EXAMINATION OF PRELIMBIC CORTEX AND NUCLEUS ACCUMBENS CORE SIGNALING DYNAMICS AS A BIOMARKER FOR COCAINE USE DISORDER IN A PRECLINICAL MODEL

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ABSTRACT

Metika Laya Ngbokoli: Examination of Prelimbic Cortex and Nucleus Accumbens Core Signaling Dynamics as a Biomarker for Cocaine Use Disorder in a Preclinical Model
(Under the direction of Regina M. Carelli)

The prelimbic cortex (PrL) and nucleus accumbens (NAc) core, brain regions implicated in higher order processes such as decision making and behavioral flexibility, undergo neuroadaptations following prolonged abstinence from cocaine. Critically, impairments in these processes are also observed in individuals living with substance use disorders (SUDs) and is thought to be linked with these neuroadaptations. Furthermore, there is evidence that using noninvasive brain stimulation (NIBS) techniques may be a promising treatment for SUDs. However, it is not quite clear how these abstinence-related disruptions in PrL and NAc core signaling are related to drug seeking and taking behaviors. Recent work in the Carelli lab has shown that using transcranial alternating current stimulation (tACS, a form of NIBS) was effective in reversing cocaine-induced deficits in PrL-NAc core circuit signaling and restoring impaired behavioral flexibility. The following document consists of three specific aims that used electrophysiological recording methods to investigate signaling dynamics in the PrL and NAc core following prolonged abstinence from cocaine, their relationships to drug seeking and taking behavior, and the effectiveness of tACS to restore cocaine-induced deficits in signaling and behavior.

To all the little Black girls out there, dream big.

In loving memory of Pierre Ngbokoli

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PREFACE

This dissertation was prepared within the guidelines set forth by the University of North Carolina at Chapel Hill Graduate School. This dissertation is comprised of a general introduction, three chapters of original data, and a general discussion chapter. Each original data chapter includes an introduction, methods, results, and discussion section. All figures referenced are embedded within the text of each corresponding section. A complete list of references cited throughout the document can be found at the end. References follow APA format.

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LIST OF ABBREVIATIONS

BLA	Basolateral amygdala
CS	Conditioned stimulus
CTA	Conditioned taste aversion
cTBS	Continuous theta burst stimulation
CUD	Cocaine use disorder
EEG	Electroencephalograph
fMRI	Functional magnetic resonance imaging
LFP	Local field potential
LiCl	Lithium chloride
mPFC	Medial prefrontal cortex
NAc	Nucleus accumbens
NIBS	Noninvasive brain stimulation
OFC	Orbitofrontal cortex
PFC	Prefrontal cortex
PrL	Prelimbic cortex
PSD	Power spectral density
rsFC	Resting state functional connectivity
SUD	Substance use disorder

tACS	Transcranial alternating current stimulation
TMS	Transcranial magnetic stimulation

CHAPTER 1

GENERAL INTRODUCTION

The 2020 US National Survey on Drug Use and Health reported roughly 40 million people ages 12 and older living with a substance use disorder (SUD) in the past year with about 1.3 million of those individuals reporting having cocaine use disorder (CUD) specifically (Center for Behavioral Health Statistics, 2021). Furthermore, individuals living with SUDs are also more likely to experience other mental health issues including, but not limited to mood and anxiety disorders (Grant et al., 2004; Pasche, 2012). Almost 74 million adults (ages 18+) reported having either an SUD or a mental illness and of those, 17 million reported having both in the past year (Center for Behavioral Health Statistics, 2021). Additionally, although rates of drug use are similar across race and ethnicity, Black and Latinx communities are disproportionately criminalized compared to white people (Burlew et al., 2021) and there is evidence of discrepancies in treatment for historically underrepresented groups which is often attributed to lack of access and stigma (Center for Behavioral Health Statistics, 2021; Pinedo & Villatoro, 2020). Importantly, the ongoing COVID-19 pandemic has not only exacerbated these discrepancies, but those with SUDs are also at higher risk for developing severe COVID-19 infection (Czeisler et al., 2020; McKnight-Eily et al., 2021; Ornell et al., 2020; Rubin, 2020). In short, research and education surrounding substance use and importantly accessible treatments is timely, relevant and necessary.

SUDs are defined as cognitive, behavioral, and physiological impairments due to the repeated use of a substance (American Psychiatric Association, 2013). The brain's reward system is disrupted, and this eventually leads to a cycle of drug use characterized by preoccupation-

anticipation with drug, followed by a binge-intoxication, then withdrawal-negative affect during abstinence from drug use (Koob & Le Moal, 1997). Additionally, SUDs have been shown to cause deficits in higher order processing including cognitive flexibility, executive function, and impulsivity (Butler & Le Foll, 2019; Jentsch et al., 2002; Porter et al., 2011; Verdejo-Garcia et al., 2019).

This dissertation aims to examine signaling dynamics within the brain's reward system before and after prolonged abstinence from cocaine self-administration in rats. This introductory chapter provides an overview of the neurobiology of SUDs including altered signaling in brain reward circuitry and associated cognitive deficits, the use of resting state activity as a biomarker for SUDs, and potential treatments using noninvasive brain stimulation techniques (NIBS), specifically transcranial alternating current stimulation (tACS). **Chapter 2** examines resting state neural activity in the prelimbic (PrL) cortex and nucleus accumbens (NAc) core, part of the brain's reward system, during prolonged (1-month) abstinence from short access (2hr) cocaine self-administration. **Chapter 3** examines resting state PrL and NAc core signaling before and after prolonged abstinence from extended access (6hr) cocaine self-administration and relationships with drug seeking and taking behaviors. **Chapter 4** examines the effects of high frequency (80Hz) tACS on activity in the PrL and behavioral flexibility following prolonged abstinence from short access cocaine self-administration. Finally, the general discussion will incorporate findings from these 3 chapters and implications of these results.

Neurobiology of Substance Use Disorder

Substance use disorder (SUD) is a chronically relapsing illness characterized by cycles of drug consumption, abstinence from drug use, and resumption of drug taking (relapse) (Ahmed & Koob, 1998; Gawin, 1991; Koob & Volkow, 2016). The PrL, part of the prefrontal cortex (PFC),

and its projections to the NAc core are key structures within the brain's reward system and abstinence from repeated cocaine use produces disruptions in their baseline activity and function (Goldstein & Volkow, 2011; Robinson et al., 2001). It has also been well documented that this circuit is functionally linked to drug seeking and taking behavior (Chen et al., 2013; McFarland et al., 2003). Taken together, these data suggest that altered PrL-NAc core activity following prolonged abstinence from cocaine self-administration is likely linked to cognitive deficits associated with CUD.

Prefrontal Hypoactivity in SUDs

One particular maladaptive brain pathology linked to cognitive impairments in SUD is reduced function of frontal cortical regions associated with executive control (Aharonovich et al., 2006; Garavan & Hester, 2007; Goldstein & Volkow, 2002, 2011; Hanlon et al., 2013; Jentsch & Taylor, 1999). Neuroimaging studies in individuals with SUD exhibit reduced gray matter volume (Franklin et al., 2002; Liao et al., 2011; Tanabe et al., 2009) and brain glucose metabolism in the PFC compared to non-SUD controls that persists during abstinence from drug (Tomasi et al., 2019; Volkow et al., 1991). Given the critical role of the PFC in higher order cognitive processes such as impulsivity and decision making (Goldstein & Volkow, 2002, 2011), it is likely that this decrease in activity of the PFC is linked to cognitive deficits seen in individuals with SUD. For example, decreased resting cerebral blood flow in the PFC of individuals with CUD has been associated with impairments in decision making (Adinoff et al., 2003; Bolla et al., 2003). Furthermore, there is evidence that individuals with CUD who exhibit deficits in PFC dependent tasks are more likely to relapse and less likely to remain in treatment for SUD (Aharonovich et al., 2006; Garavan & Hester, 2007).

Resting State Neural Activity as a Biomarker for SUDs

A method used to examine cortical hypoactivity and its functional consequences is by studying resting state neural activity in these regions and its relationship to drug seeking/taking behaviors. In laboratory experiments, resting state typically refers to a period free from a task with little to no sensory stimulation. Preclinical studies have shown reduced resting state activity in the medial PFC (mPFC) and NAc following prolonged abstinence from cocaine (Gozzi et al., 2011; Hammer et al., 1993; Wolf, 2016). In support, preclinical models have also shown decreased resting state connectivity and disruptions in oscillatory signaling dynamics in the PFC-NAc circuit following prolonged abstinence from cocaine associated with cocaine-induced deficits in behavior (H. Lu et al., 2014; H. Lu & Stein, 2014; McFarland et al., 2003). Furthermore, preclinical studies have also revealed correlations between resting state functional connectivity in specific brain regions and escalation of drug taking (Gozzi et al., 2011; H. Lu et al., 2014). Critically, resting state functional connectivity (rsFC) studies in humans with SUD reveal decreased resting state corticolimbic connectivity compared to non-SUD controls (Gu et al., 2010; Wolf, 2016) and this decreased activity has been correlated with drug seeking and taking behaviors (Fedota & Stein, 2015; Geng et al., 2017), evidence that rsFC may be used as a biomarker for SUDs.

Additionally, a recent study by Kearney-Ramos and colleagues (2019) assessed if activity in the striatum can serve as a biomarker for the effectiveness of continuous theta burst stimulation (cTBS, a form of NIBS) to decrease cue-elicited drug craving. Here, they determined if baseline drug cue reactivity in the striatum (measured using fMRI) predicted striatal response to medial prefrontal cortex cTBS. Interestingly, they found that individuals with a high striatal response to cocaine cues at baseline had significantly attenuated striatal activity after real but not sham cTBS. These data demonstrate that the effects of mPFC cTBS on the neural circuitry of cue-elicited

craving are not uniform across people but instead may depend on an individual's baseline frontal-striatal reactivity to drug cues. As such, these individual differences may serve as a biomarker for cue-induced drug craving and seeking that may help inform personalized treatment strategies in SUDs.

Assessing Resting State Functional Connectivity Using *In Vivo* Electrophysiology: A Preclinical Approach to Identifying Neural Biomarkers in SUDs

In vivo electrophysiology is a technique used to measure neuronal activity in awake and behaving animals. Specifically, this technique can be used to record extracellular single cell activity (see **Chapter 4**) and local field potentials (LFPs, see **Chapters 2 and 3**). LFP recordings allow for the study of neurocircuit analysis and network-level processing both within and between brain regions (van der Meer et al., 2010). While higher frequencies (~600-10000Hz) can be used to detect spiking of individual neurons (i.e., single cells), LFPs represent lower frequencies (~1-300Hz) that demonstrate the slower changes across membrane potential in a larger population of cells (van der Meer et al., 2010). Since the LFP signal is representative of the average activity of such a large group of neurons, it can be implied that the coordinated activity of the LFP signal can be influenced by external factors (e.g., drug cues) or even other frequency bands within the same structure. Several metrics can be used to analyze LFP data, including power, or the strength (i.e., amplitude) of a given frequency band or oscillation, and coherence, the degree to which that oscillatory power co-occurs in signals across different brain regions (van der Meer et al., 2010).

Neural Oscillations are Linked to Specific Functions Measured via LFPs

The first two aims of this dissertation will focus on examining if resting state LFP activity in the PrL-NAc core circuit varies across weeks of prolonged abstinence (**Chapter 2**) as well as and its relationship with subsequent drug-associated behaviors (**Chapter 3**). Importantly, the LFP

signal oscillates at different frequencies, linked to behavioral correlates. In rats, previous work has defined functionally relevant frequency bands and informed our LFP analyses as follows: delta (0.5-4 Hz), theta (4-12 Hz), beta (12-30 Hz), low gamma (30-58 Hz), and high gamma (62-100 Hz) (McCracken & Grace, 2013). Delta oscillations have been functionally linked to processing of sensory input, specifically increases to cocaine cues relative to neutral cues suggesting a role in cue-induced cocaine craving (Reid et al., 2003). Theta oscillations in the PFC have been linked to cognitive control (Cavanagh & Frank, 2014), cocaine versus natural reward choice behaviors (Guillem & Ahmed, 2020) and inhibition of cocaine seeking in extinction (Müller Ewald et al., 2022). Oscillations in the beta range have been implicated in affective processing (Lipsman et al., 2014) as well as top-down mechanisms in cognitive processing (Amemori et al., 2018) and the maintenance of current cognitive states (Engel & Fries, 2010). Finally, gamma rhythms have been quantified in the NAc and separated into two functional bands: gamma-50 and gamma-80 Hz that have distinct behavioral correlates related to, for example, reward location, reward delivery (van der Meer & Redish, 2009), decision making (Donnelly et al., 2014) and flexible behavior (West et al., 2021). Furthermore, interactions between frequency bands have also been found to be functionally relevant. For example, delta-beta and theta-gamma cross-frequency coupling are thought to be causally linked to distinct components of cognitive control (Riddle et al., 2021). Taken together, these data reveal frequency-specific correlations between LFP signaling and behaviors implicated in SUD. However, precisely *how* resting state LFP oscillations at specific frequency bands in the PrL and NAc core are linked to measures of drug seeking/taking behaviors or distinct types of cognitive functions following prolonged abstinence from short or extended access cocaine self-administration is largely unknown.

Preclinical Models of Cocaine Self-administration: Short vs Extended Access

Preclinical models of SUDs are valuable as they allow us to better understand the neurobiological mechanisms of SUDs to guide treatment development for clinical populations. Of the many preclinical models used to study SUDs, the Carelli lab uses the drug self-administration model due to its high face validity. During self-administration, drug delivery is contingent on the motivation of the animal to take drug (Kuhn et al., 2019) because animals must perform an operant behavior to receive drug reinforcement. As such, we have the unique ability to examine aspects of drug seeking/taking that more closely mimic human motivation that non-contingent (experimenter-controlled) drug administration models cannot assess. Critically, preclinical approaches allow investigators to manipulate the amount of time that animals have access to drug during their daily sessions to model different aspects of SUDs. Two models frequently used are short access (1 to 3-hour daily sessions) and long access (6 to 12-hour daily sessions) procedures, the latter of which is thought to better model SUD because it engenders escalation of drug intake and compulsive (i.e., resistant to punishment-induced suppression of) drug taking (Ahmed & Koob, 1998; Mandt et al., 2015).

Some of these key aspects of human SUDs observed in preclinical models include 'incubation of craving' and 'escalation of drug taking'. Incubation of craving refers to time-dependent increased cue-induced drug craving observed in humans with SUD during prolonged abstinence (Parvaz et al., 2016; Volkow et al., 2006). Escalation of drug taking is the increased drug intake over time, a key process thought to reflect increased drug tolerance and underlie the transition from recreational to compulsive substance use (Ahmed & Koob, 1998). In preclinical models, incubation of drug craving refers to the increased cue-induced drug seeking in extinction sessions that occurs following prolonged abstinence from self-administration of drug (L. Lu et al.,

2004; Pickens et al., 2011; Wolf, 2016). Typically, rats are trained to perform an operant task (i.e., press a lever) to receive an intravenous infusion of drug paired with a discrete cue. Following experimenter-imposed abstinence, animals are returned to their behavioral chambers and tested on drug seeking behaviors in extinction where the previously learned operant behavior delivers the discrete cue, but no infusion of drug. As the length of the abstinence period increases, the level at which the animal responds for drug in extinction (i.e., is given only the drug cue and not the drug) increases. Incubation of craving has been reported during abstinence from self-administration of many drugs including cocaine and evident following training on both short and extended access models (Pickens et al., 2011; Wolf, 2016). Escalation of drug taking in preclinical models refers to a significant and progressive increase in drug intake over time (Ahmed et al., 2003; Ahmed & Koob, 1998, 1999). During short access models, the amount of drug taken during self-administration remains relatively stable across sessions. In contrast, the extended access model engenders significantly increased drug intake over sessions (Ahmed & Koob, 1998; Mandt et al., 2015). As such, the extended access paradigm is often considered a better model to study the transition from recreational drug use to compulsive drug taking (Ahmed & Koob, 1998). Notably however, evidence for incubation of craving has been reported following both short and extended-access drug self-administration (Guillem et al., 2014; Hollander & Carelli, 2005; Loweth et al., 2014; West et al., 2014). **Chapters 2 and 3** examine LFP recordings in the PrL and NAc core circuit using short and extended access self-administration procedures.

Effects of Cocaine Experience on Behavioral Flexibility

Chapter 4 examines the effects of repeated cocaine intake (using the short access self-administration model) on a specific cognitive process that is impaired in humans with SUDs called 'behavioral flexibility'. To make optimal decisions, individuals must be able to adapt their behavior

in response to changes in the environment, a process known as behavioral flexibility (West et al., 2021). A key marker of SUDs are impairments in flexible behavior which in turn may lead to poor decision making and continued drug use (Lucantonio et al., 2012; Turner et al., 2009). One preclinical method used to measure behavioral flexibility is the reinforcer devaluation task. Here, a previously rewarding food is devalued (e.g., by conditioned taste aversion, CTA). Specifically, rats are given free access to a normally palatable food, then that food is paired with an illness inducing agent such as lithium chloride (LiCl). Following just one pairing of the food with LiCl, animals will shift behavior to avoid the previously palatable, but now devalued, food reward. Following cocaine self-administration and prolonged abstinence however, animals are unable to shift behavior and continue to respond for the now devalued reward (Schoenbaum & Setlow, 2005; West et al., 2021). Critically, the PrL and NAc core are both implicated in the acquisition of cue-outcome associations required to guide flexible behaviors (del Arco et al., 2017; Ostlund & Balleine, 2005; West & Carelli, 2016) and processing in these regions is altered following cocaine self-administration experience (Carelli & West, 2014; Saddoris & Carelli, 2014). Further, recent optogenetic work in the Carelli lab determined that the PrL-NAc core circuit is necessary for behavioral flexibility and activation of this circuit after cocaine experience restored cocaine-induced deficits in neural signaling and behavioral flexibility (West et al., 2021).

Transcranial Alternating Current Stimulation (tACS) as a Promising Treatment Strategy for SUDs.

Given the critical role of the PFC in higher order cognitive processing, and its anatomic link to brain 'reward' structures such as the NAc, new treatment strategies seek to correct drug-induced brain abnormalities to help those with SUDs shift behavior to circumvent continued drug use. While optogenetics is a powerful tool to examine the role of discrete neural circuits in maladaptive behaviors associated with SUD, its clinical relevance is currently limited due to its

invasive approach. As such, another approach to reverse cocaine-induced deficits in neural signaling and restore cognitive abilities is noninvasive brain stimulation (NIBS). Notably, there is evidence that using NIBS techniques, particularly tACS, may be a promising form of treatment for SUDs (Daughters et al., 2020; McKim et al., 2021; West et al., 2021). One type of NIBS, transcranial alternative current stimulation (tACS), is the focus of **Chapter 4**. Briefly, the cortex consists of synchronized neuronal activity that generates weak electric fields that can be measured via electroencephalography (EEG) in humans and LFPs in animals. Critically, active cortical networks are susceptible to weak perturbations of the membrane voltage of a large number of neurons by electric fields. NIBS with weak, exogenous electric fields (such as that resulting from tACS) can be used to modulate these cortical oscillations by application of a weak alternating electrical current to the scalp (Fröhlich, 2014, 2015; Fröhlich et al., 2014). Given the known disruption in synchronized neuronal activity across brain structures in individuals with SUD, tACS holds great promise as a treatment strategy for SUDs by modulating disrupted cortical oscillations.

The conceptual advantage of tACS over other NIBS approaches is that stimulation frequencies can be tailored to directly modulate specific neuronal activity patterns that our electrophysiology studies show are altered after cocaine experience (West et. al., 2021; Haake, 2021). In contrast, transcranial magnetic stimulation (TMS, of great interest in SUD research (Bellamoli et al., 2014) applies a strong magnetic field via a TMS coil. Due to the relative size differences in the coils used between humans and rodents, application of TMS in rats would result in stimulation of greater brain volume than humans making the study of specific mechanisms difficult. In a recent paper published from our lab, we showed cocaine-related dampening of PrL-NAc core oscillations at the gamma-80 frequency after cocaine experience and successfully used

tACS to entrain oscillations at this frequency and restore impaired behavioral flexibility (West et al., 2021).

Goals of Dissertation

Prolonged abstinence from chronic cocaine use produces significant neuroadaptations in both the PFC and NAc, areas implicated in executive and reward processing (Wolf, 2016). These shifts in neural activity and function are thought to be related to deficits in higher order cognitive processing such as decision-making and behavioral flexibility evident in SUDs (Goldstein & Volkow, 2011; West et al., 2021). In human neuroimaging studies, evidence also indicates that resting state functional connectivity can predict aspects of drug seeking and taking behaviors (e.g., craving) and may serve as a biomarker for SUDs (Kearney-Ramos et al., 2019), but those relationships are still not well understood.

The following three specific aims were completed to examine the signaling dynamics of the PrL and NAc core before and following prolonged abstinence from cocaine, their relationships with subsequent drug seeking and taking behaviors, and the effects of using tACS as a way to reverse cocaine-induced deficits exhibited in behavioral flexibility. By characterizing cocaine-induced disruptions in neural activity and associations to SUD-related behaviors, we will gain crucial insight into neural mechanisms underlying these processes and provide information that may have implications for optimal tACS parameters for use in clinical populations. Ideally, this information will help inform us how to better help those living with SUDs, especially those in underserved populations, as they have historically been disproportionally affected by SUDs.

Specific Aims

1. To characterize spontaneous (resting state) LFP activity in the PrL and NAc core across each week of prolonged (1 month) abstinence from short access (2 hour) cocaine self-administration in male Sprague Dawley rats.

Numerous studies have shown cocaine-induced neuroadaptations within the PrL-NAc core system, a circuit critically linked with drug seeking and taking behaviors (Ma et al., 2014; McGlinchey et al., 2016; Wolf, 2016). Recent findings from the Carelli lab have documented changes in PrL-NAc core resting state local field potential (LFP) activity following prolonged (1 month) abstinence from short access (2-hour daily sessions for 2 weeks) cocaine self-administration in male rats (Haake, 2021). Those findings revealed that a history of short access cocaine self-administration and 1 month abstinence resulted in an overall (across all frequency bands) dampening of resting state LFP activity in this circuit compared to a 1-month abstinence from saline. Critically, 1-month abstinence from cocaine resulted in a significant reduction in LFP activity in this circuit in the beta frequency range (12-30Hz) when compared to rats that underwent only 1 day of cocaine abstinence. However, it remains unclear *when* during this prolonged abstinence period these neuroadaptations begin. To address this issue, I analyzed an existing electrophysiology data set from the dissertation of Dr. Rachel Haake (Haake, 2021) to determine how resting state LFP activity in the PrL and NAc core changes as a function of time during prolonged abstinence from short access cocaine self-administration. Specifically, I examined changes in PrL and NAc core oscillatory dynamics (power) and their functional connectivity (coherence) across specific LFP frequency bands during selective time periods post cocaine self-administration. Our findings show an overall (but not significant) decrease in PrL-NAc core coherence and no changes in PrL or NAc core power following a one-month abstinence from short access (2hr) cocaine self-administration.

2. To characterize spontaneous (resting state) LFP activity in the PrL and NAc core before and following prolonged (1 month) abstinence in male Sprague Dawley rats with a history of extended access (6 hour) cocaine self-administration.

While short access (e.g., 2-hour sessions/day) cocaine self-administration studies have yielded critical insights into neurobiological mechanisms underlying cocaine seeking/taking, it has been well established that long access models (6-hour daily sessions) engender differential drug seeking/taking compared to short access procedures (Ahmed et al., 2002; Ahmed & Koob, 1998; Koob & Simon, 2009; Koob & Volkow, 2016). Not only do rats consume more drug per day, but long access paradigms are also characterized by escalation of drug consumption over repeated days (Ahmed et al., 2002; Ahmed & Koob, 1998), a hallmark of the transition to compulsive drug seeking/taking in humans. In addition, 'incubation of cocaine craving' (i.e., increased drug seeking in extinction following prolonged abstinence) is more pronounced following extended (compared to short) access cocaine self-administration in rats (Wolf, 2016). However, the dynamics of resting state LFP activity within the PrL-NAc core circuit following extended access cocaine and prolonged (1 month) abstinence, and its link to subsequent cocaine taking and seeking behaviors, are unknown. Here, I used *in vivo* electrophysiology to examine resting PrL and NAc core activity LFPs before and after extended access (6-hour sessions/day, for 2 weeks) cocaine self-administration. Resting state neural activity from the PrL and NAc core was recorded before extended access cocaine and water self-administration (controls), immediately following self-administration training, and after a 30-day experimenter-imposed abstinence period. Contrary to our original hypothesis and literature, we did not find a significant decrease in overall (broadband) LFP activity following cocaine experience and/or extended abstinence. We did however observe correlational relationships between resting state LFP activity in the PrL and NAc core and drug

seeking and taking behaviors suggesting neural activity in these regions may serve as a biomarker for CUD.

3. To determine if 80 Hz tACS can restore cocaine-related deficits in PrL single cell activity and behavioral flexibility in male and female Sprague Dawley rats.

The prior aims provided critical insight into the relationship between resting state PrL and NAc core signaling and drug seeking/taking behaviors in rats trained on short (**Chapter 2**) or extended (**Chapter 3**) access cocaine self-administration procedures. However, numerous studies in the Carelli lab have also examined the effects of cocaine history (short and extended access) and prolonged (1 month abstinence) on aspects of nondrug-related associative learning and their neural underpinnings (Moschak et al., 2018; Saddoris et al., 2016; Saddoris & Carelli, 2014; West et al., 2021). In this aim, we focused on one cognitive process termed 'behavioral flexibility'. Critically, the PrL and NAc core have been implicated in behavioral flexibility (Ostlund & Balleine, 2005; Singh et al., 2010; West & Carelli, 2016), and a history of cocaine self-administration and prolonged abstinence has been shown to lead to altered neural signaling in this circuit and impaired behavioral flexibility (West et al., 2021; Wolf, 2016). In a recent report from the Carelli lab, a history of short-access cocaine self-administration was shown to be related to a dampening of LFP coherence in the PrL-NAc core circuit, particularly at the high gamma (80 Hz) frequency (West et al., 2021). Notably, it was also revealed that reversing dampened LFP activity (either using optogenetics or tACS) restored cocaine-induced deficits in behavioral flexibility (West et al., 2021). While this published study focused on the effects of tACS on PrL-NAc core LFP activity and its relationship with behavioral flexibility, the effects of tACS on single unit activity (also recorded with our electrophysiology methods) and its relationship to flexible behavior, particularly in the PrL, has not yet been explored. As such, here, I examined PrL single unit activity during a behavioral flexibility task in male and female rats with a history of short

access (2hr) cocaine self-administration and either sham (control) or 80 Hz tACS treatment using an unpublished data set. Critically, we found that while Pavlovian conditioning was not affected by cocaine history, there were significant impairments in behavioral flexibility. Furthermore, we did not see sex differences in terms of behavior even though female rats consumed more cocaine during self-administration. However, across sex, there were significant differences in PrL phasic neural population responses that were restored to control proportions using 80Hz tACS.

CHAPTER 2

PrL AND NAc POWER AND COHERENCE DOES NOT CHANGE ACROSS PROLONGED ABSTINENCE FROM SHORT ACCESS COCAINE

Introduction

Individuals with cocaine use disorder exhibit a general dampening of neural activity in the prefrontal cortex (PFC) following extended abstinence from cocaine use (Goldstein & Volkow, 2011). Likewise, numerous preclinical (rodent) studies have shown neuroadaptations within one region of the PFC, the prelimbic cortex (PrL), and its connections to the nucleus accumbens (NAc) core, is a circuit critically linked with drug seeking and taking behaviors (Chen et al., 2013; McFarland et al., 2003; McGlinchey et al., 2016; Wolf, 2016). In support, the dissertation work of Dr. Rachel Haake from the Carelli lab (Haake, 2021) documented pronounced decreases in PrL-NAc core resting state electrophysiological activity following prolonged (1 month) abstinence from short access (2-hour daily session for 2 weeks) cocaine self-administration in male rats (Haake, 2021), compared to similar recordings in rats undergoing only 1 day of cocaine abstinence. However, it remains unclear *when* during this prolonged abstinence period these decreases in neural activity occur. This study was completed to build upon and analyze an existing data set from Dr. Haake's dissertation to determine how resting state PrL-NAc core activity changes as a function of time *during* prolonged abstinence.

The methodology used by Dr. Haake and in the present chapter involves local field potential (LFP) recordings, a type of electrophysiology that can be measured in awake, behaving rats. Here, electrophysiological recordings at lower frequencies (~1-300 Hz) reflect slower

membrane potential (voltage) changes in a larger population of neurons compared to that measured with single unit recordings detected at higher frequencies. Because the LFP signal is an average of a relatively large area, the presence of rhythmic oscillations in the LFP implies some degree of systematic, coordinated activity, which can be related to external stimuli (e.g., drug associated cues) and behavior, as well as resting state activity (i.e., measured when the animal is awake, but at rest; not engaged in a task and in the absence of discrete external stimuli) that may reflect prefrontal hypofunction. Several metrics can be used to analyze LFP data, including *power*, or the strength (i.e., amplitude) of a given frequency band or oscillation, and *coherence*, or the degree to which that oscillatory power co-occurs in signals across different brain regions (van der Meer et al., 2010).

Importantly, LFP oscillations in specific frequency bands have been associated with particular types of behaviors and/or cognitive function. In the rat, functionally relevant frequency bands include: delta (0.5-4 Hz), theta (4-12 Hz), beta (12-30 Hz), low gamma (30-58 Hz), and high gamma (62-100 Hz) (McCracken & Grace, 2013). Delta oscillations have been functionally linked to processing of sensory input, specifically increases to cocaine cues relative to neutral cues suggesting a role in cue-induced cocaine craving (Reid et al., 2003). Theta oscillations in the PFC have been linked to cognitive control (Cavanagh & Frank, 2014), cocaine versus natural reward choice behaviors (Guillem & Ahmed, 2020) and inhibition of cocaine seeking in extinction (Müller Ewald et al., 2022). Oscillations in the beta range have been implicated in affective processing (Lipsman et al., 2014) as well as top-down mechanisms in cognitive processing (Amemori et al., 2018) and the maintenance of current cognitive states (Engel & Fries, 2010). Finally, gamma rhythms have been quantified in the NAc and separated into two functional bands: gamma-50 and gamma-80 Hz that have distinct behavioral correlates related to, for example, reward location,

reward delivery (van der Meer & Redish, 2009), decision making (Donnelly et al., 2014) and flexible behavior (West et al., 2021). Together, these data suggest that there are frequency-specific relationships between LFPs in the PFC and behaviors that may be implicated in SUDs.

In the present study, an existing data set from Dr. Haake's dissertation was used to determine how resting state PrL-NAc core activity changes as a function of time during prolonged (1 month) abstinence from cocaine. Specifically, LFP activity was initially recorded immediately after 2 weeks of short access (2-hour daily sessions) of cocaine self-administration. In addition, rest LFP recordings were measured on days 9, 20 and 27 (day 28 for one animal due to noise) of the abstinence period. Due to technical difficulties, the existing data set was not expanded, so the analysis in this chapter focuses only on LFP activity across those time points (and not with drug taking or seeking behaviors).

Methods

Animals

Adult, male Sprague Dawley rats (Charles River) aged 90-120 days (300-350 grams) at the beginning of the study were used. Attempts to increase n (to 10 total) were unsuccessful due to various technical problems, so n=5 rats from Dr. Haake's dissertation were used here. All rats were housed individually and maintained on a standard 12:12 hour light-dark cycle (lights off at 07:00 AM). During preoperative behavioral training, rats were restricted to no less than 85% of their free-fed body weight by access to 20-25g of standard rat chow (Purina RMH3000) per day (*ad libitum* water). During cocaine self-administration and testing, rats were maintained on 30 ml of water/day (*ad libitum* food). All animal procedures were conducted in accordance with the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals and approved by the University of North Carolina, Chapel Hill Institutional Animal Care and Use Committee (IACUC).

Apparatus

Behavioral sessions were conducted in 43 x 43 x 53 cm custom-made Plexiglas operant chambers housed within commercial sound-attenuating cubicles (Med. Associates, Inc., St. Albans, VT, USA). Each chamber contained two retractable levers (Coulbourn Instruments, Whitehall, PA, USA) 17 cm apart, with a cue light positioned 6.5 cm above each lever. A food/water receptacle was centered between the two levers, approximately 4 cm above the floor. A house light and speaker were centrally located on the opposite wall of the chamber. White noise and ventilation were provided by a wall-mounted fan. A commutator (Crist Instruments, Hagerstown, MD, USA) was mounted to the top of the chamber, and allowed for attachment of the electrophysiological recording cable, as well as insertion of the i.v. infusion line. Drug delivery

was provided by a computer-controlled syringe pump located outside the chamber. The chamber and its components were connected to a computer interface (Med Associates) for real-time automated data collection.

Surgical Procedures

Rats were anesthetized with a ketamine hydrochloride and xylazine hydrochloride (i.m., 100 mg/kg and 10 mg/kg, respectively) mixture and implanted with custom-made intrajugular catheters (Access Technologies, Skokie, IL, USA) for i.v. cocaine self-administration. In addition, microwire electrode arrays (8 microwires/array; 50 μm diameter; NB Labs, Denison TX, USA) were implanted into the PrL (AP: +2.6, ML: ±0.6, DV: -4.0 from skull) and ipsilateral NAc core (AP: +1.5, ML: ± 1.5, DV: -7.0 from skull; sides counterbalanced) in the same surgery, described previously (Haake et al., 2019; West et al., 2021). Animals were given at least 7 post-operative recovery days during which an anti-inflammatory medication (meloxicam, 1 mg/kg, s.c.) and an antibiotic (cefazolin, 10 mg/ml, i.v.) were given daily for three and five days, respectively. Catheters were flushed daily with heparinized saline (0.1 ml of 30 U/ml, i.v.), and Taurolidine-Citrate catheter lock solution (TCS, 0.03 ml, i.v.) was used to prevent clot formation and bacterial or fungal growth. Food and water were available *ad libitum* during post-operative recovery.

Experimental Design

Food-restricted rats were initially trained in 2-5 daily ≤ 60-min sessions to lever press for sucrose pellets (45 mg delivered into the receptacle) then underwent surgery and recovery followed by reestablishment of lever pressing, using standard procedures (Haake et al., 2019; Wheeler et al., 2008). **Figure 2.1** shows a schematic diagram of the cocaine self-administration and abstinence portion of the experimental design from which data were used in this chapter, described in detail below.

Briefly, one day prior to the beginning of cocaine self-administration training, rats were placed in an operant chamber (contextually distinct from the self-administration and testing chamber) and connected to the electrophysiology recording system. Resting state LFPs in the PrL and NAc core were simultaneously recorded for 15 min while animals were not engaged in a behavioral task (Day 0, not shown). Due to noise issues, these data were not included in the following analyses. On subsequent cocaine self-administration days 1-14, rats were placed in the self-administration chamber and a lever was extended into the chamber with the cue light above it illuminated. During 2-hour daily cocaine self-administration sessions, lever depression resulted in intravenous cocaine delivery (6 s infusion, 0.33 mg/infusion, ~1 mg/kg) paired with termination of the cue light and simultaneous onset of a tone (67 dB, 1 kHz)/houselight conditioned stimulus (CS) for 20 s. One day following the final cocaine self-administration session, rats were returned to the LFP recording chamber for a 15 min post-self-administration rest LFP recording (Day 1, Fig. 1). Next, rats underwent a prolonged (1 month) abstinence. During experimenter-imposed abstinence, rats remained in their home cages for one month except during rest LFP recordings (15 min each) conducted on days 9, 20, and 27 or day 28 (for one rat) of abstinence (**Fig. 2.1**).

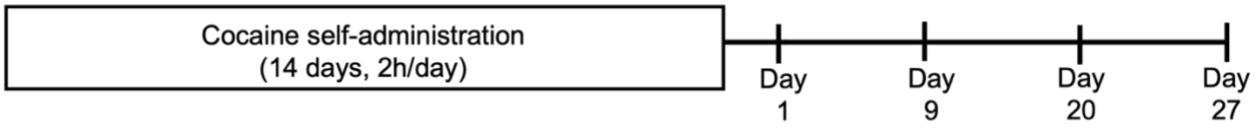

Figure 2.1. Aim 1 experimental design. Rats were trained to self-administer cocaine (0.33 mg/inf, 2 hr daily sessions over 2 weeks) followed by one-month experimenter-imposed abstinence. LFP recordings used in this chapter were conducted on days 1, 9, 20 and 27 (although one rat was recorded on day 28 instead of day 27 due to noise issues).

Electrophysiology

Electrophysiology procedures used here and in subsequent aims have been described in detail previously (Carelli, 2000; Hollander & Carelli, 2005) and in the dissertation document of

Dr. Rachel Haake (Haake, 2021). Briefly, rats were connected to a flexible recording cable attached to the commutator which allowed free movement within the chamber. Online isolation and discrimination of LFPs was accomplished using a commercially available neurophysiological system (OmniPlex system or multichannel acquisition processor [MAP] system; Plexon, Inc., Dallas, TX, USA), described previously (Haake et al., 2019; West et al., 2021). Continuous recordings from each electrode were virtually referenced (PlexControl; Plexon, Inc.) and fed into a Pentium computer. Continuous signals were low-pass filtered (≤ 200Hz) to isolate LFPs from single unit activity. LFPs were recorded and analyzed in Neuroexplorer (Plexon, Inc). Finally, an additional computer processed operant chamber input and output (Med Associates, Inc) and sent digital outputs corresponding to each task or behavioral event into the electrophysiology recording system to be time stamped along with the neural data.

Histology

Electrode placements in the PrL and NAc core were histologically confirmed and reported by Dr. Haake (Haake, 2021).

Data Analysis

LFPs were used to examine neural activity within the PrL and NAc core (power) and across the PrL-NAc core circuit (coherence). Power spectral densities (PSDs) were normalized by dividing power within each 2 Hz bin in the entire frequency band (0-100 Hz [54-68 Hz noise band excluded]) by the average power across all frequency bands. Raw electrical outputs from each region were low-pass filtered (≤200 Hz) from individual wires implanted within the PrL and NAc core. Two-way ANOVAs were used to examine if shifts in resting state LFP PrL and NAc core power and their coherence across abstinence. On each rest LFP recording day (days 1, 9, 20, 27 or 28 of abstinence), peak delta (0.5-4 Hz), theta (6-10 Hz), beta (12-30 Hz), low gamma (32-52 Hz),

and high gamma (70-100 Hz) PrL-NAc core coherence values were calculated individually for each animal. Separate two-way repeated measure ANOVAs of resting state LFP with frequency (0-100Hz) and abstinence duration (days 1, 9, 20, 27 or 28 of abstinence) as factors were used to examine shifts in PrL and NAc oscillatory power and coherence across frequencies over time. One-way ANOVAs were used to examine if shifts in rest LFP power in the PrL-NAc core peak coherence at each frequency band occurred across abstinence weeks. Rest LFP PrL and NAc core power and coherence values were exported directly from Neuroexplorer, and post hoc tests used where appropriate. All analyses were considered statistically significant at α=0.05. Statistical and graphical analyses were performed using GraphPad Prism 8.0 for Windows (GraphPad Software, La Jolla, CA).

Results

PrL-NAc core coherence, but not PrL or NAc core oscillatory power, decreased across abstinence from cocaine self-administration

Normalized power spectral densities (PSDs) in the PrL and NAc core were examined across the 4 weeks of abstinence (i.e., on days 1, 9, 20, 27 or 28 of abstinence). **Figure 2.2** shows PSD recordings in the PrL (left) and NAc core (right) across abstinence. For PSD recordings in the PrL, a 2-way ANOVA revealed a main effect of frequency ($F_{53, 212} = 160.3$, $p < 0.0001$), but no main effect of time ($F_{3,12} = 0.5812$, $p = 0.6385$) and a trending frequency x time interaction ($F_{159,636} = 1.192$, $p = 0.0741$). For the NAc core, a 2-way ANOVA revealed a main effect of frequency ($F_{53, 212} = 81.64$, $p < 0.0001$), but no main effect of time ($F_{3,12} = 0.6316$, $p = 6086$), or frequency x time interaction ($F_{159,636} = 0.5750$, $p > 0.9999$). In summary, there were no significant changes in oscillatory power (normalized PDS) in the PrL or NAc core across the 4 weeks of cocaine abstinence.

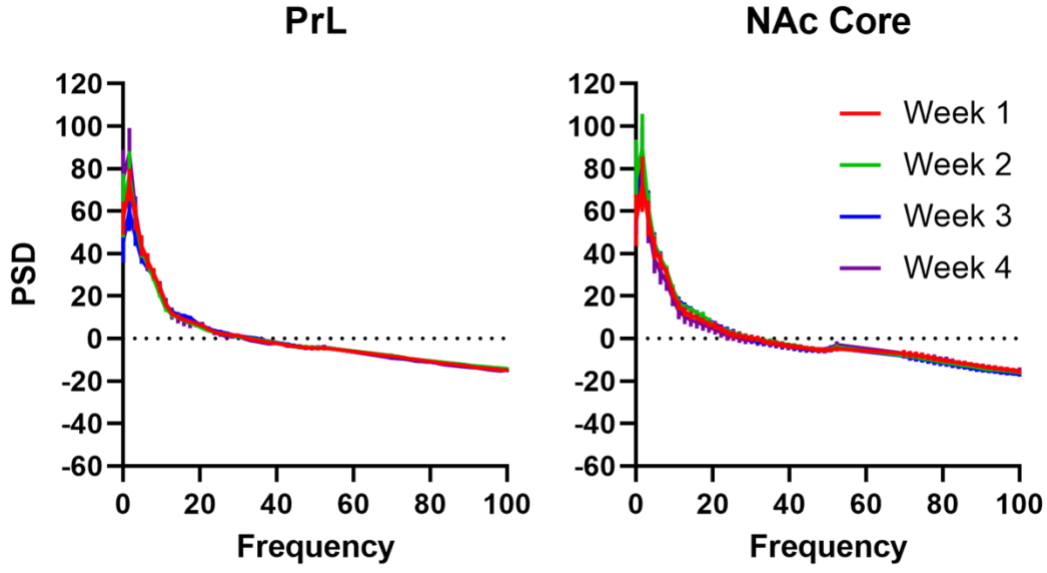

Figure 2.2. Normalized resting state power spectral density (PSD) across abstinence in the prelimbic cortex (PrL, left) and nucleus accumbens (NAc) core (right)

Next, we examined if broadband shifts in resting state PrL-NAc core coherence occurred across all frequencies during the 4 weeks of abstinence (**Fig. 2.3**). A repeated measures 2-way ANOVA with frequency and time as factors revealed a main effect of frequency ($F_{53, 212} = 5.248$, $p < 0.0001$), a trending main effect of time ($F_{3, 12} = 3.057$, $p = 0.0696$), but no frequency x time interaction ($F_{159, 636} = 0.7534$, $p = 0.9849$). Finally, we determined if shifts in peak PrL-NAc core coherence within each frequency band of interest (delta, theta, beta, low and high gamma) were evident across weeks of abstinence (**Fig. 2.4**). Separate one-way ANOVAs examining coherence in each frequency band revealed no significant changes across time, although there were trending main effects of time at the theta ($F_{3, 12} = 2.941$, $p = 0.0763$; **Fig. 2.4B**) and beta ($F_{3, 12} = 2.718$, $p = 0.0912$; **Fig. 2.4C**) ranges. These data show an overall (but not significant) decrease in PrL-NAc core coherence following a one-month abstinence from short access (2hr) cocaine self-administration.

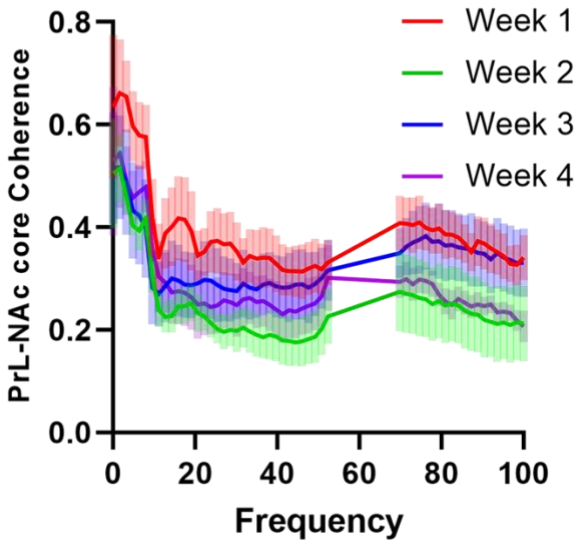

Figure 2.3. Resting state PrL-NAc core coherence across abstinence

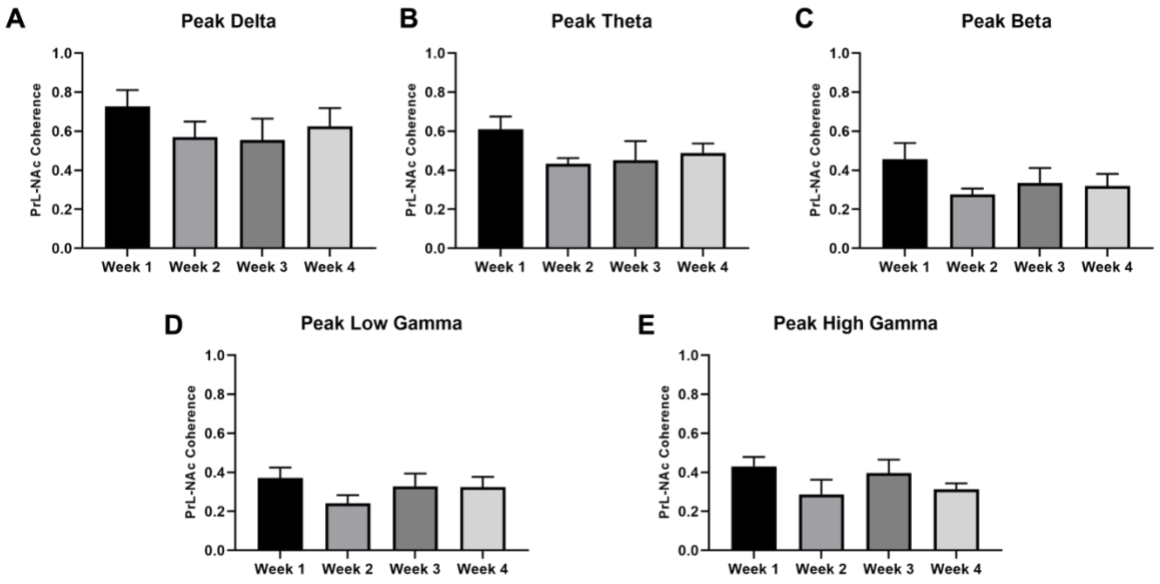

Figure 2.4. Peak PrL-NAc core coherence across abstinence. **A.** PrL-NAc core coherence at peak delta **B**. Peak theta **C.** Peak beta **D.** Peak low gamma **E.** and Peak high gamma

Discussion

The current study was designed to examine if resting state LFP activity (power and coherence) in the PrL and NAc core decrease across each week of prolonged (1-month) abstinence following short access (2hr) cocaine self-administration. This is a timely and important topic since decreased function in the human PFC has been well-documented in individuals living with substance use disorders (SUDs) (Goldstein & Volkow, 2002, 2011). We hypothesized that short access cocaine self-administration followed by a month of abstinence would lead to a generalized, broadband decrease in both PrL and NAc core oscillatory power and its functional connectivity (coherence) across all frequency bands. However, although there was a clear decrease in LFP coherence by week 4 of abstinence (in both broadband activity and peak coherence at each frequency band), these findings were not significant. Collectively, the results indicate that a significant hypofunction in resting state activity in either the PrL or NAc core or their coherence is not evident this system, despite this circuit's clear link with drug taking and seeking behaviors (Goldstein & Volkow, 2011; Wolf, 2016).

There are numerous studies in the literature supporting the view that following prolonged abstinence from cocaine, the brain undergoes neuroadaptations evident in both humans with SUDs as well as animal models of this disorder (Volkow & Morales, 2015; Wolf, 2016). One malfunction in particular is the hypofunction, or decreased activity, of the PFC which has been observed in both preclinical and clinical models of cocaine use disorder (Aharonovich et al., 2006; Garavan & Hester, 2007; Goldstein & Volkow, 2002, 2011). The PFC is critical for higher order processes such as decision making and executive function and following prolonged abstinence from cocaine, deficits are seen in these processes (Goldstein & Volkow, 2002, 2011; Wolf, 2016). As such, it has been speculated that hypofunction of the PFC is linked to these negative consequences of

repeated drug use in SUDs. When exactly during abstinence this hypofunction occurs is not well understood. Although examination of LFP activity in this chapter, particularly PrL-NAc core coherence, shows a slight decrease in activity across weeks, this was not significant. As such, a main objective of the current study, to determine when during the 4 weeks of prolonged abstinence this decrease in prefrontal cortical function begins, could not be determined. These findings may indicate that resting state LFP measurements in the PrL and NAc core system may not mirror the observed hypofunction that has been well documented using neuroimaging techniques in humans with SUDs (Franklin et al., 2002; Goldstein & Volkow, 2002).

Importantly, there are limitations of the current study that may underlie the absence of hypoactivity observed in our resting state LFP recordings. First, there was a low number of animals used in these analyses (n=5 total). By increasing the n, an increase in statistical power may reveal significant changes in resting state LFP activity in the PrL-NAc core system across weeks of abstinence. Further, the relatively low quantity of drug that was self-administered using the short access model (i.e., 2 hours/day) may have limited the amount of hypoactivity observed.

In the next chapter, I sought to build upon this current chapter to examine the role of the PrL and NAc core in drug seeking and taking behaviors in several important ways. First, since cocaine use disorders in humans involve the consumption of large quantities of drug (Ahmed & Koob, 1998; Gawin, 1991), the study in **Chapter 3** involves the use of the extended access (6hr) cocaine self-administration model. Second, a control group of rats were included that were trained to self-administer water that enabled me to determine if any changes in LFP resting state activity were cocaine-specific. Third, I increased the n in each group of rats (cocaine versus water self-administration) to increase statistical power of my analysis. Fourth, instead of completing measurements throughout abstinence, the next chapter incorporated 3 resting state recording

sessions (one before self-administration training, the second immediately after it, and the third completed after 1 month of abstinence) as well as analysis of drug taking and seeking behaviors. As such, a major goal of the study in the next chapter was to expand the current work and determine if resting state PrL and NAc core LFP activity measured at specific times in the SUD cycle could serve as predictors (biomarkers) of subsequent cocaine taking and cocaine seeking behaviors.

CHAPTER 3

RESTING STATE PrL AND NAc LFP PREDICTS ASPECTS OF COCAINE SEEKING AND TAKING BEHAVIORS

Introduction

Substance use disorders (SUDs) and animal models of this disease involve maladaptive brain pathologies that are a consequence of repeated drug use (Volkow & Morales, 2015; Wolf, 2016), including a reduction of activity in prefrontal cortical (PFC) regions (Aharonovich et al., 2006; Garavan & Hester, 2007; Goldstein & Volkow, 2002; Hanlon et al., 2013; Jentsch & Taylor, 1999). For example, neuroimaging studies in individuals with cocaine use disorder (CUD) show reduced gray matter volume (Fein et al., 2002; Franklin et al., 2002) and brain glucose metabolism in the PFC compared with matched controls, detectable even after several weeks to months of cocaine abstinence (Matochik et al., 2003; Volkow et al., 1992)**.** Given the critical role of the PFC in higher order executive functions and its anatomic link to brain 'reward' structures (e.g., nucleus accumbens, NAc), it is important to examine mechanisms underlying compromised PFC neural signaling in SUDs.

In this regard, research has focused on identifying biomarkers within PFC system that can help predict those individuals at risk for developing SUDs. For example, preclinical neuroimaging studies have shown reduced resting state activity in the medial PFC and NAc following prolonged abstinence from cocaine (Gozzi et al., 2011; Hammer et al., 1993; Wolf, 2016), and have revealed correlations between resting state functional connectivity and escalation of drug taking (Gozzi et al., 2011; H. Lu et al., 2014). These findings share similarities with human

neuroimaging studies showing reduced corticolimbic resting state functional connectivity in people with CUDs who are abstaining from cocaine use (Gu et al., 2010; McHugh et al., 2014). This work supports the view that alterations in PFC resting state functional connectivity may serve as a biomarker to identify individuals who are susceptible to develop SUDs in human populations (Fedota & Stein, 2015; McHugh et al., 2014).

Resting state neural activity in the PFC and associated regions may also help identify the individual effectiveness of specific treatment strategies for SUDs. For example, Kearney-Ramos and colleagues assessed if activity in the striatum can serve as a biomarker for the effectiveness of continuous theta burst stimulation (cTBS, a form of noninvasive brain stimulation, NIBS) to decrease cue-elicited drug craving (Kearney-Ramos et al., 2019). They examined if baseline drug cue reactivity in the striatum (measured using fMRI) predicted striatal response to medial PFC cTBS. Interestingly, they found that individuals with a high striatal response to cocaine cues at baseline had significantly attenuated striatal activity after verum but not sham cTBS. These data demonstrate that the effects of medial PFC cTBS may depend on an individual's baseline frontal-striatal reactivity to drug cues and this information may help inform personalized cTBS treatment strategies in SUDs.

In animal studies, *in vivo* local field potential (LFP) recordings can be used to measure resting state neuronal activity and allow for the study of neurocircuits and network-level processing, both within and between brain regions (Fröhlich et al., 2014; van der Meer et al., 2010). Importantly, the LFP signal oscillates at different frequencies, each linked to specific behavioral correlates (Fröhlich et al., 2014; Riddle et al., 2021). In rats, functionally relevant frequency bands include delta (0.5-4 Hz), theta (4-12 Hz), beta (12-30 Hz), low gamma (30-58 Hz), and high gamma (62-100 Hz) (Fatahi et al., 2020; Lalla et al., 2017; McCracken & Grace, 2013). Since the

LFP signal is representative of the average activity of a large group of neurons, the coordinated activity of the LFP signal may be influenced by external factors (e.g., drug cues) or even other frequency bands within the same structure (van der Meer et al., 2010; van der Meer & Redish, 2009).

Here, we used *in vivo* electrophysiology in rats to characterize spontaneous (resting state) LFP activity in the prelimbic cortex (PrL) and NAc core, and their functional connectivity, before and after cocaine self-administration experience. We focused on recordings in the PrL and NAc core since numerous studies have causally linked this circuit to drug seeking and taking behaviors (Jean-Richard-dit-Bressel & McNally, 2019; Pickens et al., 2011; Wolf, 2016) and enhanced drug seeking following prolonged abstinence (termed 'incubation of craving'; Pickens et al., 2011; Wolf, 2016). These findings were compared to rats trained to self-administer water to examine if any neural changes were unique to cocaine. The study was completed with two primary objectives. First, we studied if cocaine self-administration and/or abstinence resulted in broadband reductions in resting-state activity in the PrL and NAc core and their functional connectivity. Second, we determined if resting-state LFP could serve as predictors (biomarkers) of subsequent cocaine seeking and/or taking behaviors.

Methods

Animals

Adult male Sprague Dawley rats (Charles River, n = 20) aged 90-120 days (300-350 grams) at the beginning of the study were used. Rats were housed individually and maintained on a standard 12:12 hour light-dark cycle (lights off at 08:00 AM) and divided into two groups for cocaine (n=10 rats) or water (n=10 rats) self-administration. During self-administration and before extinction sessions, rats were maintained on 30 mL of water per day (*ad libitum* food). All animal procedures were conducted in accordance with the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals and approved by the University of North Carolina, Chapel Hill Institutional Animal Care and Use Committee (IACUC).

Apparatus

Behavioral sessions were conducted in 12 x 10 x 7 cm Plexiglas operant chambers housed within commercial sound-attenuating cubicles (Med. Associates, Inc., St Albans, VT, USA). Each chamber contained two retractable levers (Coulbourn Instruments, Whitehall, PA, USA) 17 cm apart, with a cue light positioned 6.5 cm above each lever. A food/water receptacle was centered between the two levers, approximately 4 cm above the floor. A nosepoke receptacle, houselight, and speaker were centrally located on the opposite wall of the chamber. White noise and ventilation were provided by a wall-mounted fan. Drug delivery was provided by a computer-controlled syringe pump located outside the chamber. The chamber and its components were connected to a computer interface (Med Associates) for real-time automated data collection.

Surgical Procedures

Rats were anesthetized with an intraperitoneal injection of ketamine hydrochloride and xylazine hydrochloride (i.p., 100mg/kg and 10mg/kg, respectively) and implanted with custom-

made intrajugular catheters (Access Technologies, Skokie, IL, USA) for self-administration. Microelectrode arrays (8 microwires/array; 50 μm diameter; NB Labs, Denison, TX, USA) were stereotaxically implanted into the NAc core (angled 10°, AP: +1.5; ML: ± 2.5; DV: -6.8 from skull; mm relative to bregma) and the ipsilateral PrL (AP: +2.5; ML: ± 0.6; DV: -4.0 from skull; mm relative to bregma) using established procedures (Moschak & Carelli, 2021), with hemisphere (left or right) counterbalanced across rats. Animals were given at least 7 days of post-operative care during which an inflammatory medication (meloxicam, 1 mg/kg, s.c.) and an antibiotic (cefazolin, 10 mg/ml, i.v.) were given for three and five days respectively. Catheters were flushed daily with heparinized saline (0.1 mL of 30U/mL, i.v.) and Taurolidine-Citrate catheter lock solution (TCS, 0.03 mL, i.v.) was used to prevent blood clot formation and bacterial or fungal growth. Food and water were provided *ad libitum* during post-operative recovery.

Experimental Design

Figure 3.1 shows the experimental timeline. Following recovery from surgery, rats were placed in the electrophysiology recording chamber and the first resting state LFP recording was completed over 15 minutes (Rest LFP 1). Next, rats were mildly water restricted and trained to self-administer either cocaine (n=10) or water (n=10) in a behavioral chamber (different from the electrophysiology chambers) during 6-hr daily sessions conducted for 14 consecutive days as described elsewhere (Moschak et al., 2018). Briefly, during cocaine self-administration, a lever was extended into the chamber with a cue light illuminated above it to indicate availability of a reward. Each lever press resulted in the termination of the cue light, onset of a tone-houselight conditioned stimulus (20 s) and intravenous infusion of cocaine (1 mg/kg/inf, over 6 s). Any additional responses during this 20 s period post response period were still recorded but not reinforced. In some cases, the operant response was a nosepoke, however there were no significant

differences in total drug intake ($t_8 = 1.367$, $p > 0.05$) or drug intake ($t_4 = 0.4515$, $p > 0.05$) on the final 3 days of self-administration between animals that lever pressed (n=7) versus nose poked (n=3) for cocaine, so their behavior was pooled. Rats in the water self-administration group underwent a similar training protocol but learned to press a lever for water reinforcement (250 µls into a drinking receptacle). The day following the last self-administration session, rats were returned to the electrophysiology chamber and the second 15 min resting state LFP recording was conducted (Rest LFP 2). All rats then underwent a 2-hour extinction session (Ext 1) in which they were placed back into the same behavioral chamber used for self-administration, but responses were not reinforced. Next, rats underwent a 1-month abstinence period in which they were placed in their home cages and given food and water *ad libitum*. After this period, rats were again mildly water restricted before the last 15-min LFP resting state recording (Rest LFP 3), followed by a final 2-hour extinction session (Ext 2).

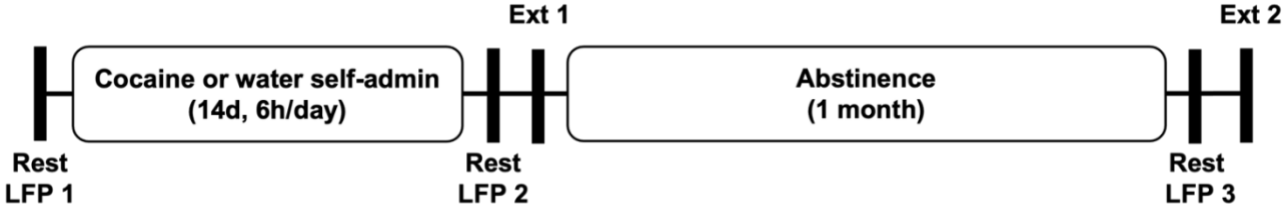

Figure 3.1. Aim 2 experimental design. Rats underwent three resting state LFP recordings (Rest LFPs). The first one (Rest LFP 1) was performed in naïve rats before the start of the experiment. Then, rats had the opportunity to self-administer either cocaine or water for 6h/day for 14 days. After self-administration, rats underwent a second LFP recording (Rest LFP 2). Then, the first extinction test (Ext 1) was performed followed by 1 month experimenter-controlled abstinence. Finally, the last LFP recording (Rest LFP 3) was performed followed by the second extinction test (Ext 2).

Electrophysiology

Electrophysiology procedures have been described in detail previously (Carelli, 2000; Hollander & Carelli, 2005) and conducted in 43 x 43 x 53 cm custom-made Plexiglas operant chambers housed within commercial sound-attenuating cubicles (Med. Associates, Inc., St Albans,

VT, USA). Briefly, rats were connected to a flexible recording cable attached to the commutator. LFP recordings conducted during the 3 resting state sessions were collected using a commercially available neurophysiological system (OmniPlex system or multichannel acquisition processor [MAP] system; Plexon, Inc., Dallas, TX, USA), described previously (West et al., 2021). Continuous recordings from each electrode were virtually referenced (PlexControl; Plexon, Inc.) and fed into a Pentium computer. Continuous signals were low-pass filtered (≤ 200Hz) to isolate LFPs and LFPs were recorded and analyzed in Neuroexplorer (Plexon, Inc). Due to noise issues, 2 animals were excluded from electrophysiological analysis (cocaine n=1; water n=1).

Histology

Electrode placements were assessed using established procedures (Moschak & Carelli, 2021). Rats were deeply anesthetized with an intraperitoneal injection of a ketamine hydrochloride (100mg/kg) and xylazine hydrochloride mixture (10mg/kg). A 15 μA current was passed through each microwire electrode for approximately 5s to mark the placement of the electrode tips. The brains were extracted and placed in a 3% potassium ferrocyanide in 20% formalin solution. Following post-fixing and freezing, 40μm coronal brain sections were mounted and viewed under a 1X microscope lens. Placement of an electrode tip was determined by examining the relative position of the potassium ferrocyanide reaction to visual landmarks and anatomical organization of the PrL and NAc core (Paxinos & Watson, 2006). Only rats with electrode placements within the PrL and NAc core were used in the analysis (**Figure 3.7**).

Data Analysis

Self-Administration:

To determine if the extended access self-administration procedure resulted in escalation of cocaine or water taking, simple linear regressions of the daily average reward intake were

examined. Incubation of craving was analyzed using separate paired t-tests that compared extinction responses before (Extinction 1) versus after 1-month abstinence (Extinction 2) for rats with a history of cocaine versus water self-administration.

Electrophysiology:

LFPs were used to examine neural activity within the PrL and NAc core (power) and across the PrL and NAc core circuit (coherence). Power spectral densities (PSDs) were normalized by dividing power within each 2 Hz bin by the average power across all frequency bands (0-100 Hz [58-62 Hz noise band excluded]). Repeated measures 2-way ANOVAs were used to examine changes in PrL and NAc core normalized peak PSDs and their coherence for both groups of animals using recording session (Rest LFP 1-3) and frequency band (delta, 0.5-4 Hz; theta, 4-12 Hz; beta, 12-30 Hz; low gamma, 30-58 Hz; and high gamma, 75-85 Hz) as factors, followed by Tukey's multiple comparisons when appropriate. Pearson correlations and simple linear regressions were used to determine if normalized power in the PrL or NAc core and/or PrL-NAc core coherence at specific rest LFP recording sessions correlated with subsequent total cocaine or water intake, escalation of cocaine intake, or incubation of cocaine or water seeking. For these analyses, escalation of intake was determined by subtracting the number of responses for reward during early (Day 3) self-administration from responses made during late (Day 14) self-administration sessions. Incubation of craving was determined by subtracting the number of extinction responses immediately after self-administration training from extinction responses following abstinence (Extinction 2 -Extinction 1). LFP power and coherence values were exported directly from Neuroexplorer. All analyses were considered to be statistically significant at α=0.05. Statistical and graphical analyses were performed using GraphPad Prism 9.0 for Windows (GraphPad Software, Inc., La Jolla, CA).

Results

Self-Administration Behavior

All rats acquired self-administration behavior (**Figure 3.2**). To assess escalation of reward intake, simple linear regressions revealed a significant escalation of cocaine ($F_{1, 118}$ =11.47, p = 0.0010; **Figure 3.2A**) but not water ($F_{1, 118}$ = 0.5644, p = 0.4540; **Figure 3.2B**) intake across sessions. To examine incubation of craving, responses during Extinction 1 and 2 were analyzed. Paired t-tests revealed animals that self-administered cocaine (t_9 = 4.218, p = 0.0022; **Figure 3.2C**) and water (t_9 = 3.811, p = 0.0041; **Figure 3.2D**) had significantly more responses during Extinction 2 than Extinction 1, showing that both groups of animals displayed elevated reward seeking after abstinence (i.e., incubated reward seeking).

Resting state LFP power in the PrL and NAc core and PrL-NAc core coherence did not show overall (broadband) hypoactivity following self-administration training and prolonged abstinence

One objective of the present study was to determine if resting state LFP power (PSD) in the PrL and NAc core, and their functional coherence, vary as a function of cocaine self-administration experience and one month drug abstinence. **Figure 3.3** shows normalized PSDs in the PrL (left) and NAc core (right) across the three LFP rest sessions for cocaine (top) and water (bottom) rats. For cocaine rats PrL activity (**Fig. 3.3A**, left), a 2-way ANOVA of normalized peak PSDs across frequency bands revealed a main effect of frequency ($F_{1.536, 12.29}$ = 500.5, p < 0.0001), but no main effect of session ($F_{1.790, 14.32}$ = 0.9889, p = 0.3872) or frequency x session interaction ($F_{2.537, 20.30}$ = 2.705, p = 0.0801). In the NAc core, analyses of normalized peak PSDs in cocaine rats (**Fig. 3.3A**, right) revealed a main effect of frequency ($F_{2.322, 18.58}$ = 916.9, p < 0.0001), no main effect of session ($F_{1.918, 15.34}$ = 2.658, p = 0.0.1037) but a significant frequency x session interaction ($F_{2.687, 21.50}$ = 3.816, p = 0.0280). Post hoc tests only revealed a significant difference between

normalized peak LFPs at the delta frequency for Rest LFP 1 compared to Rest LFP 2 and Rest LFP 3. For water rats, LFP activity in either the PrL (**Fig. 3.3B**, left) or the NAc core (**Fig. 3.3B**, right) were not altered across the 3 resting state periods (all analyses, $p > 0.05$). Finally, LFP PrL-NAc core functional connectivity (i.e., coherence) did not vary across the 3 resting state periods for cocaine or water rats (all p's > 0.05; data not shown).

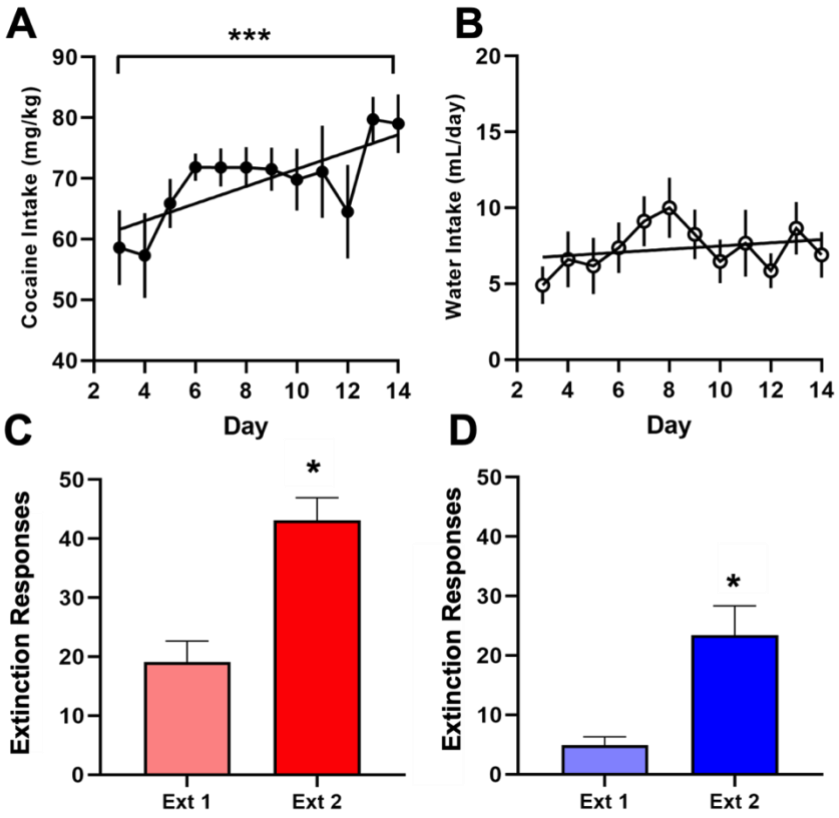

Figure 3.2. Behavior during self-administration and extinction sessions. A. Mean ± SEM of cocaine intake (mg/kg/day) across self-administration sessions. Rats showed escalation of cocaine intake during across sessions indicated by significant difference from zero in slope of linear regression line. *** $p = 0.001$. B. Rats did not show escalation of water intake indicated by no significant difference from zero in slope of linear regression line. C. Mean ± SEM in the number of extinction responses for cocaine rats during extinction test 1 (Ext 1) and extinction test 2 (Ext 2) D. Mean ± SEM number of extinction responses for water rats during extinction test 1 (Ext 1) and extinction test 2 (Ext2). * indicates significant differences between Ext1 and Ext 2. *$p < 0.05$.

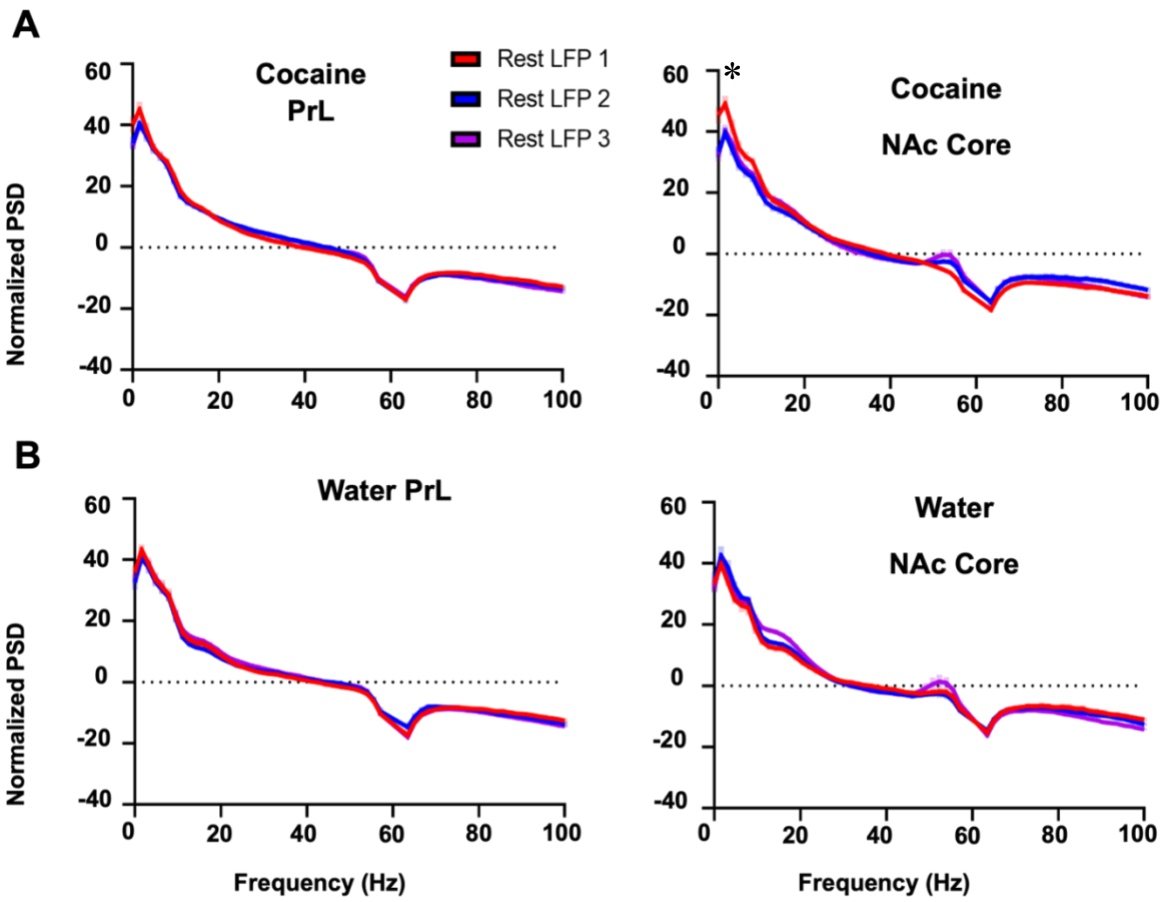

Figure 3.3. PrL and NAc core normalized power spectral density (PSD) across frequencies during rest LFP recording sessions. A. Normalized PSD during the 3 rest LFP recording sessions in the PrL (left) and NAc core (right) across the frequency spectrum (0-100Hz [58-62Hz excluded]; 2Hz/bin) for animals with a history of cocaine self-administration. B. Normalized PSD during the 3 rest LFP recording sessions in the PrL (left) and NAc core (right) across the frequency spectrum (0-100Hz [58-62Hz excluded]; 2Hz/bin) for animals with a history of water self-administration. Error bars represent mean + SEM. * indicates significant difference during Rest LFP 1 compared to Rest LFP 2 and Rest LFP 3.

Resting state oscillatory signaling dynamics in the PrL and NAc core *prior to* self-administration training (Rest LFP 1) differentially predicts total amount of cocaine and water intake

Another objective of the present study was to identify potential biomarkers associated to resting state LFP activity that can predict subsequent cocaine seeking and/or taking behaviors. To this end, we completed a series of correlational analyses of resting state oscillatory activity during

the 3 LFP recording sessions with the following behavioral measures: total amount of cocaine or water consumed, escalation of cocaine intake, and incubation of cocaine or water craving following one month abstinence. **Figure 3.4A** shows the timepoint for the LFP 1 recording and self-administration behaviors (highlighted in red). Interestingly, in cocaine rats (**Figure 3.4B**), a significant *positive* correlation at beta frequency was observed between normalized LFP power during LFP1 and subsequent cocaine taking during self-administration in the PrL (top left), but not NAc core (top middle), suggesting that the higher the power at this frequency band prior to self-administration, the higher the cocaine intake during self-administration. However, PrL-NAc core coherence did not correlate with drug taking behavior (top, right). Notably, for water rats (**Figure 3.4C**), there was a significant *negative* correlation between normalized PSDs at the low gamma frequency range in the PrL and NAc core with total water intake during self-administration, suggesting that the less power at this frequency band prior to water self-administration training, the more water intake during training. No significant correlations were evident at any frequency between PrL-NAc core coherence and total water intake. Taken together, these data suggest that rest PrL and NAc core power at distinct frequencies (but not PrL-NAc core coherence) differentially predicts subsequent total cocaine (beta frequency) or water (low gamma frequency) intake.

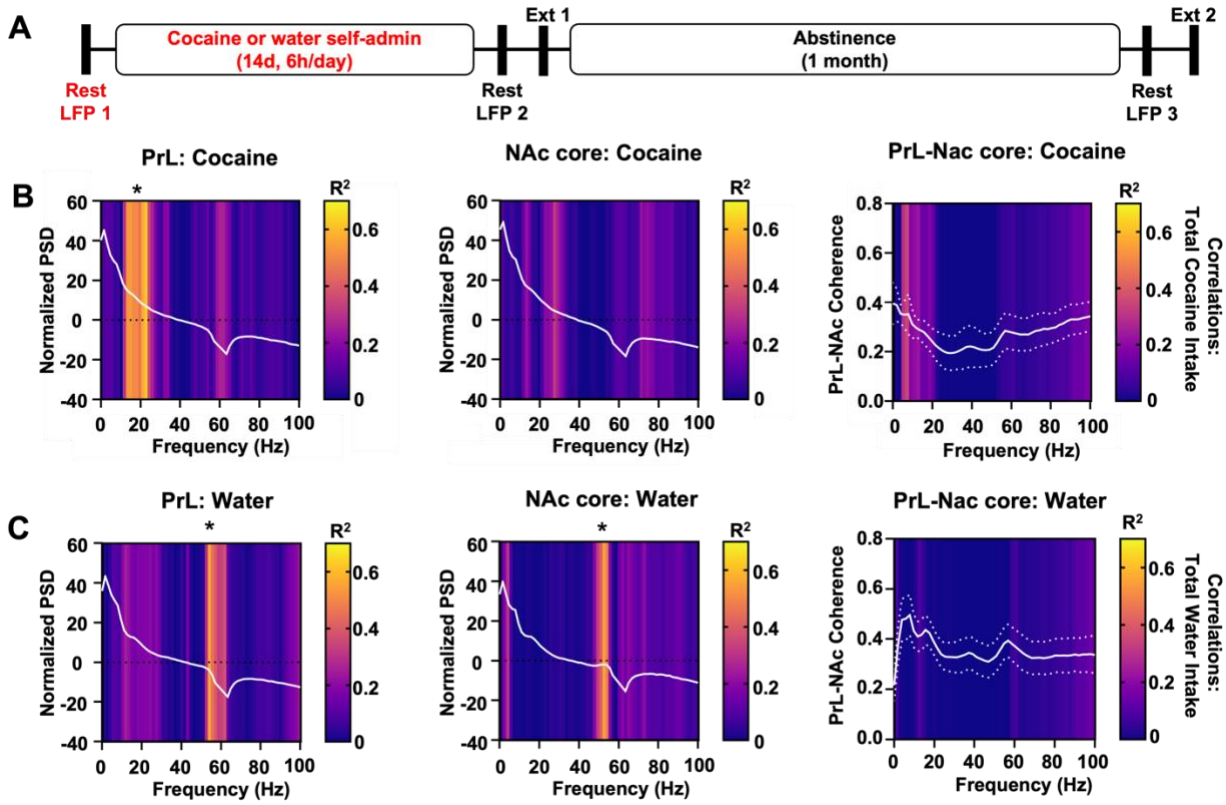

Figure 3.4. Relationship between rest PrL and NAc core power and PrL-NAc core coherence before self-administration (Rest LFP 1) and total cocaine intake. **A.** Experimental timeline with red text indicating timepoint used for the analysis. **B.** PrL (left) and NAc core (middle) normalized PSDs and PrL-NAc core coherence (right) across frequencies during Rest LFP 1 in cocaine rats. Power (normalized PSDs) and coherence values at each frequency were correlated with individual rats' total cocaine intake during self-administration. R^2 values for each of these correlations are represented in the color overlay (R^2 values that are significant, $p < 0.05$, are indicated by yellow-orange in overlay). **C.** PrL (left) and NAc core (middle) normalized PSDs and PrL-NAc core coherence (right) across frequencies during Rest LFP 1 in cocaine rats. Correlations and color coding as in B. Mean and SEM indicated by white bars and dotted lines.

Resting state PrL power but not NAc core power or PrL-NAc core coherence *prior* to self-administration training (Rest LFP 1) predicts subsequent escalation of cocaine taking

Next, we examined if rest PrL and NAc core power or PrL-NAc core coherence prior to cocaine self-administration predicted subsequent escalation of cocaine taking during self-administration. **Figure 3.5A** shows the timepoint (red highlighted text) for the LFP 1 recording and escalated cocaine intake during cocaine self-administration. Here, escalation of cocaine taking

was quantified by subtracting the number of responses made during an early session (Day 3) from those that occurred during a later self-administration session (Day 14). As shown in **Figure 3.5B**, while no correlation was observed in the NAc core or coherence, only normalized rest PrL PSD at the beta frequency range was significantly *positively* correlated with escalation of drug taking ($p < 0.05$)**.** This finding indicates that increased power at the beta range prior to cocaine self-administration training can later predict which individuals will escalate cocaine use.

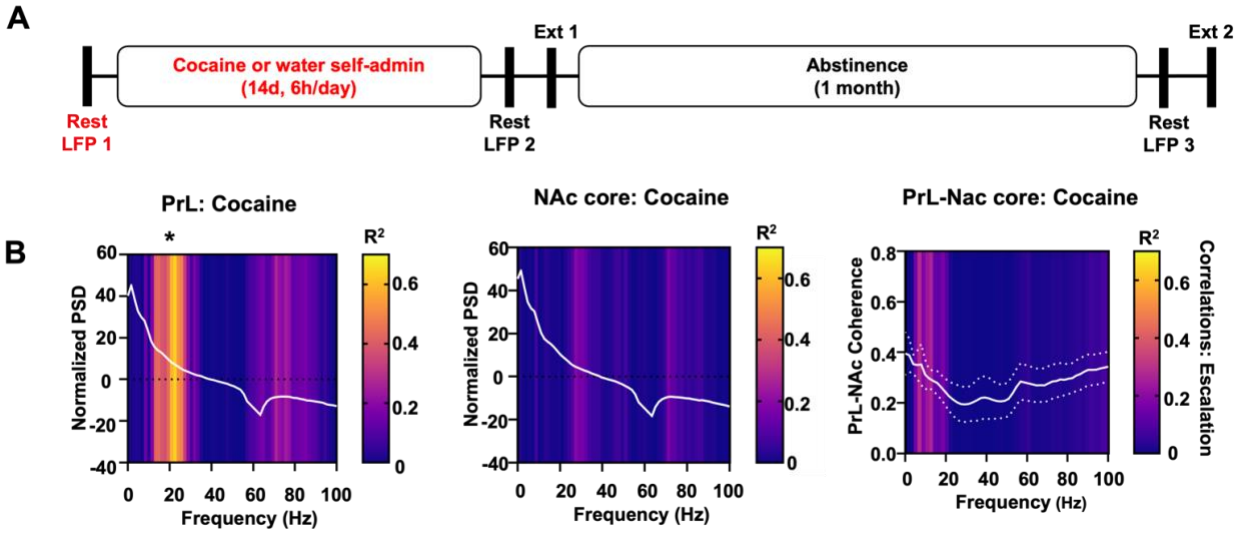

Figure 3.5. Relationship between rest PrL and NAc core power and PrL-NAc core coherence before self-administration (Rest LFP 1) and escalation of cocaine taking. **A.** Experimental timeline with red text indicating timepoint used for the analysis. **B**. PrL (left) and NAc core (middle) normalized PSDs and PrL-NAc core coherence (right) across frequencies during Rest LFP 1 in cocaine rats. Power (normalized PSDs) and coherence values at each frequency were correlated with individual rats' escalated cocaine intake during self-administration and the R^2 values for each of these correlations are represented in the color overlay (correlations and color coding as in **Figure 3.4**). Mean and SEM indicated by white bars and dotted lines.

Rest PrL and NAc core power and PrL-NAc core coherence *after* self-administration training *but before* prolonged abstinence (Rest LFP 2) predicts incubation of cocaine, but not water craving

Another goal of the present study was to determine if power in the PrL and/or NAc core and/or its coherence recorded after two weeks extended access self-administration (Rest LFP 2) can predict subsequent incubation of drug seeking (craving) one month later. The timepoints for

these analyses are highlighted in red in **Figure 3.6A**. **Figure 3.6B** shows cocaine normalized PSDs in the PrL and NAc core and their coherence correlated with individual rats' incubation of cocaine craving (number of responses during Extinction 2 minus number of responses during Extinction 1). Here, there were significant *negative* correlations were observed between PrL and NAc core PSD and incubation of cocaine seeking at the low gamma frequency range (**Figure 3.6B**, left and middle). Further, Pearson's correlations revealed that incubation of cocaine seeking was *negatively* correlated ($p < 0.05$) or trended toward negative correlation ($p < 0.10$) with rest PrL-NAc core coherence at the following frequencies: 6-10 Hz (theta, $p < 0.05$, yellow) and 12-24 Hz (beta, $p < 0.10$, orange; **Figure 3.6B**, right). Of note, the predictive relationship between rest PrL and NAc core power and PrL-NAc core coherence and subsequent incubation of cocaine craving did not remain when LFPs were measured 1 month following abstinence (Rest LFP 3, all p values > 0.05, data not shown). In contrast, for water rats (**Figure 3.6C**, bottom) Pearson's correlations revealed that neither rest PrL or NAc core PSD or PrL-NAc core coherence were predictive of subsequent incubation of water seeking at any frequency examined.

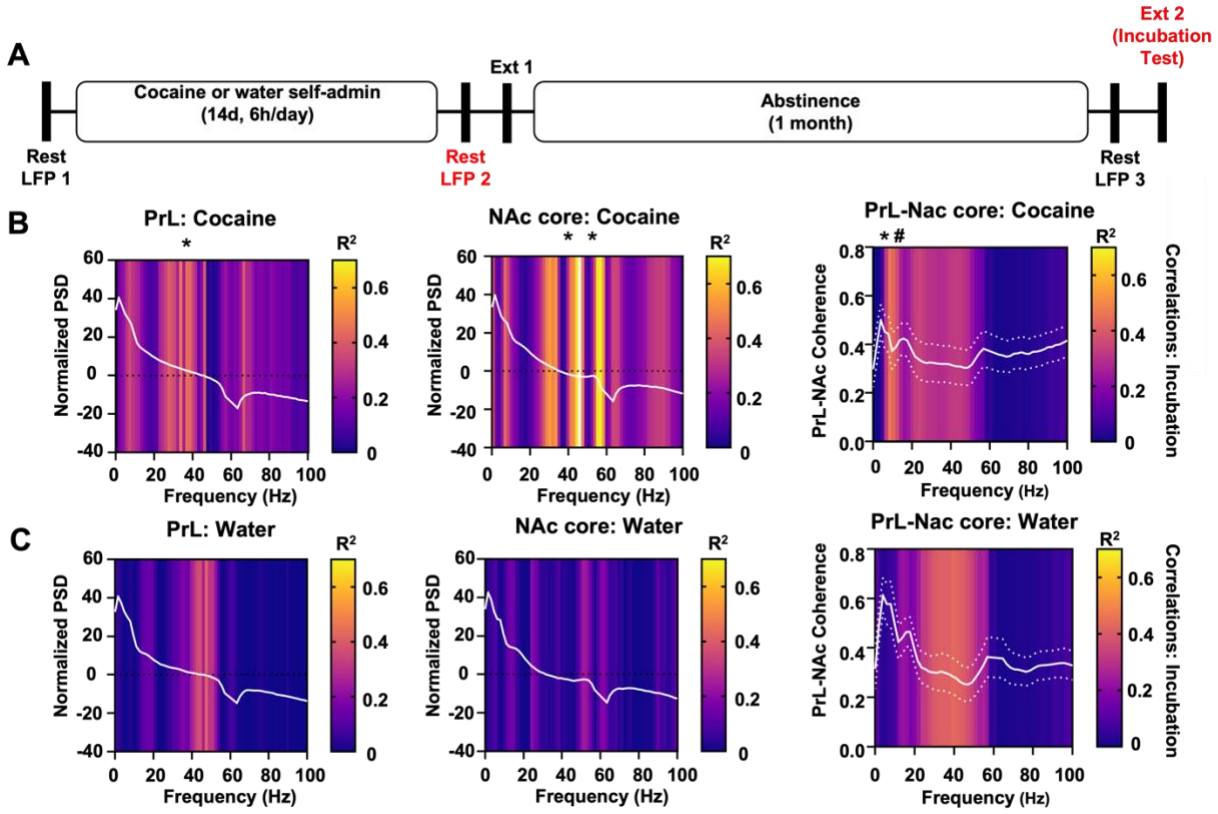

Figure 3.6. Relationship between rest PrL and NAc core power and PrL-NAc core coherence following self-administration (Rest LFP 2) and incubation of reward seeking. **A.** Experimental timeline with red text indicating timepoint of rest LFP recordings and incubation (extinction 2) test session. **B.** PrL (left) and NAc core (middle) normalized PSDs and PrL-NAc core coherence (right) across frequencies during Rest LFP 2 in cocaine rats. Power (normalized PSDs) and coherence values at each frequency were correlated with individual rats' incubated responding (Ext2 responses – Ext1 responses). R2 values for each of these correlations are represented in the color overlay (correlations and color coding as in Figure 2.4). **C.** PrL (left) and NAc core (middle) normalized PSDs and PrL-NAc core coherence (right) across frequencies during Rest LFP 1 in water rats. Correlations and color coding as in B. Error bars (dotted white lines) represent mean ± SEM.

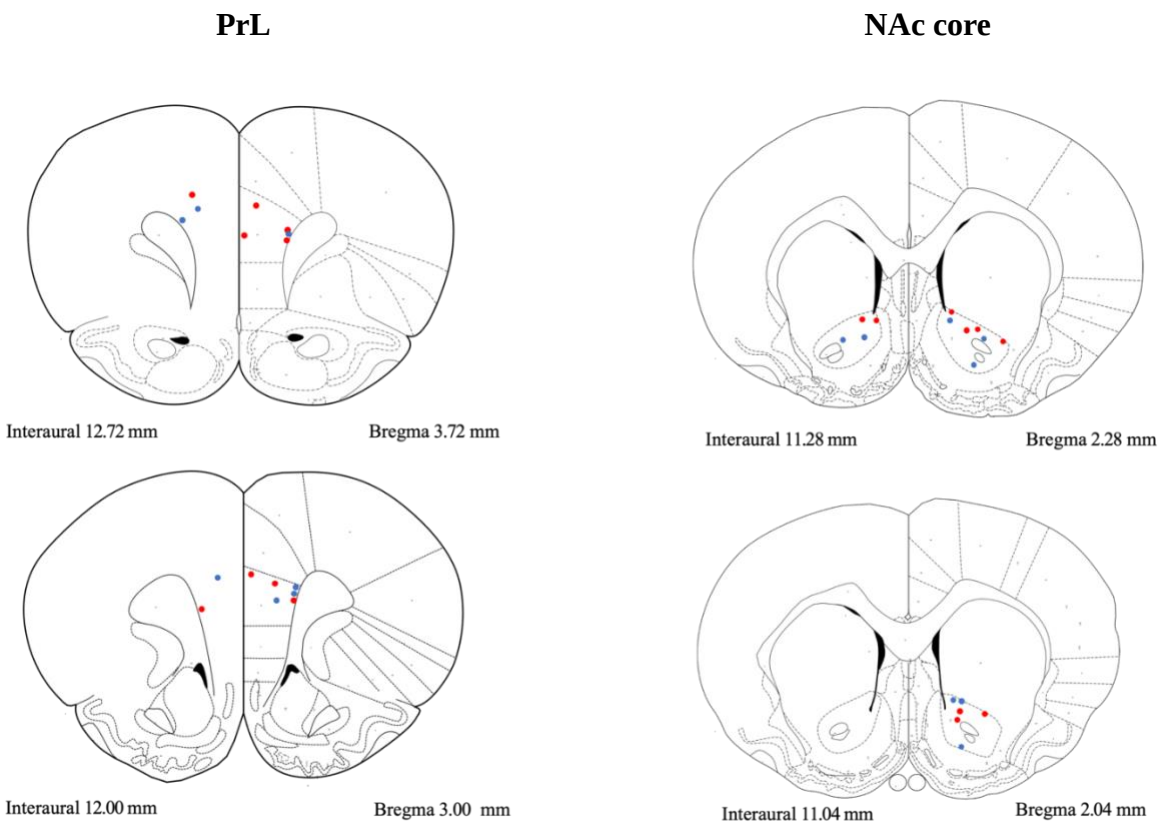

Figure 3.7. Histological verification of electrode placements. Red dots signify animals with a history of cocaine self-administration, blue dots signify animals with a history of water self-administration.

Discussion

In vivo LFP electrophysiology was used to characterize spontaneous (resting state) activity within the PrL and NAc core, and its functional connectivity, at specific time points relative to cocaine self-administration training and abstinence with two primary objectives. First, we examined if resting state LFP reflects prefrontal hypoactivity (overall decreased prefrontal activity) in cocaine experienced rats, a phenomenon typically observed in humans suffering from SUDs (Aharonovich et al., 2006; Garavan & Hester, 2007; Goldstein & Volkow, 2002; Hanlon et al., 2013; Jentsch & Taylor, 1999). We hypothesized that 2 weeks of extended access (6 hr/day) cocaine self-administration and 1 month abstinence would result in lower overall resting state activity, particularly in the PrL. Contrary to our predictions however, we did not observe broadband hypofunction in resting state power in the PrL or NAc core, or their coherence. Second, we examined if resting state LFP activity is predictive (can serve as a biomarker) of subsequent drug seeking and/or drug taking behaviors. A summary of these findings is illustrated in **Table 3.1.** Here, we found that resting state LFP power (PSD) in the PrL recorded prior to training (Rest LFP 1) was *positively* correlated with total cocaine intake and escalation of cocaine taking during cocaine self-administration at the beta frequency range. Additionally, rest PSD recorded immediately after self-administration training (Rest LFP 2) in the PrL and NAc core at gamma frequency, and PrL-NAc core coherence at the theta frequency, were *negatively* correlated with incubation of cocaine craving. Interestingly, for rats trained on water reinforcement, Rest LFP 1 was only correlated with total water intake at the low gamma range. Together, these data show that although broadband decreases in resting state activity across the 3 recording sessions was not observed, examination of individual differences using correlational analyses revealed that PrL and NAc core power and its functional coherence can serve as unique biomarkers for subsequent

cocaine seeking and taking behaviors. These findings are considered in detail below.

Resting state LFPs in the PrL and NAc core, and their functional coherence, do not exhibit broadband hypoactivity following cocaine self-administration and prolonged abstinence

It has been well-established that following prolonged abstinence from cocaine, the brain undergoes numerous neuroadaptations, evident in both animal models (Goldstein & Volkow, 2002; Volkow & Morales, 2015; Wolf, 2016) and humans that suffer from SUDs (Aharonovich et al., 2006; Hanlon et al., 2013). One type of neuroadaptation, known as hypofrontality refers to an overall decrease in activity in the prefrontal cortex following cocaine experience and prolonged abstinence (Garavan & Hester, 2007; Goldstein & Volkow, 2002, 2011). Given the role of the prefrontal cortex in higher order cognitive processes and executive function, hypofrontality has been linked to negative consequences associated with chronic cocaine use such as impaired decision-making, deficits in behavioral flexibility, and increased impulsivity (Goldstein & Volkow, 2002, 2011). As such, one objective of the present study was to determine if broadband decreases would be observed in resting state activity in rats with a history of extended access cocaine self-administration and prolonged experimenter-imposed abstinence. Interestingly however, although there was a significant decrease in delta frequency oscillatory activity in the NAc core following cocaine self-administration training and abstinence, this was not observed in the PrL, or in PrL-NAc core coherence. As such, the absence of overall (broadband) reductions in resting state activity in PrL or NAc core indicates that the resting state LFP measures used in the present study do not reflect hypofrontality typically observed in neuroimaging studies of people with SUDs (Garavan & Hester, 2007; Goldstein & Volkow, 2002).

The absence of hypofrontality in the current study was unexpected since previous work in our lab has shown that PrL-NAc core power and their coherence significantly decreased across frequencies after one month abstinence from cocaine self-administration experience, compared to

water/yoked saline rats (West et al., 2021). In that prior study, rats were exposed to 2-hour daily cocaine self-administration sessions for 2 weeks, followed by 1 month abstinence. We hypothesized that since animals in the current study consumed more drug in the daily 6-hour sessions than animals experiencing the short access (2hr/day) drug self-administration procedure, even greater reductions in power and coherence across our 3 resting state recording sessions would be evident here, particularly after extended 1 month abstinence from cocaine. However, it is important to note that the broadband hypoactivity observed by West and colleagues was relative to Pavlovian cues linked to food (nondrug) rewards, not resting state activity as recorded in the present study. Further, the use of the extended access cocaine self-administration in which more drug is consumed, may have reduced activity in other prefrontal brain regions that project to the NAc core (e.g., OFC) that in turn, may underlie the functional hypoactivity and associated decreased executive function observed in humans with SUDs (Goldstein & Volkow, 2002; Volkow et al., 2003).

Resting state LFP activity prior to self-administration training (Rest LFP 1) may serve as unique predictors (biomarkers) of cocaine seeking and/or taking behaviors

An ongoing goal of SUD research is to uncover potential biomarkers within the prefrontal cortex and associated regions that can help predict those at risk for developing SUDs. For example, animal studies have revealed correlations between resting state functional connectivity and escalation of drug taking (Gozzi et al., 2011; H. Lu et al., 2014) similar to neuroimaging studies in humans showing reduced corticolimbic resting state functional connectivity in people with cocaine use disorders (Gu et al., 2010; McHugh et al., 2014). In the present study, we first examined correlations between rest LFP activity (power and coherence) prior to self-administration training (rest LFP 1) and various aspects of drug seeking and taking behaviors. We focused our analysis on particular frequency bands (theta, beta, low/high gamma) which have been

linked to specific behaviors in both humans and animal models (although the ranges for each band vary slightly across species, (Buzsaki & Draguhn, 2004). For example, the theta frequency band (4-12Hz) has been associated with behaviors such as reward anticipation, cognitive control, and avoidance (Donnelly et al., 2014) while beta frequency (12-30Hz) is often linked to top-down cognitive processing (Engel & Fries, 2010). Gamma frequencies have been functionally divided into low (< 30 Hz) and high (e.g., 80 Hz) ranges and are associated with behaviors such as reward receipt and movement initiation (van der Meer & Redish, 2009).

Interestingly, we found that rest PrL power prior to self-administration (Rest LFP 1) was predictive of subsequent reward taking behaviors. Specifically, in animals destined to self-administer cocaine, there was a *positive* relationship between PrL power *before* self-administration and total cocaine intake and escalation of cocaine seeking during cocaine self-administration at the beta frequency range. In other words, the higher the PrL power at beta from the overall PrL mean power *before* drug exposure, the more drug the animal subsequently took and the greater the escalation of drug intake during self-administration. These findings may appear inconsistent with literature suggesting that an increased risk of developing an SUD is associated with a *reduction* in baseline oscillatory activity (power) and hypoconnectivity (coherence) in prefrontal and striatal pathways (Goldstein & Volkow, 2002; Jentsch & Taylor, 1999; Volkow et al., 1992), however additional evidence in the literature links increased activity at lower frequency bands (e.g., theta and beta) with cocaine craving to drug cues (Kearney-Ramos et al., 2019; Reid et al., 2003, 2008; D. Zhao et al., 2021).

To determine the specificity of correlational data between resting state activity and cocaine seeking behavior, we completed similar analyses in rats trained to self-administer water. In water rats, we observed a *negative* correlation between both the PrL and NAc core power at rest LFP 1

and total water intake. Specifically, the lower the PrL and NAc power at the low gamma frequency range from the overall mean, the more water the animal took during water self-administration. Note that this is a different frequency range and opposite direction than was seen in animals self-administering cocaine. Taken together, these data indicate that the correlations between LFP power and drug taking and seeking are reward type and frequency specific.

Resting state LFP activity after self-administration training (Rest LFP 2) also serves as unique predictors (biomarkers) of cocaine seeking behaviors

We also examined if resting state LFP activity recorded after self-administration training but prior to prolonged abstinence would be a significant predictor of subsequent escalation of reward seeking, termed 'incubation of craving' (Wolf, 2016). Enhanced drug seeking following prolonged abstinence has been well-established in animal models (L. Lu et al., 2004; Wolf, 2016), linked to PrL-NAc core circuitry (Goldstein & Volkow, 2011; Koob & Volkow, 2016), and observed in humans with SUDs (Gawin & Kleber, 1986; Parvaz et al., 2016). In the present study, we observed a *negative* correlation between PrL and NAc core power at rest and incubation of cocaine craving as well as a *negative* correlation between rest PrL-NAc core coherence and incubated drug seeking. That is, the *lower* the PrL and NAc core power at low gamma the *more* incubated cocaine seeking that occurred in extinction following abstinence. Furthermore, the *weaker* the rest PrL-NAc core coherence at theta (and trending at beta) following cocaine self-administration but before abstinence, the greater responding in extinction for the drug following abstinence (i.e., incubation). Of note, while both cocaine and water self-administration groups displayed incubation of craving during abstinence, only animals self-administering cocaine exhibited this relationship between Rest LFP 2 and incubation of craving. Further, this relationship did not remain for cocaine animals during Rest LFP 3 (following 1-month abstinence) indicating a limited time period to observe this relationship. Interestingly, Müller Ewald and colleagues

reported a similar relationship between theta band activity and cocaine seeking in extinction in the infralimbic (IL) cortex, another prefrontal cortical region (Müller Ewald et al., 2022). Collectively, these data suggest that the lower the resting state activity (power) or synchronization (coherence) at specific lower frequency bands in prefrontal regions, the more elevated drug seeking behavior in extinction following prolonged drug abstinence.

The finding of negative correlations between LFP 2 and escalated drug seeking following cocaine abstinence may also have clinical implications with respect to potential SUD treatments. For example, stimulation of the PrL-NAc core circuit during rest LFP 2 time period may serve to restore dysregulated neural activity related to repeated cocaine use and reduce later incubated cocaine seeking. This hypothesis is consistent with optogenetic studies by Ma and colleagues (2014) and from our laboratory (Moschak & Carelli, 2021) showing that theta frequency optogenetic stimulation of the PrL-NAc core pathway reduces incubated cocaine seeking in rats following daily 6-hour cocaine self-administration and 1 month abstinence. Of note, recent work in the Carelli lab showed that one form of noninvasive brain stimulation, transcranial alternating current stimulation (tACS) had the ability to reverse cocaine-induced reductions in PrL-NAc core coherence and restore one form of cocaine-induced cognitive impairment, behavioral flexibility (West et al., 2021). It may therefore be the case that restoring dysregulated neural signaling in the PrL and NAc core circuit after extended access cocaine self-administration and prior to abstinence (during the Rest LFP 2 period) using tACS may in turn decrease incubated cocaine seeking behaviors following prolonged abstinence in humans. Indeed, non-invasive theta burst stimulation of prefrontal regions is under active investigation as a treatment strategy for humans with cocaine use disorders (Hanlon et al., 2017; Kearney-Ramos et al., 2019).

Concluding Remarks and Future Directions

The present study provides foundational information on relationships between resting state LFP activity in the PrL and NAc core, and drug taking and seeking behaviors. While interesting, there are a few limitations to the current work. First, only male rats were used in the present study and similar studies using female rats will reveal if the present findings are sex specific. Second, the majority of the present findings are correlational and while some optogenetics studies link the PrL-NAc core circuit to incubated drug seeking following abstinence (Ma et al., 2014; Moschak & Carelli, 2021), additional studies are needed to confirm causality with respect to rest LFP recordings and behavior. Finally, resting state neural activity in the PFC and associated regions may also help identify the individual effectiveness of specific treatment strategies for SUDs, and/or provide insight into whether other variables, such prenatal exposure to cocaine, known to increase risk of developing later SUDs in humans (Glantz & Chambers, 2006; Richardson et al., 2013), show altered resting state PFC activity linked to subsequent drug seeking behaviors.

	Cocaine			Water		
	PrL PSD	NAc Core PSD	PrL-NAc core Coherence	PrL PSD	NAc Core PSD	PrL-NAc core Coherence
LFP 1: Total Intake	↑ beta	—	—	↓ low gamma	↓ low gamma	—
LFP 1: Escalation	↑ beta	—	—			
LFP 2: Incubation	↓ low gamma	↓ low/high gamma	↓ Theta	—	—	—

Table 3.1. Summary of Aim 2 findings.

CHAPTER 4

80HZ tACS RESCUES COCAINE-INDUCED SHIFTS IN PrL SINGLE UNIT ACTIVITY AND RESTORES BEHAVIORAL FLEXIBILITY

Introduction

In order to achieve a desirable reward, individuals must be able to predict the outcome of choices, use that information to enforce appropriate actions and adjust behavior to changes in the environment, a phenomenon often referred to as behavioral flexibility (Brown & Tait, 2014; Stalnaker et al., 2009). A key marker of substance use disorders are impairments in flexible behavior such as poor decision making and continued drug use observed in both human and rodent populations (Jentsch & Taylor, 1999; Turner et al., 2009). Importantly, the prelimbic cortex (PrL, rodent functional homolog of the human mPFC) and nucleus accumbens (NAc) core, two brain regions implicated in higher order processes such as decision making, are also thought to be involved in behavioral flexibility (del Arco et al., 2017). A history of drug followed by a period of abstinence can lead to altered neural signaling in this circuit as well as impairments in flexible behavior (Saddoris & Carelli, 2014; West et al., 2021).

One method used to measure behavioral flexibility is a reinforcer devaluation task. Here, first animals must associate a particular cue with a specific reward. Then, the previously rewarding food is devalued (e.g., by conditioned taste aversion, CTA). Last, the ability of the animal to shift behavioral responding toward the cue previously paired with the now devalued reward is measured (i.e., behavioral flexibility). Following cocaine self-administration and prolonged abstinence however, animals are unable to shift behavior and continue to the cue

previously paired with the devalued outcome (Schoenbaum & Setlow, 2005). A recent paper from the Carelli lab aimed to examine the effects of a type of noninvasive brain stimulation called transcranial alternating current stimulation (tACS) on PrL-NAc core circuit LFP activity and its relationship to flexible behavior in male rats with a history of cocaine self-administration. Critically, previous cocaine experience caused impairments in behavioral flexibility as well as dampened PrL-NAc core functional connectivity (coherence), particularly at the high gamma (80Hz) frequency range. Interestingly, using either optogenetics or tACS reversed these cocaine-induced deficits in behavioral flexibility and restored LFP coherence to control levels (West et al., 2021).

In addition to these altered signaling dynamics following abstinence from drug, sex differences in behaviors and neural effects of substance use disorders have also been observed. Women have shown increased drug intake and are more vulnerable to developing substance use disorders; this has been replicated in preclinical research as well (Lynch et al., 2002; W. Zhao & Becker, 2010). Female rats exhibited a decrease in basal prefrontal activity linked with deficits in cognitive flexibility faster than male rats following self-administration (Anderson et al. (2021) . It is not known however, if there are sex differences in behavioral flexibility following a history of cocaine self-administration and abstinence in rats.

In this chapter, we aim to build off the published work by West et. al. (2021) that examined the effects of tACS to restore cocaine-induced deficits in behavioral flexibility and reverse alterations in PrL and NAc core neural activity in male rats. Here, we examined the effects of tACS on PrL single unit activity and its relationship to flexible behavior in male and female rats with a history of cocaine self-administration and abstinence.

Methods

Animals

Long-Evans male and female rats (Charles River) aged 90-120 days (male= 31, female n= 35; 250-350 grams) at the beginning of the study were used. All subjects were housed individually and maintained on a standard 12:12 hour light-dark cycle (lights off at 7:00 am). During behavioral training and testing, rats were restricted to no less than 90% of their preoperative body weight by limiting food access to 20-25 g of standard rat chow (Purina RMH3000) per day (*ad libitum* water*)*. During self-administration, rats were maintained on 20-30 ml of water/day (*ad libitum* food). All animal procedures were approved by the University of North Carolina at Chapel Hill Institutional Animal Care and Use Committee (IACUC).

Surgical Procedures

Rats were implanted with microwire electrode arrays in the PrL (AP: +2.6, ML: ±0.6, DV: -4.0 from skull), described previously (West et al., 2014). In the same surgery, a Linear Stimulus Isolator (World Precision Instruments, Sarasota, FL) attached to two leads (stainless steel skull screws; Fine Science Tools, Foster City, CA) in direct contact with, but without penetrating the outer surface of, the skull was used. Screws were positioned 2 mm apart, at midline at the level of the PrL (bregma +2 mm and +4 mm).

Cocaine Self-Administration

An experimental timeline can be found in **Figure 4.1A**. Rats underwent 14 days of cocaine self-administration in standard operant chambers in which a nosepoke led to an intravenous infusion of cocaine (6-s infusion, 0.33 mg/infusion, ~0.75 mg/kg) followed by the presentation of a tone-house light cue for 30 seconds, described previously (Cameron & Carelli, 2012). Self-administration was performed before the behavioral flexibility task in these groups.

tACS Procedures

Rats underwent tACS or sham treatment on days 18 to 20 of cocaine abstinence in a distinct treatment chamber. A computer-generated sine-wave was input into the stimulator at the desired frequency (80 Hz) such that the stimulation oscillated between a + 18 μA and -18 μA current across the screws. An electric swivel was employed to allow free movement during stimulation periods, which lasted ~ 7 minutes per day. Stimulations consisted of 20 cycles of 10 seconds on then 10 seconds off for 3 consecutive days. Sham rats received identical headcaps and treatments, except that no current was delivered.

Behavioral Flexibility Task

Rats were trained on a behavioral flexibility (reinforcer devaluation) task consisting of three distinct phases: 1. Pavlovian Conditioning 2. Devaluation and 3. Post Devaluation Test (**Figure 4.1A**). During Pavlovian conditioning, one CS+ consisted of the illumination of two cue lights located to the left and right sides of the food receptacle (i.e., solid CS+). The other CS+ consistent of the flashing illumination (5 Hz) of the two cue lights located to the left and right side of the food receptacle (i.e., flashing CS+). The food paired with the two CS+ was counterbalanced such that a subset of rats received the food pellets paired with the solid CS+ (and the sugar pellets paired with the flashing CS+) and the other half received the reverse. Importantly, the two distinct CS- consisted of either a solid or flashing cue light located above the food receptacle (i.e., solid CS- and flashing CS-). The CS+ paired with food (regardless of solid or flashing) is designated as CS+1 and the CS+ paired with sugar is designated as CS+2. Each rat received 10 presentations of each stimulus and the order of the presentation were pseudorandomized. The inter-trial interval for the stimuli was pseudorandom and variable (75, 90, 105, or 120 seconds). There were three

different sessions with different stimuli presentation order, however, the same order was used for Day 1, Day 10, and the Post-Devaluation Test Day.

During the devaluation phase, rats were habituated for two days in a standard empty rat cage which would be used for the subsequent devaluation procedure (30 mins/day: on the last two days of Pavlovian conditioning,). After habituation, rats began the devaluation procedure. Here, rats were allowed 30 minutes to eat one of the rewards (food or sugar) ab libitum in the empty cage. Following consumption of the sucrose pellets, rats received an injection of LiCl (0.3 M, 7.5 ml/kg, i.p., while following consumption of the food pellets rats received an injection of saline (i.p.). At least 48 hours later, the same two-day procedure was repeated. At least 48 hours after the completion of devaluation, rats were tested in Phase 3, the post-devaluation test session to measure behavioral flexibility. Here, rats were given the same paradigm in Pavlovian conditioning (10 presentations of each stimulus, CS+1, CS+2, CS-1, CS-2), however, no reinforcer was delivered during testing (extinction). The ability to shift behavior away from the CS+ previously paired with the now devalued reinforcer is a canonical measure of "behavioral flexibility". To confirm successful devaluation post-testing, rats were given 30 minutes free access to food and sugar pellets (food choice test) on the following day. In this study we exclusively devalued the sucrose pellets as the food pellets were similar to what the rats received in their home cages, and we wanted to avoid artificially enhancing their motivational state (if they avoided their daily food). Critically, the devaluation of multiple reinforcers can be transferred to the reward predictive cues using similar methods (West et al., 2012; West & Carelli, 2016).

Electrophysiology

Online isolation and discrimination of single unit and LFP activity was accomplished using a commercially available neurophysiological system (OmniPlex system; Plexon), described

previously (Haake et al., 2019; West et al., 2014). Continuous recordings from each electrode were virtually referenced (PlexControl, Plexon) and fed into a Pentium computer. Continuous signals were high-pass filtered (300Hz) to identify individual spike events, or low-pass filtered (200Hz) to isolate local field potentials (LFPs) an 60Hz noise was removed using a notch filter. The analysis in this chapter will focus exclusively on single unit recordings (LFP data was previously published in West et al., 2021). Discrimination of individual waveforms began by setting a threshold level for each wire. Individual waveforms corresponding to a single cell were discriminated using template analysis procedures and time–voltage boxes provided by the neurophysiological software system. Cell recognition and sorting was finalized after the experiment using the Offline Sorter program (Plexon, Inc). This allowed neuronal data to be further assessed based on the principal component analysis of the waveforms, cell firing characteristics such as autocorrelograms and interspike interval distribution to ensure that putative cells showed biologically appropriate firing refractory periods, and cross-correlograms to ensure that multiple cells recorded on the same wires showed firing independently of each other. Waveform and spontaneous firing rates were examined to identify putative glutamatergic pyramidal neurons in the PrL in the analysis (West et al., 2014; Moorman & Aston-Jones, 2014). Finally, an additional computer processed operant chamber input and output (Med Associates, Inc) and sent digital outputs corresponding to each event into system to be time stamped along with the neural data.

Histology

Verification of electrode placement for electrophysiology was confirmed to be in the PrL and presented in detail in West et al., 2021.

Data Analysis

Behavior

To determine sex differences in behavior on days 1 and 10 of Pavlovian conditioning, separate 2-way ANOVAs using sex (male vs. female) and stimuli (CS+1, CS+2, CS-1, CS-2) as factors were performed. To assess the degree of conditioning across all experiments, the percent of time spent in the food cup during the stimuli was measured for each animal on day 1 of Pavlovian training and the last day of Pavlovian conditioning (day 10) and analyzed using repeated-measures two-way analysis of variance (ANOVA) with day (day 1 vs. day 10) and stimuli (CS+1, CS+2, CS-1, CS-2) as factors. We calculated a devaluation index (DI) (West et al., 2021) on the first half of the CS+ trials on the test day (trials 1-5) using the following formula:

[nondevalued-devalued] / [nondevalued +devalued]

To normalize this to day 10 preferences, we used the same formula as above on day 10 and subtracted it from the index on the test day. A normalized DI equal to 1 represents an animal that, prior to devaluation, entered the food cup in response to both CS+ equally and, after devaluation, only entered the food cup in response to CS+ that predicted the nondevalued reinforcer (maximally flexible). A normalized DI equal to 0 represents an animal that did not successfully alter its response to CS+ between predevaluation and post-devaluation sessions (maximally inflexible).

Electrophysiology

Changes in neuronal firing patterns relative to task events were analyzed by constructing peri-event histograms (PEHs) surrounding each cue presentation using commercially available software (Neuroexplorer for Windows version 4.034; Plexon, Inc.). PEHs (200-ms bins; 20 s total)

will be constructed on the first and last day of Pavlovian conditioning to all cues. The activity of each cell was examined relative to cue (0–10 s following cue presentation) for all trials. Individual units were categorized as showing either a decrease (inhibition) or an increase (excitation) in firing rate compared to baseline (i.e., termed 'phasic' activity) or no difference in activity from baseline (termed 'nonphasic'). Specifically, cells were classified as phasic if during cue presentation the firing rate is greater than (excitation) or less than (inhibition) the 99.9% confidence interval projected from the baseline period for at least one 200-ms time bin. This confidence interval is selected such that only robust responses are categorized as excitatory or inhibitory, following established procedures (Saddoris & Carelli, 2014; West et al., 2014). Units that exhibit both excitations and inhibitions within the same epoch were classified by the response that is most proximal to the event. Fisher's exact tests were performed to compare neural population responses between groups.

Results

Self-Administration Behavior

First, we examined self-administration of cocaine in both male and female rats. **Figure 4.1B** displays the number of reinforced responses animals received during saline or cocaine self-administration. A 2-way repeated measures ANOVA using Session (self-administration day) and group (saline/sham, cocaine/sham, cocaine/tACS) as factors revealed a main effect of session ($F_{13, 728} = 8.773$, $p < 0.0001$), main effect of group ($F_{2, 56} = 7.832$, $p < 0.0001$), as well as a session x group interaction ($F_{26, 728} = 2.690$, $p < 0.0001$). Tukey's post hoc tests revealed that animals self-administering saline responded more for reward compared to animals responding for cocaine destined for sham tACS treatment on days 3-14 of self-administration and also compared to animals responding for cocaine destined for 80Hz tACS treatment specifically on days 8-14 of self-administration (all p's < 0.05). Regardless of group, all animals acquired self-administration behavior, and critically, there was no difference in the amount of cocaine that rats that were destined to receive sham or tACS treatment earned throughout the session. **Figure 4.1C** shows the amount of cocaine intake during each day of self-administration analyzed by sex. We combined the sham and tACS groups since all rats received cocaine prior to sham or tACS treatment and there was no difference between these two groups as shown in **Figure 4.1B**. For animals self-administering cocaine, a 2-way ANOVA using session and sex as factors revealed a main effect of session ($F_{13, 494} = 5.244$, $p < 0.0001$), a main effect of sex ($F_{1, 38} = 21.03$, $p = 0.0005$), and a session x sex interaction ($F_{13, 494} = 2.654$, $p = 0.0013$). Sidak's multiple comparisons post hoc tests revealed that females self-administered more cocaine than males specifically on days 1, 2, 5, 6, and 8 of self-administration (all p's < 0.05). However, by the end of self-administration, both groups took in similar amounts of cocaine.

No sex differences in Pavlovian learning

One main objective of the current study was to determine if there were sex differences in behavior following self-administration and subsequent tACS treatment. On Day 1, separate 2-way ANOVAs with CS (CS+1, CS+2, CS-1, CS-2) and sex (male vs. female) as factors revealed no main or interaction effects in the saline group (all p's > .05), a main effect of CS in the cocaine/sham group (p = .0081), and a main effect of sex in the cocaine/tACS group. However, post hoc tests revealed no significant differences between male and female rats in the cocaine/tACS group. On Day 10, separate 2-way ANOVAs revealed a main effect of CS in all three groups (all p's < .0001), however there were no main effects of sex found for any group (data not shown). These data suggest that there are no differences in behavior following cocaine self-administration and subsequent tACS treatment between male and female rats. As such, male and female data was pooled together in each group for the remainder of this study.

No effect of cocaine on Pavlovian conditioning, but impaired ability to shift behavior away from cue paired with devalued reward, restored by 80Hz tACS

Next, we determined if a history of cocaine and/or tACS treatment had any effect on the animals' ability to discriminate between the CS+ and CS-. **Figure 4.1D** shows the amount of time spent in the food cup to the CS+ compared to the CS- after 10 days of conditioning. Separate 2-way ANOVAs revealed that regardless of group (saline/sham, cocaine/sham, cocaine/tACS), animals spent significantly more time in the food cup to the CS+ than they did the food cup associated with the CS- on Day 10 compared to Day 1 of conditioning (**Figure 4.1D**, all p's < 0.0001). **Figure 4.1E** displays the conditioning index (CI) during Pavlovian conditioning. A one-way ANOVA revealed there was no significant difference in conditioning between groups ($F_{2, 53}$ = 1.665, p = 0.1990). While there were no differences in the ability to discriminate cues, there was however a difference in the ability of rats to update their behavior towards reward predictive cues

following devaluation of the reward. Specifically, a 2-way ANOVA using group (Saline/sham, cocaine/sham, cocaine/tACS) and Devaluation (Devalued vs. NonDevalued) as factors revealed no main effect of group ($F_{2, 55} = 0.02648$, $p = 0.9739$), a main effect of devaluation ($F_{1, 55} = 23.65$, $p < 0.0001$), but critically, a group x devaluation interaction ($F_{2, 55} = 9.805$, $p = 0.0002$, **Figure 4.1F**). Post hoc tests revealed that both the saline/sham and cocaine/tACS groups were able to successfully shift their behavior following devaluation ($p < 0.001$). Finally, a one-way ANOVA examining at the devaluation index (DI) revealed a significant difference between groups ($F_{2, 53} = 18.39$, $p < 0.0001$). Post hoc tests revealed again both the saline/sham and cocaine/tACS groups had a significantly higher DI compared to the cocaine/sham group (**Figure 4.1G**). Together, these findings indicate that although cocaine had no effect on Pavlovian learning, there were cocaine-induced deficits in behavioral flexibility as revealed by their inability to shift behavior following devaluation that was rescued using 80Hz tACS.

80Hz tACS shifts PrL neural population following cocaine

Finally, we examined how PrL neurons encode Pavlovian conditioning and how cocaine history and treatment with tACS affects this encoding. **Figure 4.2A** displays representative PrL neurons that are either excitatory (top) or inhibitory (bottom) to the CS+. The neural population of PrL neurons were examined on Day 1 and Day 10 of Pavlovian conditioning. On Day 1, there was no significant difference in the proportion of phasic (excitatory and inhibitory) PrL neurons to the CS+ between groups. However, by Day 10, Fisher's exact test analysis revealed that the PrL neural population in animals with a history cocaine/sham tACS treatment was significantly different from the cocaine/tACS group ($p = 0.0029$), trending significant difference between saline/sham and cocaine/sham groups ($p = 0.0714$) and no difference between saline/sham and cocaine/tACS ($p = 0.3423$, **Figure 4.2B**). Furthermore, animals with a history of cocaine and sham treatment had a

majority PrL neurons inhibitory to the CS+ by day 10 while animals with a history of saline were majority excitatory and restored to majority excitatory in animals with a history of cocaine and tACS treatment. These data suggest that tACS restores neural populations closer to control proportions.

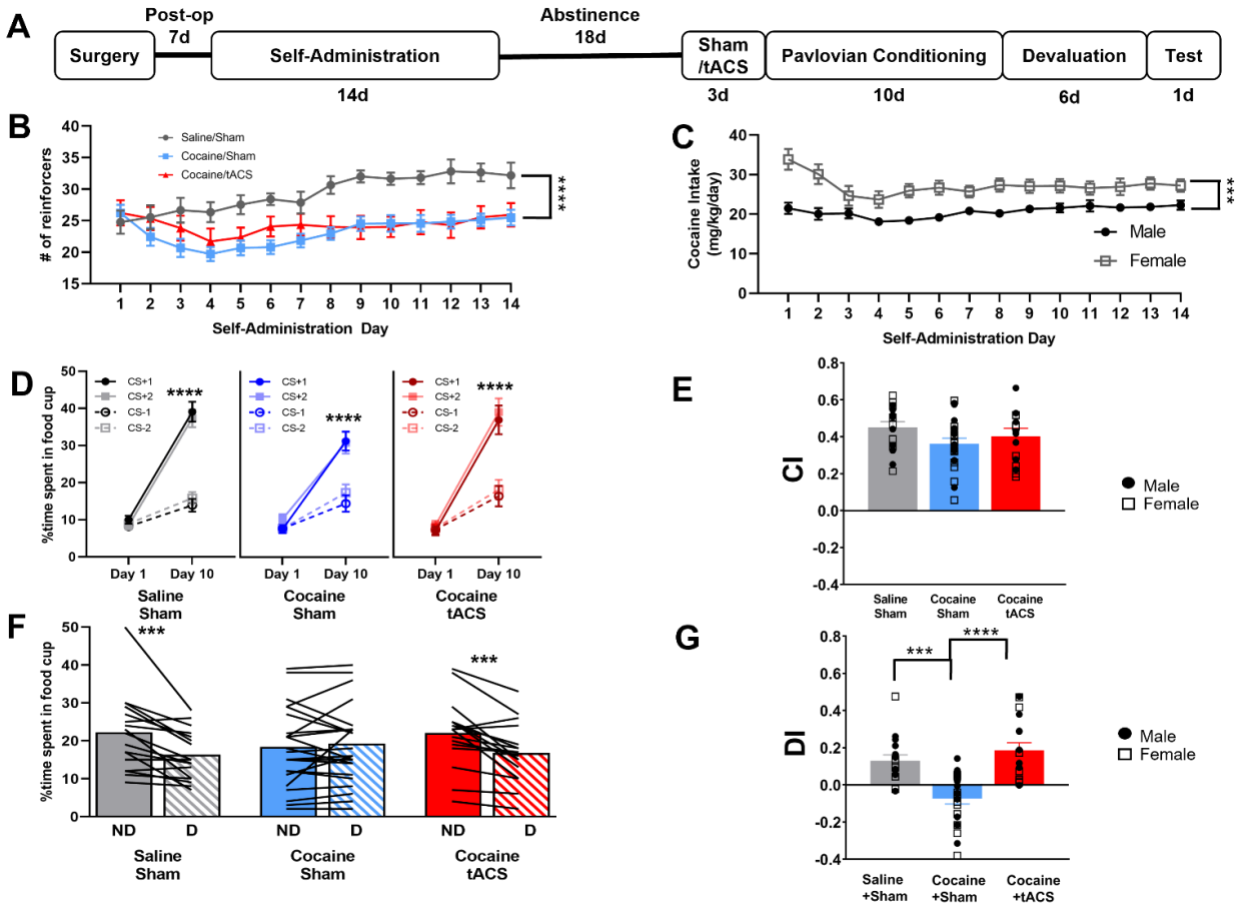

Figure 4.1. Aim 3 Experimental design and behavioral results. A. Experimental timeline B. Reinforced responses received during self-administration (**** $p < 0.0001$) C. Cocaine concentrations for male and female rats (*** $p < 0.001$) D. Percentage of time spent in food cup on Days 1 and 10 of Pavlovian conditioning (**** $p < 0.0001$) E. Conditioning Index (CI) during Pavlovian conditioning F. Percentage of time spent in devalued vs non devalued food cup (*** $p < 0.001$) G. Devaluation Index (DI) (*** $p < 0.001$, **** $p < 0.0001$).

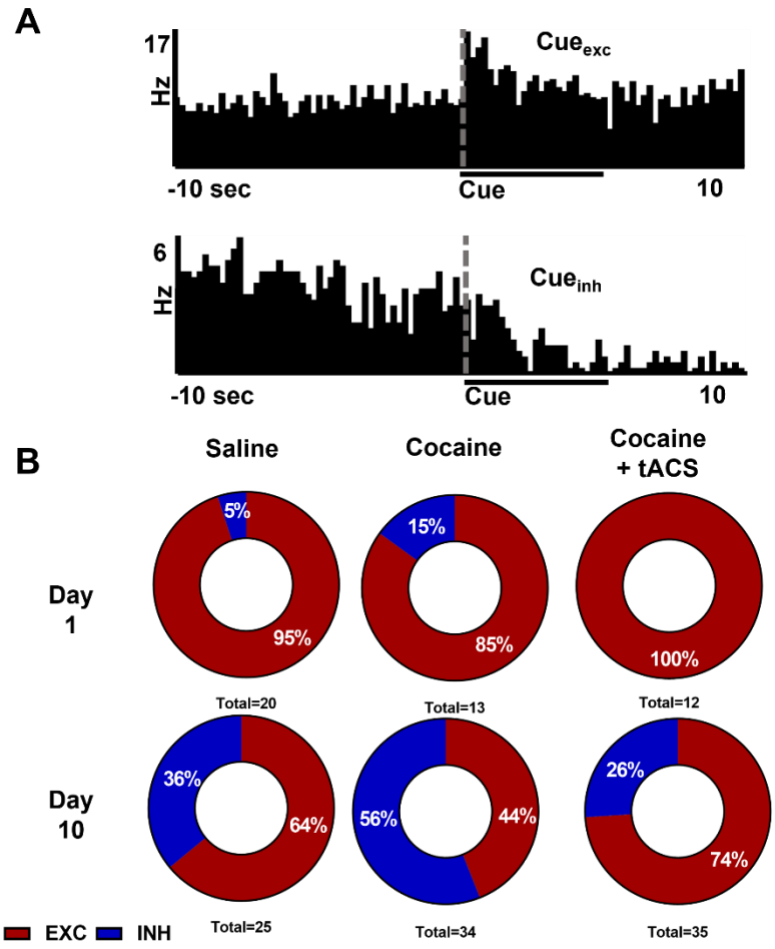

Figure 4.2. Electrophysiology results. A. Representative phasic PrL excitatory (top) and inhibitory (bottom) neurons. B. Phasic PrL neuron population on Day 1 (top) and Day 10 (bottom) of Pavlovian conditioning (total= total number of neurons).

Discussion

In the current study, we examined the effects of 80Hz tACS on PrL single unit activity in male and female rats following a history of cocaine self-administration and prolonged abstinence. Following prolonged abstinence from cocaine, numerous neuroadaptations occur that have been linked with cognitive deficits (Wolf, 2016). Critically, we found that while Pavlovian conditioning was not affected by cocaine history, there were significant cocaine-induced deficits in behavioral flexibility. This is consistent with our other work showing that cocaine history does not impact learning during conditioning but impairs the ability of rats use this information during higher order conditioning (Saddoris & Carelli, 2014) and following outcome devaluation (West et al., 2021). Here, we extended that work by showing that tACS, a noninvasive brain stimulation technique applied after self-administration, had the ability to prevent the shifts in PrL population responses (from excitatory to inhibitory profiles) to the CS signaling reward that result from a history of cocaine.

Another important goal of the present study was to determine if a history of cocaine and prolonged abstinence differentially alters behavioral flexibility across sex. Female rats exhibit decreased basal activity in the prelimbic cortex that were linked to deficits in behavioral flexibility sooner than male rats after opioid self-administration (Anderson et. al. (2021). Critically, there is also evidence supporting increased drug seeking behavior and drug intake among females compared to male rats and that incubation of craving is particularly higher during specific parts of the estrous cycle (Corbett et al., 2021; Nicolas et al., 2019). While we also found that female rats self-administered more cocaine than males, our current findings indicate no sex differences with respect to the ability of cocaine history to alter behavioral flexibility in our task. This is likely

because our rats all are all maximally impaired at this time point so even though the females took more cocaine, it was sufficient to induce behavioral deficits.

As noted above, the current findings are consistent with literature showing that while a history of cocaine may not affect initial learning, the ability to shift behavior is impaired, consistent with prior studies (Saddoris & Carelli, 2014; West et al., 2021). The present findings also support a role of the PrL in this process, although precisely how it does so remain to be examined further. For example, previous work in the Carelli lab showed a generalized loss of phasic neurons following learning by cocaine history (Saddoris & Carelli, 2014), while in the current study we observed a shift in the neural population responses (from primarily excitatory responses to the CS+ to mostly inhibitory activity) after cocaine. While the functional consequences of this shift in neural population remains to be determined, it is important to note that the loss of phasic neurons observed in the higher order conditioning task (Saddoris & Carelli, 2014) was observed in the NAc (a major efferent of the PrL). Future studies are needed to examine how the PrL and NAc circuit may encode aspects of tasks involving learned shifts in behavior, such as behavioral flexibility.

Regardless, the current study showed that behavioral flexibility was restored to controls levels in rats treated with 80Hz tACS after cocaine self-administration but before conditioning in the current study. It would be interesting to see if this same restoration in behavior occurs when tACS is applied at a different timepoint (i.e., after conditioning but before devaluation). Additionally, the larger prefrontal cortex (PFC) is of particular interest given its importance in higher order processing such as executive function and decision making. Studies have shown that following cocaine, there is a generalized decrease in baseline (resting state) activity in the PFC and this hypofunction has been linked to deficits in cognitive function such as poor decision making and increased impulsivity (Gozzi et al., 2011; Wolf, 2016). Future studies are needed to

determine if resting state hypofunction may also be directly linked to cocaine-induced deficits in behavioral flexibility and tACS actions across sex.

CHAPTER 5

GENERAL DISCUSSION

SUD is a chronically relapsing illness characterized by cycles of drug consumption, abstinence from drug use, and resumption of drug taking (relapse) (Ahmed & Koob, 1998; Gawin, 1991; Koob & Volkow, 2016). There are numerous neuroadaptations found in individuals w/SUDs, specifically hypoactivity in the PFC and disruption in PFC and NAc signaling, which are thought to be linked to cognitive deficits associated with SUD (Koob & Volkow, 2010; Volkow & Morales, 2015; Wolf, 2016). A method of interest to examine hypoactivity and its functional consequences is by studying resting state neural activity in these regions and their relationship to drug seeking and taking behaviors (Fedota & Stein, 2015; Gozzi et al., 2011; Hammer et al., 1993; Kearney-Ramos et al., 2019; H. Lu & Stein, 2014). In this dissertation, three specific aims were completed to examine signaling dynamics in the PrL and NAc core following prolonged abstinence from short or extended access cocaine-self-administration and their relationship to drug seeking and taking behavior, as well as the effects of tACS to restore cocaine-induced deficits in PrL neural signaling and behavioral flexibility in male and female rats.

Summary of Experimental Findings

Chapter 2 examined the resting state LFP activity of the PrL and NAc core during prolonged abstinence from short (2hr) access cocaine self-administration using an existing data set from the dissertation of Dr. Rachel Haake (Haake, 2021). Briefly, *in vivo* electrophysiology was used to record resting state LFP neural activity in the PrL and NAc core at weekly timepoints during prolonged (1 month) abstinence from cocaine self-administration. We hypothesized that

both PrL and NAc core power and their functional connectivity (coherence) would be reduced during late (weeks 3 and 4) of abstinence. While PrL and NAc core oscillatory power did not change as a function of time, PrL-NAc core coherence was reduced across frequency bands, although not significant, during abstinence. Specifically, we saw trending significant decreases in peak theta and beta frequency bands across weeks of abstinence. This decrease in coherence following prolonged abstinence is consistent with previous findings from our lab (West et al., 2021; Haake, 2021).

Chapter 3 built upon our findings in **Chapter 2** and examined resting state LFP activity in the PrL and NAc core before and after prolonged abstinence from extended (6hr) access cocaine or water self-administration and their relationship with cocaine seeking and taking behavior. Here, we used *in vivo* electrophysiology to again record resting state LFP activity in the PrL and NAc core before and after abstinence from self-administration. Behaviorally, we hypothesized that both groups (cocaine vs. water self-administration) would exhibit incubation of craving, but only animals self-administering cocaine would show escalation of reward taking. We found that both groups indeed did incubate craving in extinction for their respective rewards but only animals self-administering cocaine escalated reward taking during self-administration. These findings are consistent with the literature and previous findings from our lab (Ahmed & Koob, 1998; Moschak 2014 & Carelli, 2021). In terms of electrophysiology, we hypothesized that we would see significant decreases in PrL and NAc core power and their functional connectivity following abstinence in animals self-administering cocaine compared to water controls. Interestingly, although we saw a decrease in power at the delta frequency range for the NAc core in animals with a history of cocaine, there were no other significant changes in either power or coherence as a function of abstinence. Finally, we hypothesized that resting state LFP activity in the PrL and NAc

core would be correlated with drug seeking and taking behaviors, particularly at lower frequencies (i.e., theta and beta) given their importance in top-down cognitive processing and link to incubated cocaine seeking (Donnelly et al., 2014; Engel & Fries, 2010; Ma et al., 2014; Moschak & Carelli, 2021). We found that resting state PrL power before self-administration (Rest LFP 1) was *positively* correlated with total cocaine intake and escalation at the beta frequency range and *negatively* correlated with total water intake. Furthermore, we found *negative* correlations between Rest LFP 2 (after self-administration, but *before* abstinence) and PrL and NAc core power at low gamma and PrL-NAc core coherence at theta (with trending significance at beta). Taken together, these data suggest that resting state LFP activity (power and coherence) differentially predict specific aspects of cocaine versus water seeking and taking behaviors.

Chapter 4 shifted focus to the effects of tACS on single unit neural activity of the PrL and behavioral flexibility following abstinence from short access cocaine self-administration in male and female rats. Previous work in the Carelli lab showed that a history of cocaine self-administration significantly causes impairments in nondrug-related associative learning and cognition (Cameron & Carelli, 2012; Moschak et al., 2018; Saddoris & Carelli, 2014). Recently, the Carelli lab reported that these neural deficits are related to a cocaine-induced dampening of LFP activity in the PrL -NAc core circuit (West et al., 2021). Importantly, we also revealed that reversing this dampened activity using optogenetics or tACS restored cocaine-induced deficits in behavioral flexibility (West et al., 2021). Briefly, *in vivo* electrophysiology was used to record single unit activity in the PrL and NAc core during a behavioral flexibility task following prolonged abstinence from short (2hr) access cocaine self-administration and subsequent tACS or sham treatment in male rats. In this chapter, we built upon that work by examining single unit activity in the PrL in both male and female rats. We hypothesized that there would be sex

differences in cocaine self-administration behavior and subsequent Pavlovian conditioning given evidence in sex differences in self-administration and behavior (Anderson et al., 2021; Lynch et al., 2002). We found that female rats consumed more cocaine, but no sex differences were found in terms of Pavlovian conditioning, or behavioral flexibility so data were pooled across sex for the remainder of the study. We further hypothesized that cocaine would have no effect on Pavlovian conditioning but would affect the animal's ability to shift behavior and that this deficit would be restored using tACS as previous work from the lab supports this (West et al., 2021). We found that animals with a history of cocaine self-administration that received 80Hz tACS treatment were able to shift behavior, similar to controls (saline). Furthermore, although no difference in PrL neural population data was observed on Day 1 of Pavlovian training, by Day 10, the PrL neural population in animals with a history cocaine/sham tACS treatment was significantly different from the cocaine/tACS group, trended significant difference between saline/sham and cocaine/sham groups, but showed no difference between saline/sham and cocaine/tACS groups. Together, these data suggest that 80Hz tACS has the ability to restore cocaine-induced deficits in behavioral flexibility as well as restore neural population dynamics in the PrL similar to those observed in rats without any cocaine experience.

General Implications, Limitations, and Future Directions

In **Chapter 2**, we sought to examine if broadband decreases in resting state activity in the PrL and NAc core occurred across weeks of prolonged (1 month) experimenter-imposed abstinence from cocaine self-administration in a within subject design. We found a general (although not significant) broadband decrease in resting state PrL-NAc core coherence across frequencies with trending significant decreases at the theta and beta frequency bands, consistent with previous work in the lab (West et al., 2021; Haake, 2021). A broadband decrease in PrL-NAc

core functional connectivity is consistent with findings in humans that speculate that prefrontal hypoactivity seen in individuals with SUD contributes to continued drug use and cognitive deficits evident in this disorder (Goldstein & Volkow, 2002, 2011; Hanlon et al., 2013). Of note however, we did not observe a decrease in oscillatory power in either the PrL or NAc core. As mentioned previously, attempts were made to increase the n from the original data set from the dissertation of Dr. Rachel Haake but were unsuccessful for numerous reasons. This may be why there were no significant decreases found in resting state LFP activity during abstinence. As such, future experiments should increase the number of animals and as well as correlate resting state LFP activity to subsequent drug seeking and taking behaviors to determine if these measurements may be useful as a biomarker for SUD.

In **Chapter 3**, we incorporated the extended access (6 hr/day) cocaine self-administration model and LFP resting state recordings at different time points in the study. We observed significant decreases in NAc core power at the delta frequency range in animals with a history of cocaine but no broadband decreases in PrL or NAc core power or their coherence across recording sessions (i.e., immediately after self-administration training or following 1 month abstinence). As with **Chapter 2**, the lack of a clear decrease in resting state LFP activity either immediately after cocaine self-administration training and/or following abstinence are inconsistent with data showing that prefrontal hypoactivity is observed in humans with SUDs that is thought to be linked with cocaine-induced cognitive deficits (Goldstein & Volkow, 2002, 2011). However, we speculated that regardless of our lack of finding of prefrontal hypoactivity, it was possible that potentially interesting relationships of these neural signals may exist with drug seeking and taking behaviors.

As such, we performed correlational analyses with resting state LFP and cocaine seeking and taking behaviors to determine if these neural signaling dynamics could predict aspects of behavior (i.e., serve as biomarkers for subsequent drug-related behaviors). Interestingly, we found *positive* correlations between resting state PrL power at beta *prior* to self-administration (Rest LFP 1) and total cocaine intake and escalation of cocaine taking. This suggests that increased power at beta is predictive of more cocaine intake and more escalation of drug taking during self-administration. Critically, there is a lack of preclinical studies examining the effects of drug on resting state activity which include a pre drug baseline. Interestingly, when included, there was no effect of cocaine (or sucrose) self-administration on the strength of connectivity (Orsini et al., 2018). Furthermore, the current study used water self-administration as controls instead of yoked saline which may have affected behavior. As such, future studies should instead use yoked saline controls to ensure they experience similar operant behavior as the cocaine self-administration group and include female rats to determine if the relationships found between rest LFP and subsequent behaviors are sex specific.

Chapter 4 was designed to expand previously published work on the effects of high frequency (80 Hz) tACS on restoring cocaine-induced deficits in single unit PrL signaling and flexible behavior, in male and female rats. **Chapter 4** revealed no sex differences in Pavlovian conditioning across groups. However, it was previously reported that female rats exhibit decreases in basal PrL firing and deficits in behavioral flexibility sooner than male rats (Anderson et al., 2021) following opioid self-administration. In the present study, regardless of sex, all animals showed a deficit in flexible behavior following cocaine self-administration however, it may be the case that our female rats show this deficit sooner than male rats and future studies could be employed to investigate these differences further. Finally, we observed a significant shift on Day

10 of Pavlovian conditioning in predominately inhibitory PrL neurons in animals with a history of cocaine/sham tACS treatment. This shift in population dynamics we believe may be similar to the hypofrontality observed in humans with SUDs (Gu et al., 2010; Hu et al., 2015).

Both **Chapters 2 and 3** aimed to examine signaling dynamics in the PrL and NAc core and their functional connectivity measuring LFPs via *in vivo* electrophysiology. However, it is still unclear *when* during abstinence these pronounced changes occur as we were underpowered using a short-access self-administration model (**Chapter 2**). As such, future studies will need to increase the n and measure oscillatory dynamics before, during, and after prolonged abstinence from short access cocaine self-administration. Interestingly however, when using the extended access model with more animals per group (**Chapter 3**), we still did not observe any significant decreases in either oscillatory power or coherence. As such, it may be the case that cocaine induced changes that reflect hypoactivity occur in other neural circuits beyond the PrL-NAc core (e.g., basolateral amygdala [BLA]-NAc core or orbitofrontal cortex [OFC]-NAc core) that in turn, play a critical role in the development of incubated cocaine craving and escalation of drug taking (Stefanik & Kalivas, 2013; Winstanley, 2007). Future studies should assess and compare resting state LFP activity and single unit activity in both male and female rats in short versus extended access models in order to completely examine the use of oscillatory dynamics of the PrL and NAc core and their relationship with subsequent drug seeking and taking behaviors.

Concluding Remarks

Prolonged abstinence from cocaine self-administration has been shown to lead to significant neuroadaptations in the brain's reward circuitry and associated cognitive deficits in individuals living with SUDs (Goldstein & Volkow, 2002, 2011; Wolf, 2016). Measuring resting state neural activity has been employed in order to help identify potential biomarkers of SUDs in

both clinical and preclinical populations (Fedota & Stein, 2015; Gu et al., 2010; Hammer et al., 1993; H. Lu & Stein, 2014). Additionally, noninvasive brain stimulation techniques such as tACS have been identified as a promising treatment strategy for SUDs and has been shown to be successful in restoring cocaine-induced deficits in neural activity and behavioral flexibility (West et al., 2021). The present work described in this document further indicate that oscillatory signaling dynamics of the PrL and NAc core may be useful as a biomarker for CUD, and tACS may be an appropriate treatment for SUDs in both sexes.

In closing, it is important to note that preclinical work such as that described in this dissertation can help characterize cocaine-induced alterations in neural activity in the PrL and NAc core, and thus provide a better understanding of the neurobiology of SUDs. Further, this and related work can also provide important insight regarding optimal tACS parameters to use in clinical populations of those living with SUDs. As mentioned previously, SUDs disproportionally affect those in underserved populations (Burlew et al., 2021; Pinedo & Villatoro, 2020) and has only been exasperated during the current COVID-19 pandemic (Czeisler et al., 2020; McKnight-Eily et al., 2021). Furthermore, historically underserved populations are more skeptical towards medical treatments due to issues such as distrust and poor communication from medical providers and promoting informed decision making surrounding medical procedures can be achieved partly through education surrounding disparities in medicine and potential treatment options (Nguyen et al., 2021; Shungu & Sterba, 2021). The noninvasive nature of tACS and relative efficiency of treatment strategy may encourage those disproportionally affected by SUDs to seek treatment that otherwise may not due to issues such as time constraints, as lack of time can be a barrier in receiving healthcare (George et al., 2014; Lindsay et al., 2020). As such, education and research surrounding potential treatment options and optimal parameters is crucial in closing SUD

treatment gaps. Given this critical health crisis, it is important for preclinical research such as that described in this dissertation to continue in order to not only make information surrounding SUDs more accessible, but to make treatments for this devastating disorder more accessible as well.

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