; Tunquist et al., 2008; Weisenhaus et al., 2010). Additionally, recent evidence suggests that AKAP150 expression is upregulated in the nucleus accumbens after cocaine self-administration and extinction (Reissner et al., 2011).

We sought to examine the role of AKAP150 in the reinstatement of cocaine seeking behavior. Following self-administration and extinction, we expressed a humanized dominant negative isoform of AKAP150 lacking the PKA binding domain and co-expressing GFP (HSV-AKAP79 $\Delta$ PKA) in the accumbens shell. As outlined in experimental timeline illustrated in Figure 3A, the reinstatement session occurred 3 days following viral microinjections (HSV-eGFP vs. HSV-AKAP79 $\Delta$ PKA), at peak HSV expression (Neve et al., 2005) (Figure 3B). Total active and inactive lever responses (mean $\pm$ SEM) from the cocaine reinstatement session are shown in Figure 3C (n = 10-13/group). These data were analyzed using a two-way ANOVA (virus treatment was a between-subjects factor and lever a within-subject factor), which revealed no effect of virus treatment ( $F_{(1,20)} = 3.166$ ,  $p=0.09$ ), a significant effect of lever responding ( $F_{(1,20)} = 111.7$ ,  $p<0.0001$ ), and a significant interaction between these variables ( $F_{(1,20)} = 8.423$ ,  $p<0.01$ ). Subsequent pairwise analyses indicated that the total active lever responses between the HSV-eGFP and HSV-AKAP79 $\Delta$ PKA treatments were significantly different (Bonferroni,  $p<0.01$ ).

In order to determine if the disruption of PKA binding to AKAP150 affects general operant behavior, we tested the effects intra-accumbal shell injections of HSV-AKAP79 $\Delta$ PKA on sucrose reinstatement. Total active and inactive lever responding (mean $\pm$ SEM) from the sucrose reinstatement session are presented in Figure 3D (n = 7/group). These data were analyzed using a two-way ANOVA which revealed no effect of virus treatment ( $F_{(1,12)} = 0.01814$ ,  $p=0.89$ ), a significant main effect of lever responding ( $F_{(1,12)} = 26.99$ ,  $p<0.001$ ), and no interaction between these variables ( $F_{(1,12)} = 0.01413$ ,  $p=0.91$ ). The cannula placements are shown in Figure 3E.

*Expression of HSV-AKAP79 $\Delta$ PKA in the accumbens shell attenuates PKA-mediated phosphorylation of GluA1 Ser845*

Previous work showed that intra-accumbal injections of St-Ht31 attenuated GluA1 surface expression (Reissner et al., 2011). Since it is well-established that open probability and surface expression of GluA1 are regulated by PKA phosphorylation (Malinow, 2003), we examined the phosphorylation state of Ser845, a PKA phosphorylation site on GluA1 AMPAR subunits, following intra-accumbal shell administration of HSV-AKAP79 $\Delta$ PKA.

Total active lever responses (mean  $\pm$  SEM) from the 30 min reinstatement session are shown in Figure 4A. These data were analyzed using a two-way ANOVA (both virus and treatment were between-subject factors), which revealed significant main effects of virus ( $F_{(1,36)} = 16.29$ ,  $p < 0.001$ ), treatment ( $F_{(1,36)} = 47.31$ ,  $p < 0.0001$ ), and a significant interaction between these variables ( $F_{(1,36)} = 22.06$ ,  $p < 0.0001$ ). Post hoc analyses showed that total active lever responses were significantly different between GFP/Coc groups and all other groups (Tukey,  $p < 0.0001$ ;  $n = 9-11$ /group).

The average intensity for pSer845 in the nucleus accumbens shell was expressed as percent change from control and is shown in Figure 4B. Percentages were analyzed by two-way ANOVA (both virus and treatment were between-subject factors), which revealed no effect of virus ( $F_{(1,36)} = 2.397$ ,  $p = 0.13$ ), no effect of treatment ( $F_{(1,36)} = 3.384$ ,  $p = 0.07$ ), but a significant interaction between these variables ( $F_{(1,36)} = 4.332$ ,  $p < 0.05$ ). *Post hoc* analyses showed that the GFP/Coc group was significantly different from GFP/Sal and AKAP/Coc groups (Tukey,  $p < 0.05$ ;  $n = 9-11$ /group).

Since the AKAP79/150  $\alpha$ -helical motif binds to the PKA-RII regulatory subunit dimer near the AKAP C-terminus (Sanderson and Dell'Acqua, 2011), we wanted to be sure that deletion of this anchoring domain did not affect overall expression of the PKA-RII subunit in the accumbens shell. The average intensity for PKA RII in the nucleus accumbens shell was expressed as percent change from control and is shown in Figure 4C (n = 7/group). Percentages were analyzed by two-way ANOVA (both virus and treatment were between- subject factors), which revealed no effects of virus ( $F_{(1,24)} = 0.3518$ ,  $p=0.56$ ), treatment ( $F_{(1,24)} = 0.1112$ ,  $p=0.74$ ), or interaction between these variables ( $F_{(1,24)} = 0.00374$ ,  $p=0.95$ ).

*Expression of HSV-AKAP79 $\Delta$ PKA in the accumbens shell attenuates AMPAR currents*

Given our biochemical findings that intra-accumbal shell administration of HSV-AKAP79 $\Delta$ PKA attenuated phosphorylation of GluA1-Ser845, we sought to examine the effects on AMPAR eEPSCs after reinstatement. Representative traces of eEPSCs from AKAP+ and AKAP- cells are shown in Figure 5A. Quantification of this experiment is depicted in Figure 5B. These data were analyzed with a two-sample t-test, which revealed a significant difference in the size of the AMPA eEPSCs between the two groups at 2x intensity (AKAP+: n = 9; AKAP-: n = 9;  $p=0.008$ ) and 3x intensity ( $p = 0.03$ ). The rectification index was also assessed in both AKAP+ and AKAP- cells, which revealed no significant differences between the two groups ( $p = 0.18$ , two- sample t- test). Additionally, we analyzed the AMPAR eEPSCs of rats treated with HSV-GFP. A one-way ANOVA indicated no significant differences between AKAP- (n = 9), GFP+ (n = 7), and GFP- (n = 8) neurons at 2x ( $F_{(2,21)} = 0.92$ ,  $p=0.41$ ) or 3x ( $F_{(2,21)} = 1.23$ ,  $p=0.31$ ) intensities.

## Discussion

Our results indicate that disruption of PKA binding to AKAPs, specifically AKAP150, in the nucleus accumbens shell attenuates the reinstatement of cocaine seeking. We show that AKAP150 promotes cocaine reinstatement by facilitating D1DR-induced, PKA-mediated phosphorylation of GluA1-containing AMPARs. Collectively, these results suggest that AKAP150 can bridge the dopamine and glutamate systems in the nucleus accumbens to promote cocaine seeking.

The current findings support previous findings that nonspecifically disrupting PKA binding to all AKAP isoforms in the nucleus accumbens attenuate cocaine reinstatement (Reissner et al., 2011). Our results expand upon the previous findings as we show that disruption of PKA binding specifically to the AKAP150 isoform attenuates cocaine reinstatement. Additionally, our findings show that AKAP signaling is required for the D1DR agonist-induced reinstatement of cocaine seeking. D1DR stimulation in the nucleus accumbens, particularly the shell subregion, promotes cocaine reinstatement (Schmidt et al., 2006) by increasing transmission through GluA1-containing AMPARs. The C-terminal region of the GluA1 subunit of AMPARs can be phosphorylated by PKA, Protein Kinase C (PKC), and calcium/calmodulin-dependent kinase II (CaMKII) (Derkach et al., 2007; Anggono and Huganir, 2012), all of which contribute to the reinstatement of cocaine seeking (Self et al., 1998; Anderson et al., 2008; Schmidt et al., 2013). In part, D1DR stimulation reinstates cocaine seeking via serial activation of L-type calcium channels and CaMKII (Anderson et al., 2008). Cocaine reinstatement is also associated with D1DR-dependent increases in GluA1-pSer831, a PKC/CaMKII phosphorylation site, as well as increased surface expression of GluA1-containing AMPARs (Anderson et al., 2008). However, D1DR stimulation leads to cAMP and subsequently PKA activation,

which is linked to cocaine seeking (Self et al., 1998). Surprisingly, intra-accumbal administration of a PKA inhibitor, Rp-cAMP, promotes the reinstatement of cocaine seeking (Self et al., 1998). A potential explanation for this unexpected result is that Rp-cAMPs can also inhibit other cAMP-activated targets, such as exchange factors directly activated by cAMP (Epacs) (Bos, 2006). Epac activation leads to increased levels of the GTPase, Rap, which can interact with the Ras/ERK cascade to modulate ERK-dependent processes (Lin et al., 2003; Johnson-Farley et al., 2005). There is considerable evidence linking ERK activation in the accumbens core with cocaine seeking (Edwards et al., 2011; Fricks-Gleason and Marshall, 2011). Our findings suggest that AKAP150 is required for the appropriate subcellular targeting of PKA during D1DR-mediated reinstatement of cocaine seeking.

In addition to a PKA binding domain, AKAP150 also contains a membrane-associated guanylate kinase (MAGUK) motif that promotes its interaction with AMPA and NMDA receptors via binding to scaffolding proteins, PSD-95 and SAP97 (Colledge et al., 2000; Robertson et al., 2009; Sanderson and Dell'Acqua, 2011). Furthermore, AKAP150 enhances PKA-mediated phosphorylation of AMPARs, especially Ser845 on GluA1 subunits (Colledge et al., 2000; Tavalin et al., 2002). Our findings show that disrupting the binding of PKA to AKAP150 attenuates GluA1-Ser845 phosphorylation and reduces AMPAR eEPSCs. PKA phosphorylation of GluA1 at Ser845 leads to increased open probability of AMPARs and increases surface expression of GluA1-containing AMPARs (Banke et al., 2000; Malinow, 2003). In cultured accumbal neurons, D1DR stimulation increases both Ser845 phosphorylation and GluA1 surface expression (Chao et al., 2002a; 2002b). Consistent with these findings, cocaine reinstatement is attenuated by intra-accumbal shell administration of AAV10-GluA1-C99, which impairs the trafficking of



GluA1-containing AMPA receptors to the cell surface (Anderson et al., 2008). Moreover, withdrawal from cocaine self-administration leads to both increased GluA1 surface expression, Ser845 phosphorylation, and increased rectification, which suggests an increase in GluA1-containing, GluA2-lacking calcium permeable AMPARs (CP-AMPARs) (Conrad et al., 2008; McCutcheon et al., 2011b). Our findings also support previous work demonstrating that disrupting AKAP signaling in the nucleus accumbens reduces GluA1 surface expression (Reissner et al., 2011). Surprisingly, in that study, no change was observed in the phosphorylation status of GluA1-Ser845. However, there are several methodological differences between the previous and current study, most notably the biochemical findings of the previous study were observed in drug-naïve animals. Additionally, stimulus-driven changes in GluA1-Ser845 phosphorylation can enhance AMPA-mediated excitatory synaptic transmission and increase synaptic localization of GluA1, partially through AKAP150-PKA binding (Lee et al., 2003; Man et al., 2007; Qiu et al., 2014), whereas unstimulated changes in GluA1 surface expression and AMPA transmission can be independent of basal Ser845 phosphorylation (Lee et al., 2003; Lu et al., 2008; Sanderson et al., 2016).

Our findings also reveal decreases in AMPAR eEPSCs after disruption of PKA binding to AKAP150. This is consistent with previous work showing that synaptic plasticity is impaired in GluA1 KO mice, as well as S845A mutant mice that have impaired Ser845 phosphorylation and PKA-deficient AKAP150-D36 mice (Lee et al., 2003; Lu et al., 2008). Additionally, disruption of PKA binding to AKAPs leads to downregulation of AMPAR currents (Tavalin et al., 2002). Several studies also suggest that PKA binding to AKAP150 and phosphorylation of GluA1-Ser845 can lead to increased surface expression of GluA1-containing AMPARs, particularly CP-AMPARs (Qiu et al., 2014;

Sanderson et al., 2016). However, we did not observe any significant reduction in rectification index. Typically, CP-AMPA accumulation in the nucleus accumbens is linked to long-access (Mameli et al., 2009; Ferrario et al., 2010; McCutcheon et al., 2011a; 2011b), but not short-access cocaine self-administration (Purgianto et al., 2013) or experimenter-delivered cocaine (McCutcheon et al., 2011b). However, a recent study illustrated that blocking CP-AMPA receptors in the nucleus accumbens attenuates the reinstatement of cocaine seeking following a short-access paradigm (White et al., 2015), though this effect was likely mediated through transient increases in GluA1 surface expression as seen previously (Anderson et al., 2008; Schierberl et al., 2011). These data demonstrate that AKAP150 facilitates GluA1-Ser845 phosphorylation and increased AMPAR transmission, promoting cocaine reinstatement. Furthermore, this suggests that during cocaine reinstatement there is synaptic incorporation of GluA1-containing AMPARs, some of which may form CP-AMPA receptors, but most will likely form GluA1A2 heteromers.

In addition to associating with AMPARs and NMDARs via its MAGUK domain, AKAP150 can also bind PKC (Klauck et al., 1996). PKC signaling plays a critical role in cocaine reinstatement. Cocaine reinstatement is associated with increased PKC activation and can be attenuated by intra-accumbal administration of PKC inhibitors (Schmidt et al., 2013). Moreover, cocaine reinstatement is associated with increased GluA1-Ser831 phosphorylation (Anderson et al., 2008). PKC phosphorylates GluA1 subunits at Ser831, facilitating GluA1 insertion into the membrane (Song and Huganir, 2002). AKAP150 can also interact with L-type calcium channels via interaction with a leucine zipper domain at its C-terminus (Oliveria et al., 2007). AKAP150 facilitates PKA phosphorylation of L-type calcium channels at Ser1928, thereby increasing channel activity (Gao et al., 1997). This

channel plays a major role in cocaine-induced synaptic plasticity and cocaine reinstatement. Specifically, intra-accumbal shell administration of diltiazem, an L-type calcium channel antagonist, attenuates the reinstatement of cocaine seeking precipitated either by systemic cocaine injection or intra-accumbal shell administration of the D1DR agonist SKF-81297 (Anderson et al., 2008). Though we did not investigate the interactions between PKC, L-type calcium channels, and AKAP150 in this study, these findings underscore the importance of AKAP150 as a major regulator of cocaine-induced plasticity and cocaine craving.

The present results contribute to the growing body of literature indicating that increased transmission through GluA1-containing AMPARs in the nucleus accumbens shell promotes the reinstatement of cocaine seeking. Moreover, these data demonstrate a compelling role for AKAP150 as a biochemical bridge linking the dopamine and glutamate systems in the nucleus accumbens during cocaine reinstatement. These findings suggest that AKAP150 may be a potential novel target for the development of cocaine addiction pharmacotherapies.

## Figures

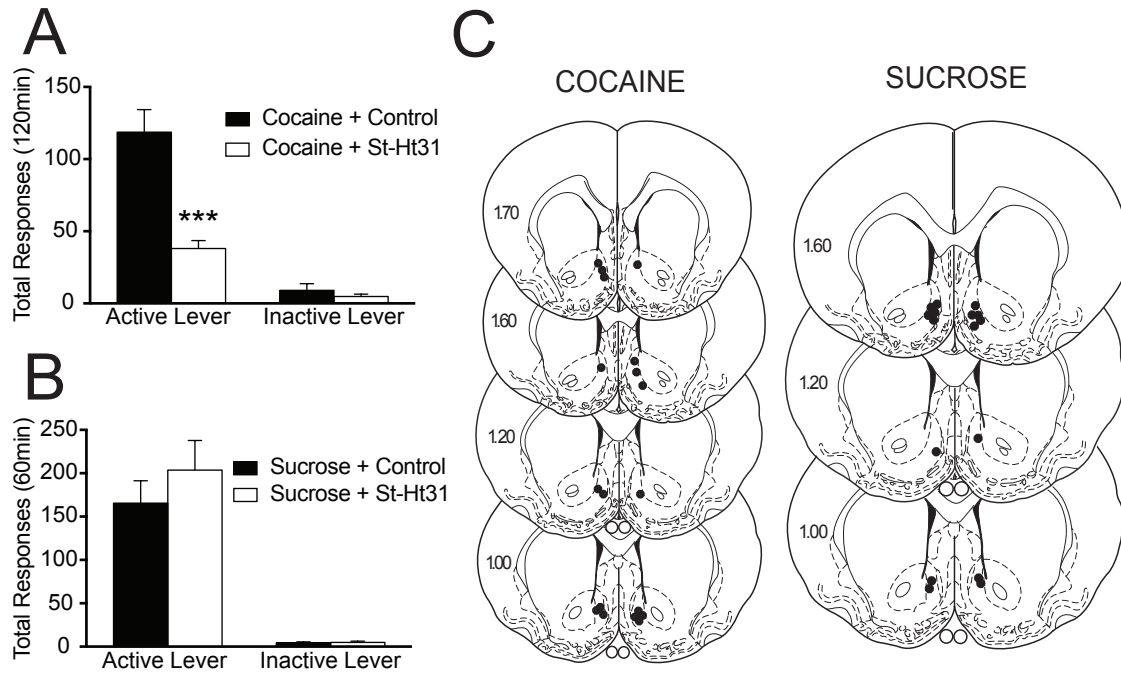


Figure 4. 1 Intra-accumbal shell microinjections of St-Ht31 attenuates cocaine, but not sucrose, reinstatement. Mean ( $\pm$ SEM) active and inactive lever responses from (A) cocaine reinstatement session, (B) sucrose reinstatement session. (C) Cannula placements from the nucleus accumbens shell (dark circles). The values are in millimeters, relative to bregma. \*\*\* $p < 0.001$  St-Ht31 compared to St-Ht-31P Control. There were 8-9 animals per group.

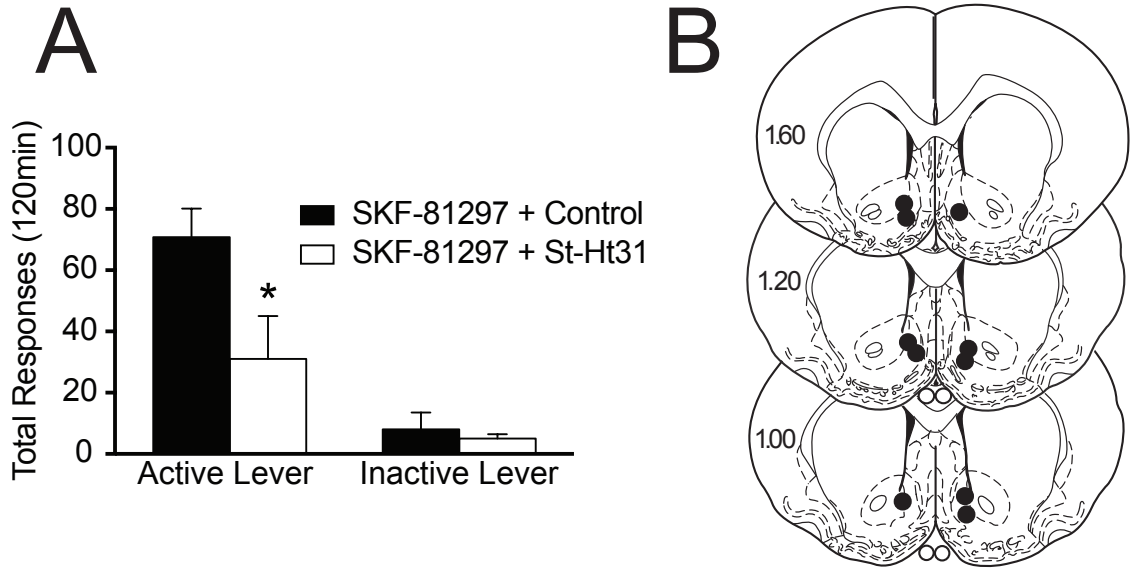


Figure 4. 2 Intra-accumbal shell microinjections of St-Ht31 attenuates D1DR-agonist induced reinstatement of cocaine seeking. (A) Mean ( $\pm$ SEM) active and inactive lever responses from reinstatement session. (B) Cannula placements from the nucleus accumbens shell (dark circles). The values are in millimeters, relative to bregma. \* $p < 0.05$  St-Ht31 compared to St-Ht-31P Control. There were 5 animals per group.

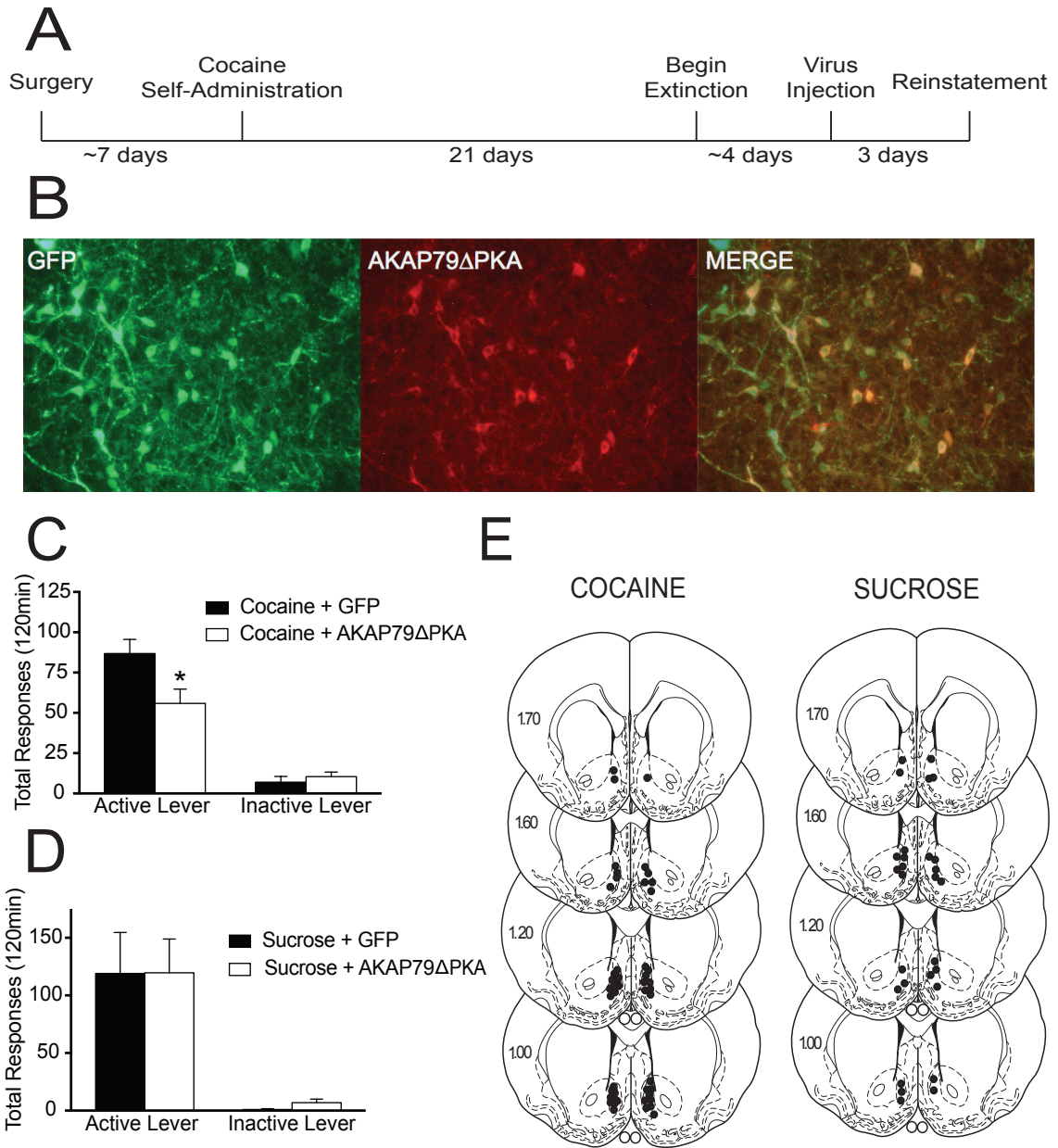


Figure 4. 3 Intra-accumbal shell expression of HSV-AKAP79ΔPKA attenuates cocaine, but not sucrose, reinstatement. (A) Schematic of experimental paradigm. (B) Representative image of peak expression in the nucleus accumbens 3 days following HSV-AKAP79ΔPKA injection. Three days prior to the reinstatement test session, all animals received either HSV-AKAP79ΔPKA or HSV-GFP injections to the nucleus accumbens shell. (C) Mean ( $\pm$ SEM) active and inactive lever responses from cocaine reinstatement session, (D) sucrose reinstatement session. (E) Cannula placements from the nucleus accumbens shell (dark circles). The values are in millimeters, relative to bregma. \* $p < 0.05$  HSV-AKAP79ΔPKA compared to HSV-GFP. There were 10-13 animals per group.

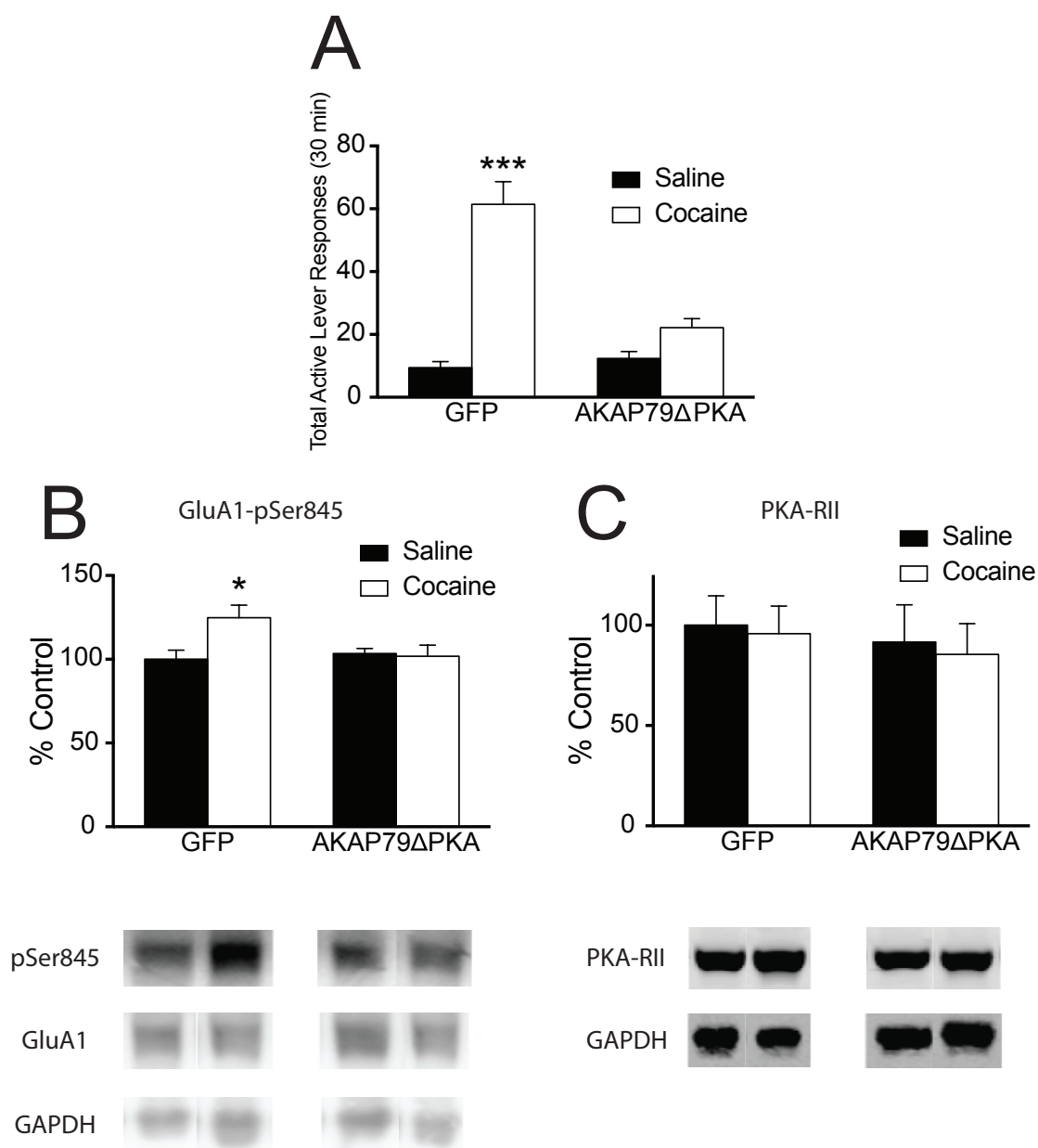


Figure 4. 4 Intra-accumbal shell expression of HSV-AKAP79 $\Delta$ PKA attenuates GluA1-Ser845 phosphorylation. All animals received either HSV-AKAP79 $\Delta$ PKA or HSV-GFP injections to the nucleus accumbens shell three days prior to the reinstatement test session. At the reinstatement test session, all animals received either a saline or cocaine priming injection. (A) Mean ( $\pm$ SEM) active and inactive lever responses from 30 min reinstatement session. (B) Decreases in GluA1-Ser845 phosphorylation are measured by Western blot (see insets). (C) No change in PKA-RII expression is observed by Western blot (see insets). \*\*\* $p < 0.001$ , \* $p < 0.05$  HSV-AKAP79 $\Delta$ PKA compared to HSV-GFP. There were 9-11 animals per group.

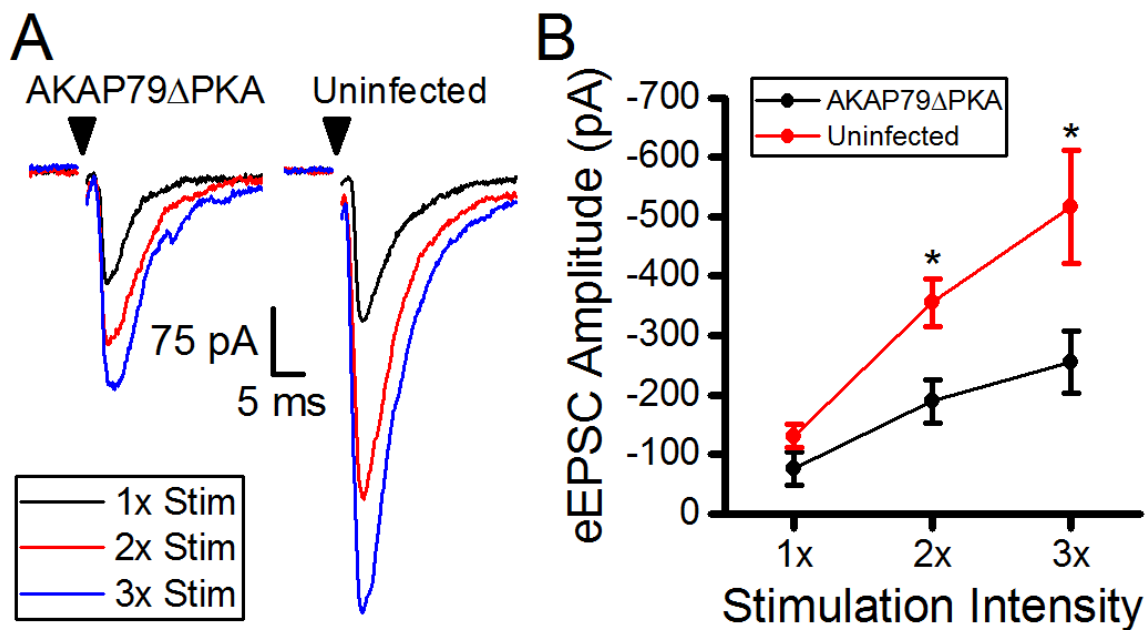


Figure 4. 5 HSV-AKAP79 $\Delta$ PKA reduces the recruitment of AMPA receptors following cocaine reinstatement. (A) Representative traces from an AKAP79 $\Delta$ PKA-positive neuron (left) and an uninfected neuron (right). A minimal intensity for eEPSC recruitment was obtained (1x) and then increased to 2x and 3x this intensity. Arrowheads represent stimulation and the stimulation artifacts of been removed for visual clarity. (B) Summary of the recruitment curves for AKAP79 $\Delta$ PKA-positive (black circles, n = 9) and uninfected (red circles, n = 9) neurons. \* p < 0.05.



## **Chapter 5**

### GENERAL DISCUSSION AND FUTURE DIRECTIONS

Leonardo Antonio Guercio

#### **Overview**

Cocaine abuse poses a significant public health concern both in the United States and across the globe. Cocaine is the fourth most commonly abused illegal drug in the world, with the United States as the global leader in cocaine demand (Crime, 2016).

Approximately 1.5 million Americans aged 12 or older are regular users of cocaine, comprising about 0.5% of the US population (Sarra L Hedden, 2015). Cocaine use is also responsible for nearly 500,000 emergency room visits annually (National Institute on Drug Abuse, 2016). A critical concern with cocaine abuse is the discouragingly high rate of relapse among addicts following detoxification and abstinence, making it a continuing public health concern that impacts our society, government, and healthcare system (O'Brien, 1997).

Preclinical research using rodent cocaine self-administration and reinstatement paradigms can elucidate the neurobiological underpinnings of human cocaine addiction and relapse, potentially leading to the development of novel therapeutic interventions that can reduce the burden of drug addiction on our society. The research presented in this doctoral dissertation examined the influence of local and circuit-wide modulation of the mesocorticolimbic reward system on cocaine reinstatement, an animal model of relapse. In sum, the findings presented herein expand our understanding of the neurobiological mechanisms underlying cocaine seeking and identify both a non-pharmacological application, deep brain stimulation (DBS), and a novel biochemical target, AKAP150, for potential therapeutic interventions in cocaine addiction and craving.

The data presented in the second and third chapters of this dissertation demonstrated that DBS may serve as a possible non-pharmacological therapeutic intervention in the treatment of cocaine addiction. In Chapter 2, I showed that DBS of the nucleus accumbens shell attenuated the cue-induced reinstatement of cocaine seeking. This expands upon our previous work showing that accumbal shell DBS attenuated priming-induced reinstatement of cocaine seeking (Vassoler et al., 2008; 2013). In Chapter 3, I demonstrated that DBS of the medial prefrontal cortex (mPFC), but not the basolateral amygdala (BLA) or the ventral hippocampus (vHipp) selectively attenuated the reinstatement of cocaine seeking. Moreover, this effect was constrained to the infralimbic subregion of the mPFC as DBS in the prelimbic or anterior cingulate cortices had no effect on cocaine reinstatement. My results also showed that infralimbic mPFC DBS decreased *zif268* immunoreactivity in the nucleus accumbens shell, suggesting inactivation of glutamatergic cortico-accumbal projections. These findings bolster a wealth of evidence suggesting that addiction causes a maladaptive response in the circuit between the mPFC and the nucleus accumbens, and that this maladaptation can be corrected through non-pharmacological manipulation (Chen et al., 2013; Stefanik et al., 2013; Vassoler et al., 2013). Moreover, my findings demonstrate that in addition to the nucleus accumbens, the mPFC may be an effective target for DBS in the treatment of cocaine craving and relapse.

The data presented in Chapter 4 of this dissertation support a substantial body of evidence demonstrating that increased transmission through GluA1-containing AMPA receptors (AMPA receptors) in the nucleus accumbens shell promotes cocaine reinstatement. Moreover, these data revealed the novel role of the protein, AKAP150, in the reinstatement of cocaine seeking. My findings indicate that AKAP150 promotes cocaine reinstatement by facilitating D1-like dopamine receptor (D1DR)-induced, PKA-mediated

phosphorylation of GluA1-containing AMPARs. Taken together, these findings suggest that AKAP150 may serve as a biochemical bridge linking the dopamine and glutamate systems in the nucleus accumbens during cocaine reinstatement.

### **The development of novel pharmacotherapies for cocaine addiction**

Over the past decade, remarkable advancements have been made in both the neuroscience of mental health and society's awareness of mental illness. Despite these advances, the treatment of mental illness remains a significant challenge. Mental illness remains the leading cause of morbidity and mortality and psychiatric disorders constitute five of the top ten causes of disability and premature death (Insel, 2009; Collins et al., 2011; Bloom et al., 2012). Additionally, in 2010, the global cost of mental health disorders was \$2.5 trillion, an estimate that is projected to balloon to \$6.5 trillion by 2030 (Bloom et al., 2012). Drug addiction is a complex mental health disorder and is among the most prevalent neuropsychiatric disorders affecting modern society (Crime, 2016). Discouragingly, despite decades of research, the core pathophysiological mechanism of drug addiction remains unknown. A greater understanding of the neurobiological circuitry and mechanisms underlying drug addiction will lead to improved pharmacotherapies.

Drugs of abuse mediate their initial reinforcing properties through dopaminergic modulation of the mesocorticolimbic reward system. Despite their differing mechanisms of action, all classes of addictive drugs (e.g. opiates, psychostimulants, alcohol, nicotine, cannabinoids) increase dopamine (DA) transmission in mesocorticolimbic nuclei (Di Chiara and Imperato, 1988), including the nucleus accumbens (Pierce and Kumaresan, 2006). The increase of mesocorticolimbic DA transmission as a common pathway for drugs of abuse is consistent with the findings that DA encodes reward-prediction error (Keiflin and Janak, 2015). However, the initial, acute actions of addictive drugs dissipate

as the drugs are metabolized by the brain and therefore cannot explain the long-term development of addictive behaviors. In 2001, a seminal study showed that a single injection of cocaine sufficed to induce a long-lasting potentiation of excitatory glutamatergic synapses onto VTA DA neurons (Ungless et al., 2001). Two years later, this finding was also observed with other drugs of abuse, specifically amphetamine, morphine, alcohol, and nicotine (Saal et al., 2003). These findings led to numerous studies highlighting the importance of glutamatergic transmission and synaptic plasticity in response to exposure to addictive drugs (Kauer and Malenka, 2007; Kalivas, 2009; Schmidt and Pierce, 2010; Lüscher and Malenka, 2011). Taken together, these findings revealed that both the dopamine and glutamate systems underlie the addictive properties of nearly all drugs of abuse.

Currently, there exist effective, FDA-approved treatments for opioid, alcohol, and nicotine addiction. However, despite decades of focused research, there are no FDA-approved pharmacotherapies for cocaine addiction. Initially, drugs that modulate the dopaminergic system were assessed in both preclinical and clinical studies for the treatment of cocaine addiction, based on the fact that all drugs of abuse increase DA transmission in the mesocorticolimbic reward system. There was particular interest in the D1DR antagonists, as they lacked the sometimes dangerous extrapyramidal side effects observed with D2-like dopamine receptor (D2DR) antagonists (Haney and Spealman, 2008). These studies revealed that acute administration of D1DR antagonists attenuated the reinforcing effects of cocaine (Romach et al., 1999; Platt et al., 2002). However, clinical use of a D1DR antagonist requires repeated administration and cocaine addicts treated with a D1DR antagonist, ecopipam, actually increased cocaine self-administration (Haney et al., 2001). Another potential therapeutic for cocaine addiction that showed promise was N-acetylcysteine (NAC). NAC, an FDA-approved treatment for

acetaminophen overdose, reduced glutamate levels in the nucleus accumbens following cocaine self-administration and cocaine reinstatement (Baker et al., 2002; 2003b). A similar reduction in glutamate levels was observed in the prefrontal cortex of cocaine-dependent patients treated with NAC (Schmaal et al., 2012). However, in a double-blind, placebo-controlled clinical trial, NAC failed to reduce cocaine use in actively using cocaine-dependent individuals, though there was some evidence suggesting that it may delay cocaine relapse in abstinent addicts (LaRowe et al., 2013). Collectively, these clinical findings demonstrate that broad-based manipulations on dopamine or glutamate signaling are not efficacious in the treatment of cocaine addiction.

Recent preclinical work has begun to examine a more nuanced interaction between accumbal dopamine and glutamate systems in cocaine craving and relapse. The nucleus accumbens is predominately made up of medium spiny neurons that express either D1DRs or D2DRs (Gangarossa et al., 2013). Neurons in the nucleus accumbens integrate information from dopaminergic and glutamatergic inputs stemming from cortical and limbic structures to generate appropriate goal-directed behavioral responses (Papp et al., 2012; Scofield et al., 2016). Stimulation of D1DRs in the nucleus accumbens led to increased phosphorylation and surface expression of GluA1-containing, or GluA2-lacking, calcium-permeable AMPARs (CP-AMPARs) (Chao et al., 2002a; 2002b; Anderson et al., 2008; Ferrario et al., 2011; Hobson et al., 2013). Interestingly, withdrawal from cocaine self-administration caused an increase in the rectification index of D1- but not D2-containing medium spiny neurons in the accumbens, suggesting an increase in CP-AMPARs in those cells (Pascoli et al., 2014). It should be noted that while these studies have not identified effects in D2-containing medium spiny neurons, previous work has shown that D2DRs and D2-containing medium spiny neurons in the accumbens can regulate glutamatergic transmission and contribute to

cocaine seeking (Bamford et al., 2004; Dani and Zhou, 2004; Bock et al., 2013; Ortinski et al., 2015).

Signaling pathways activated by the dopamine and glutamate converge at the post-synaptic density, a highly organized, macromolecular complex of synaptic proteins that serve to process and integrate neural signals to the nucleus (Kennedy, 2000; de Bartolomeis and Fiore, 2004). Synaptic proteins have been implicated in many psychiatric disorders, including drug addiction (Kalivas and Volkow, 2005) and may provide novel therapeutic targets (de Bartolomeis et al., 2014). The work encompassed in Chapter 4 of this dissertation adds to the growing body of literature highlighting the importance of synaptic proteins in the nucleus accumbens during cocaine reinstatement (Reissner et al., 2011; Wiggins et al., 2011; Schmidt et al., 2013; Briand et al., 2014; White et al., 2015). I showed that disruption of PKA binding specifically to the AKAP150 isoform attenuated cocaine reinstatement by reducing transmission through GluA1-containing AMPARs in the nucleus accumbens shell. These findings, coupled with the fact that AKAP150 is most highly expressed in the striatum (Ostroveanu et al., 2007) and can also interact with both PKC and L-type calcium channels, both of which are critically involved in cocaine seeking (Anderson et al., 2008; Schmidt et al., 2013), highlight the potential therapeutic value of AKAP150 in the treatment of cocaine craving and relapse.

### **Non-pharmacological brain stimulation in the treatment of cocaine addiction**

Over the past few years, the global pharmaceutical industry has significantly curtailed its investment in drug development for psychiatric disorders, with some companies shuttering their research programs entirely (Miller, 2010). Surprisingly, this retreat has come despite the fact that psychiatric drugs have been extremely profitable for the pharmaceutical industry, with 1 in 5 American adults currently taking at least one

psychiatric drug (Hyman, 2013). This retreat can perhaps be explained by the significant issues plaguing translational neuroscience and psychiatry: cellular and molecular underpinnings of disease remain elusive, animal models provide poor face and predictive validity, and validated biomarkers or other objective tests to aid in diagnosis and treatment have not been found (Nestler and Hyman, 2010; Hyman, 2012). There are several concerns with developing novel pharmacotherapies for substance use disorders (Paul et al., 2010). First, the estimated development costs range from \$4-11 billion, with the entire process taking an average of over 13 years to complete. Second, the return on investments for these new medications is poor, with large pharmaceutical companies expecting to recover only 26 cents on the dollar, or a <0.3% return on new product revenues. Finally, and perhaps most importantly, the lack of developmental efficiency is troubling, with a measly ~7% approval rate of CNS compounds that make it to clinical development. In fact, in 2015, the FDA approved 45 novel drugs, only 3 of which had indications for nervous system disorders (CDER, 2016). Since drug addiction is a highly prevalent neuropsychiatric disorder, these issues are a major cause for concern.

Currently, there are FDA-approved medications for drug addiction, specifically opiate, alcohol, and nicotine addiction. While these medications are effective, they target only certain aspects of the addictive process (Koob et al., 2009) and work best when used in combination with social and behavioral interventions (Douaihy et al., 2013). Importantly, there are no effective, FDA-approved pharmacotherapies for cocaine addiction, despite rational drug development and well-controlled clinical trials (Pierce et al., 2012). Drug addiction is a significant financial burden on our society, healthcare system, and criminal justice system, with total costs exceeding \$600 billion annually in the United States alone (National Drug Intelligence Center, 2011). This underscores a

critical need for effective non-pharmacological treatments, particularly for cocaine addiction.

In April 2013, President Barack Obama announced the beginning of the BRAIN Initiative (Brain Research through Advancing Innovative Technologies), a national, collaborative, public-private research initiative, with the goal of supporting the development of innovative technologies that would enhance our dynamic understanding of the human brain. Importantly, this initiative also aims to further illuminate our understanding of brain disorders in hopes of developing better treatments and improving outcomes for patients with these disorders. The United States is not alone: the BRAIN Initiative is part of a global effort by the G8 countries and others to further our understanding of neuroscience and brain disorders (Grillner et al., 2016). One of the high priority research areas for the BRAIN Initiative is the continued improvement of existing technologies and development of novel technologies to modulate neural activity (HHS, 2014).

Modulation of neural activity by electromagnetic brain stimulation is an active area of neuropsychiatric research. Since pharmacotherapeutics for psychiatric disorders have limited effectiveness and are becoming increasingly expensive to develop (Hyman, 2013), recent efforts have focused on brain stimulation as a potential treatment modality. At present, there are several different types of brain stimulation used medically. Chief among them are electroconvulsive therapy (ECT), transcranial magnetic stimulation (TMS), transcranial direct current stimulation (tDCS), and deep brain stimulation (DBS). Electroconvulsive therapy (ECT), also known as shock therapy, works by electrically inducing seizures in patients suffering from psychiatric disorders, particularly depression. Unfortunately, ECT is associated with adverse events including cognitive impairments and retrograde amnesia, and is thus used as a last resort intervention for psychiatric



disorders (Rudorfer et al., 2003). TMS has its roots in early 20<sup>th</sup> century Vienna, when psychiatrists Pollacsek and Beer filed a patent to treat depression and other neuroses with an electromagnetic device that resembled current TMS machines. However, the modern TMS era began in the 1980s, when Tony Barker and colleagues developed a focal electromagnetic device that could induce currents in the spinal cord (Barker et al., 1985). They soon realized that this technology could allow them to non-invasively stimulate the human brain. Briefly, TMS works by placing an electromagnetic coil on the scalp of a patient. The electricity flowing through the coil creates a powerful (~1.5 Tesla), but brief (microseconds) magnetic field that can pass through the skull and modulate the electrical properties of neurons. Like TMS, tDCS is another non-invasive form of brain stimulation. Though the principles underlying tDCS date back to the time of Luigi Galvani, it was not seriously considered as a technique to modulate brain activity until 2000 (Nitsche and Paulus, 2000). tDCS works by applying constant weak (~1 mA) electrical current through scalp electrodes. Unlike TMS, which can elicit action potentials in cortical neurons, tDCS induces subthreshold changes in the membrane potential of neurons, thereby more subtly increasing or decreasing the probability of eliciting action potentials. DBS, originally developed in the 1950s, first achieved recognition in the 1980s as a potential therapeutic intervention for Parkinson's disease and other movement disorders (Benabid et al., 1987). Briefly, DBS involves implanting an electrode deep in the brain and connecting it to a generator, located in the chest wall, which sends constant electrical current into the brain. While DBS is an invasive procedure, unlike TMS and tDCS, it allows physicians and scientists to target structures deep in the brain, which is not possible with TMS and tDCS. Both TMS and DBS are FDA-approved for treating and investigating neuropsychiatric disorders, while tDCS remains an experimental treatment.

These methods of brain stimulation have become increasingly examined as potential therapeutic modalities for psychiatric disorders, particularly depression (Kammer and Spitzer, 2012; George et al., 2014). TMS is FDA-approved for the treatment of major depressive disorder. In 2010, a sham-controlled, randomized, multi-site clinical trial demonstrated that TMS of the dorsolateral prefrontal cortex (dlPFC), as a drug-free monotherapy, produced a significant anti-depressant effect in patients (George et al., 2010) and lasted several weeks to months (McDonald et al., 2011; Mantovani et al., 2012). A similar, albeit smaller, effect was seen using tDCS of the dlPFC. Patients showed an improvement in mood as well as improvements in attention and working memory (Loo and Martin, 2012). DBS of subcortical structures, including the nucleus accumbens, has also been shown to demonstrate significant antidepressant effects (Mayberg et al., 2005; Bewernick et al., 2010). In addition to treating depression, these brain stimulation modalities have also been demonstrated to be effective in treating other psychiatric disorders as well (George et al., 2014), particularly drug addiction.

There is a growing body of evidence supporting brain stimulation as a potential therapeutic modality for drug addiction (Salling and Martinez, 2016). TMS, tDCS, and DBS have a wide range of effects across nearly all drug classes (e.g. alcohol, nicotine, heroin, cocaine, and cannabis). However, since there are no effective pharmacotherapeutic interventions for cocaine addiction, it is critically important to examine whether brain stimulation can serve as a potential therapeutic modality. TMS of the dlPFC and mPFC reduced both craving for cocaine and cocaine use (Camprodon et al., 2007; Politi et al., 2008; Hanlon et al., 2015; Terraneo et al., 2016). Additionally, TMS of the mPFC was shown to inhibit stimulus-evoked activity in the mPFC and decrease activity in the striatum (Hanlon et al., 2015). The tDCS studies on cocaine addiction have

primarily focused on cortical excitability and cognitive function (Conti et al., 2014; Gorini et al., 2014). However, a recent randomized, double-blind clinical trial showed that tDCS in the dlPFC reduced cocaine craving (Batista et al., 2015). These findings suggest that TMS and tDCS may be preferable to DBS in treating drug addiction since they are non-invasive, unlike DBS. However, neither TMS nor tDCS can effectively target subcortical structures. Furthermore, DBS is a one-time implantation surgery that can allow for uninterrupted, hands-off, chronic stimulation as opposed to TMS and tDCS, which only offer acute stimulation, with each session having to be done in the presence of a trained medical professional. To date, there has been only one clinical study investigating the effects of DBS on cocaine addiction, which showed that in a single patient, accumbens shell DBS reduced his cocaine dependence based on both objective and subjective measures (Gonçalves-Ferreira et al., 2016). The patient displayed significant clinical improvement, in line with improvements seen with accumbal DBS in alcohol, heroin, and nicotine addiction (Kuhn et al., 2009; Müller et al., 2009; Kuhn et al., 2011; 2014). Collectively, these findings emphasize that brain stimulation may be a highly valuable therapeutic modality in the treatment of drug addiction, especially cocaine addiction.

While there has only been a single, pilot clinical study investigating the role of DBS in cocaine addiction, a wealth of evidence from animal studies suggest that DBS may help treat cocaine craving and relapse. DBS of the nucleus accumbens shell, but not the core or dorsal striatum, attenuated priming-induced reinstatement of cocaine seeking (Vassoler et al., 2008; 2013). DBS of the nucleus accumbens also suppressed locomotor sensitization to cocaine (Creed et al., 2015), another behavioral task that reflects aspects of plasticity related to drug craving (Robinson and Berridge, 2001; Steketee and Kalivas, 2011). The work encompassed in Chapter 2 of this dissertation expands upon these findings, demonstrating that accumbal shell DBS also attenuates

cue-induced reinstatement of cocaine seeking (Guercio et al., 2015). While nearly all preclinical and clinical studies investigating DBS as a treatment for drug addiction have focused on the nucleus accumbens, evidence from clinical studies using TMS and tDCS suggest that the PFC may be a potentially effective target for DBS in treating cocaine addiction. The work encompassed in Chapter 3 of this dissertation reveals that DBS of the mPFC selectively attenuated the reinstatement of cocaine seeking. These findings support previous claims that in addition to the nucleus accumbens, the mPFC may be another effective target for DBS in the treatment of cocaine craving and relapse (Luigjes et al., 2012).

### **The societal benefits of improved treatments for drug addiction**

It is estimated that the total costs of substance abuse including productivity, health, and crime-related costs, exceed \$600 billion annually in the United States alone (National Drug Intelligence Center, 2011). Given these prohibitively high costs, it is imperative to enact sensible drug policies and legislation based on well-substantiated scientific and public health research. In 2008, President Barack Obama reignited the Office of National Drug Control Policy in an attempt to counteract the decades long, failed “war on drugs” of previous administrations. The goal of the Obama administration was to restore balance to drug control efforts by organizing an unprecedented coordination government-wide public health, public safety, and scientific approaches to reduce drug use and its consequences.

The unfortunate connection between drug use and crime is well known (National Institute on Drug Abuse, 2014). There are 7 million adults involved with the criminal justice system in the United States, both incarcerated and on probation (Glaze and Herberman, 2013). Slightly more than half of all federal prisoners had a drug offense as

their most grievous offense (National Institute on Drug Abuse, 2014). Further, 70% of state prisoners and 64% of federal prisoners regularly used drugs prior to incarceration, with 25% of violent offenders in state prisons having committed their crimes while intoxicated (Mumola and Karberg, 2007). Untreated substance using offenders are far more likely to relapse into drug use and criminal behavior, further taxing the public health and criminal justice systems (National Institute on Drug Abuse, 2014). Treatment in correctional settings followed by aftercare in the community when offenders are released leads to substantial reductions in the rates of re-incarceration and the associated criminal justice costs (McCollister et al., 2003a; 2003b). Additionally, the Obama administration has reformed the judicial branch by creating drug courts. These are special dockets designed specifically for non-violent offenders with a history of substance abuse, allowing for continued, court-managed participation in treatment programs to help reduce the rate of relapse associated with jail-time and recent parole (Anon, 2015). In addition to relieving the criminal justice costs, improved drug policies and drug treatment may also help to normalize racial disparities in drug convictions, as African-Americans are far more likely than Caucasians to be convicted for drug-related offenses (AnnCarson et al., 2015), despite equal rates of drug use (Sarra L Hedden, 2015).

Substance use also places a major financial burden on our healthcare system, both for payers and providers. Patients with histories of substance use disorders have elevated hospital and psychiatric admissions, substantially increasing total health care costs (Clark et al., 2009). In 2010, the Obama administration passed the Affordable Care Act, which includes substance use rehabilitation as an essential component of health care. This is a significant benefit for people with substance use disorders as they are far more likely to be uninsured compared to the national average (Bouchery et al., 2012).

Since the loss of insurance benefits is associated with restricted access to care and the closure of drug abuse treatment centers (Bret E Fuller et al., 2006; Deck et al., 2006), the Affordable Care Act is a welcome respite to those struggling with substance use disorders. Further, treatment for substance use disorders can lead to reduction in the utilization and cost of medical care (Walter et al., 2005).

Improving treatments for those struggling with addiction is not only our moral and ethical responsibility, it is also financially prudent. The economic benefits of investing in drug treatment exceed the costs of treatment, with cost-benefit analyses showing that every dollar spent on care returns 7 dollars in benefits (Ettner et al., 2006). These treatment benefits include increases in employment income and decreases in avoided costs of criminal activities, incarceration, and hospitalization (Ettner et al., 2006).

### **Concluding Remarks**

The work encompassed in this doctoral dissertation demonstrates that local and circuit-wide manipulations of the mesocorticolimbic reward system can modulate the reinstatement of cocaine seeking. There is a substantial body of literature indicating that DBS of the nucleus accumbens may be a potential therapeutic modality in the treatment of cocaine addiction. Specifically, DBS of the nucleus accumbens shell, but not the core or dorsal striatum, attenuates the priming-induced reinstatement of cocaine seeking (Vassoler et al., 2008; 2013). The present results expand upon this finding by showing DBS of the nucleus accumbens shell also attenuated cue-induced reinstatement of cocaine seeking (Guercio et al., 2015). The work herein also revealed a novel target brain region for DBS in the treatment of cocaine addiction: DBS of the infralimbic mPFC selectively attenuated the reinstatement of cocaine seeking. These findings are consonant with results from other therapeutic modalities, such as TMS and tDCS,

suggesting that mPFC stimulation can reduce cocaine use and craving (Batista et al., 2015; Hanlon et al., 2015; Terraneo et al., 2016).

It is now clear that cocaine reinstatement is associated with changes in dopamine and glutamate transmission in the nucleus accumbens (Schmidt et al., 2005; Schmidt and Pierce, 2010). Further, it has been shown that cocaine reinstatement promotes modifications of glutamatergic receptors and associated proteins, causing altered excitatory transmission in the nucleus accumbens, (Pierce and Wolf, 2013). The results of this dissertation expanded upon this knowledge identifying a necessity for the protein, AKAP150, which acts as a biochemical bridge between the dopamine and glutamate systems to promote the reinstatement of cocaine seeking. These findings present AKAP150 as a potential novel pharmacotherapeutic target in the treatment of cocaine craving and relapse. Since broad-based manipulations of dopamine and glutamate transmission have failed to produce effective pharmacotherapies for cocaine addiction, drugs that manipulate synaptic proteins to indirectly modulate receptor functioning may prove more fruitful in selectively combating addictive behaviors.

Finally, this dissertation addressed why we, as a nation, must invest in improved drug treatments and advocate for sensible drug policies. Improving treatments for those struggling with addiction is not only our moral and ethical responsibility, it is also financially prudent (Ettner et al., 2006). Given the significant burden drug addiction places on our society, healthcare, and criminal justice systems, it is imperative that we tackle this challenge using scientific, legal, and socioeconomic approaches. It is assuredly a difficult task, but as Benjamin Franklin, founder of this great University wisely stated, “without continual growth and progress, such words as improvement, achievement, and success have no meaning.”

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