THE EFFECTS OF TRANSCRANIAL ALTERNATING CURRENT STIMULATION ON PRELIMBIC CORTEX TO NUCLEUS ACCUMBENS CORE OSCILLATORY DYNAMICS AND COCAINE SEEKING

Rachel McDonnell Haake

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Approved by:

Regina M. Carelli

Stacey Daughters

Flavio Fröhlich

Donald T. Lysle

Todd E. Thiele

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ABSTRACT

Rachel McDonnell Haake: The Effects of Transcranial Alternating Current Stimulation on Prelimbic Cortex to Nucleus Accumbens Core Oscillatory Dynamics and Cocaine Seeking
(Under the direction of Regina M. Carelli)

Prolonged abstinence from cocaine leads to profound changes in brain reward circuitry that contribute to relapse vulnerability. Neuroimaging studies reveal a generalized (resting state) dampening of prefrontal cortex and nucleus accumbens activity which persists after extended periods of abstinence and is linked with impaired executive control in substance use disorder (SUD). Interestingly, however, these same brain regions also show heightened activation to cocaine-related stimuli following prolonged abstinence concomitant with increased drug seeking. Opposing context-dependent shifts in neural signaling following prolonged cocaine abstinence are also observed at the neurocircuit and network level. Given these findings, an emerging strategy for treating SUD uses noninvasive brain stimulation to normalize drug-induced disruptions in brain activity. Our lab recently published data showing the efficacy of transcranial alternating current stimulation (tACS) for reversing cocaine-induced deficits in neural signaling and restoring behavioral flexibility. Three specific aims were completed to investigate the role of the prelimbic cortex to nucleus accumbens core circuit in cocaine-seeking behaviors following short or prolonged abstinence and the feasibility of tACS to modulate oscillatory dynamics and incubated cocaine seeking.

For my given and chosen family.

In loving memory of John Schall.

“Focus on today, you’ll find a way.”

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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

AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
AMPAR	AMPA receptor
BLA	Basolateral amygdala
CP-AMPAR	Calcium permeable AMPA receptor
CUD	Cocaine use disorder
DA	Dopamine
DLPFC	Dorsolateral prefrontal cortex
EEG	Electroencephalography
fMRI	Functional magnetic resonance imaging
IL	Infralimbic cortex
LFP	Local field potential
LPP	Late positive potential
LTD	Long term depression
mPFC	Medial prefrontal cortex
MSN	Medium spiny neuron
NAc	Nucleus accumbens
PFC	Prefrontal cortex
PrL	Prelimbic cortex
rsFC	Resting state functional connectivity
rTMS	Repetitive transcranial magnetic stimulation
SUD	Substance use disorder
tACS	Transcranial alternating current stimulation
tDCS	Transcranial direct current stimulation
TMS	Transcranial magnetic stimulation
VTA	Ventral tegmental area

CHAPTER 1

GENERAL INTRODUCTION

Substance use disorder (SUD) is a chronically relapsing disease characterized by compulsion to seek and take drug, reduced control over intake, and negative emotional states that emerge during periods of abstinence (Koob & Le Moal, 1997; Koob & Volkow, 2010). According to the National Survey on Drug Use and Health, 21.6 million people aged 12 or older in the United States qualified as 'needing SUD treatment' in 2019 (defined by having an SUD or receiving SUD treatment at a specialized facility in the past year), but only 4.2 million individuals (19.4%) received it (SAMHSA, 2020). The most frequently-cited reasons for not receiving SUD treatment included not knowing where to go for treatment, and inability to afford the cost of treatment or healthcare coverage (SAMHSA, 2020), reflecting large-scale systemic shortcomings in SUD treatment accessibility.

Critically, recent nation-wide screenings reveal that mental health challenges, including initiation or escalation of substance use, have dramatically increased since the onset of the SARS-CoV-2 pandemic, with Black and Hispanic communities, young adults (18-25 years), essential workers, and unpaid caregivers being disproportionately impacted (Czeisler et al., 2020; Czeisler et al., 2021). Additional studies indicate heightened risk for COVID-19 and its adverse outcomes in individuals with SUDs (Wang, Kaelber, Xu, & Volkow, 2021), while trust in the COVID-19 vaccines and stigma-related barriers to healthcare access are current obstacles to SUD and COVID-19 treatment for people who use drugs (Mellis, Kelly, Potenza, & Hulsey, 2021; Stowe et al.,

2020). Collectively, these data demonstrate the crucial need for the development of effective and accessible SUD treatments not only for pandemic recovery but also for sustained improvements in the health and wellbeing of US residents.

This dissertation seeks to examine the efficacy of noninvasive brain stimulation, specifically transcranial alternating current stimulation (tACS), to reverse cocaine-induced deficits in brain activity and reduce drug seeking following prolonged abstinence in rats. This introductory chapter provides a review of the neurobiological mechanisms underlying distinct features of SUDs including impaired cognition, cue-evoked drug craving, and persistent vulnerability to relapse, all of which are attributed to dysfunction in brain reward circuitry. Chapters 2 and 3 examine whether high (80 Hz) or low-frequency (16 Hz) tACS reverse cocaine-induced disruptions in neural activity and reduce cocaine seeking following prolonged (one-month) cocaine abstinence. Next, Chapter 4 examines the effects of short versus prolonged cocaine abstinence on resting state and cocaine cue-evoked neural activity in the prefrontal cortex to nucleus accumbens core pathway and their relationships with cocaine seeking. Finally, the implications of these results will be integrated and discussed in the General Discussion.

The Neurobiology of Cocaine Use Disorder

Decades of research have revealed profound disruptions in multiple brain regions and their associated circuitry contributing to the behavioral phenotype observed in individuals with SUD. For example, neuroimaging studies in individuals with cocaine use disorder (CUD) and non-human primates reveal long-lasting structural and functional abnormalities in prefrontal cortical and limbic circuitry (Goldstein & Volkow, 2002, 2011; Hanlon, Beveridge, & Porrino, 2013) associated with impaired behavioral inhibition, emotion regulation and decision-making, as well

as heightened vulnerability to relapse (Goldstein & Volkow, 2011; Wolf, 2016). Notably, abnormalities in brain structure and function associated with SUDs exist among converging environmental, social, and genetic influences that confer individual vulnerability for developing SUD. Despite the wealth of information uncovered from neuropsychological examinations of SUD patients, relapse to drug seeking and taking following extended periods of abstinence remains a major challenge for SUD treatment. Further, there are currently no FDA-approved pharmacotherapies for CUD.

Incubation of Cocaine Craving

Sustained vulnerability to relapse in individuals with CUD is due in part to the ability of cocaine-associated stimuli to elicit intense drug craving and seeking, which progressively intensifies throughout the initial stages of abstinence. This phenomenon, now termed 'incubation of craving' (Grimm, Hope, Wise, & Shaham, 2001; Li, Venniro, & Shaham, 2016; L. Lu, Grimm, Hope, & Shaham, 2004; Pickens et al., 2011), was first reported in clinical observations of individuals with CUD and consisted of anhedonia, anxiety, and intense cue-induced cocaine craving which often triggered relapse (Gawin & Kleber, 1986). These observations have since been empirically tested and validated extensively in preclinical studies (Conrad et al., 2008; Grimm et al., 2001; Koya et al., 2009; Lee et al., 2013; Li et al., 2016; L. Lu et al., 2004; Ma et al., 2014; Neisewander et al., 2000; Pickens et al., 2011) and more recently in humans with CUD as well (Parvaz, Moeller, & Goldstein, 2016). Critically, the incubation of craving model of relapse has high translational relevance particularly for situations wherein humans with SUDs abstain from drug taking due to hospitalization or incarceration.

In preclinical examinations of the incubation of cocaine craving, rats are typically trained to self-administer intravenous (i.v.) cocaine for ~2 weeks, then tested for cue-elicited cocaine-seeking behavior following varying durations of experimenter-imposed abstinence (L. Lu et al., 2004; Pickens et al., 2011; Wolf, 2016). As the duration of forced abstinence from short or extended access cocaine self-administration increases, so does the level of drug-seeking behavior (Grimm et al., 2001; Li et al., 2016; L. Lu et al., 2004; Ma et al., 2014; Neisewander et al., 2000; Pickens et al., 2011). This increase in cocaine seeking follows a parabolic trajectory, increasing progressively over the first two months of abstinence, plateauing, then returning toward baseline levels but remaining elevated at 6 months compared to the first day of forced abstinence in rats (L. Lu et al., 2004; Pickens et al., 2011). Interestingly, drug seeking induced by priming injection of cocaine is unchanged throughout prolonged abstinence (L. Lu et al., 2004), suggesting that incubation of craving is driven by a strengthening of the motivational impact of the cocaine cue on drug-seeking behavior. Critically, behavioral expression of incubated cocaine craving is observed following both short (2 h/day) and extended access (6 h/day) cocaine self-administration (Grimm et al., 2001; Hollander & Carelli, 2005, 2007; L. Lu et al., 2004; Ma et al., 2014; Pickens et al., 2011; West, Saddoris, Kerfoot, & Carelli, 2014; Wolf, 2016), although some reports reveal more robust incubation following extended access protocols (L. Lu et al., 2004; Wolf, 2016).

Neurobiological Mechanisms Underlying Persistent Cocaine Craving and Seeking

Preclinical studies have revealed numerous cocaine-induced neuroadaptations in reward-related circuitry that persist following extended drug abstinence and are associated with cocaine seeking (Wolf, 2016). For example, cocaine self-administration abolished long-term depression (LTD) in the nucleus accumbens (NAc) (Martin, Chen, Hopf, Bowers, & Bonci, 2006) and induced

synaptic glutamate plasticity in ventral tegmental area (VTA) dopamine (DA) neurons (Chen et al., 2008) which persisted following extended (≥3 weeks) abstinence (Chen et al., 2008; Martin et al., 2006). Additionally, extensive work from Wolf and colleagues has demonstrated synaptic changes in NAc core medium spiny neurons (MSNs; e.g., insertion of high conductance calcium-permeable AMPA receptors, CP-AMPARs) following prolonged cocaine abstinence (Conrad et al., 2008; Loweth, Tseng, & Wolf, 2014; Purgianto et al., 2013; Scheyer, Wolf, & Tseng, 2014; Wolf, 2010, 2016), which are required for incubated cocaine-seeking behavior (Conrad et al., 2008; Loweth et al., 2014). These findings are consistent with the well-established role of the NAc – which receives glutamate inputs from limbic and cortical regions and projects to motor regions – as a key motivation-to-action integrator. Indeed, synaptic incorporation of CP-AMPARs in the NAc core following prolonged cocaine abstinence increases NAc MSN responsiveness to incoming glutamate (Purgianto et al., 2013) such as that released in response to cocaine-associated stimuli, thereby increasing cue-evoked cocaine-seeking (Wolf, 2016).

In support, our lab has demonstrated that neurons in the NAc core show heightened responsiveness to cocaine-associated stimuli following prolonged (one-month) versus short (one-day) cocaine abstinence concomitant with incubated cocaine seeking (Hollander & Carelli, 2005, 2007). These same findings were observed in the prelimbic cortex (PrL) (West et al., 2014), which is part of the medial prefrontal cortex (mPFC) in rodents and sends a dense glutamatergic projection to the NAc core (Sesack, Deutch, Roth, & Bunney, 1989). In these studies, rats were trained to self-administer cocaine during 14 daily short access (2 h/day) sessions, followed by either one day or one month of experimenter-imposed abstinence wherein rats remained in their home cages. Following short or prolonged abstinence, *in vivo* electrophysiology was used to record single unit activity in the NAc (Hollander & Carelli, 2005, 2007) and mPFC (West et al., 2014)

during a cue-evoked cocaine seeking test. We reported significantly increased cocaine-seeking behaviors following prolonged versus short abstinence, as well as an increase in the proportion and strength of neurons in the NAc core (not shell) and PrL (not infralimbic cortex, IL) that showed phasic responses (i.e., significant changes in firing rate within seconds of behavioral/task events) to cocaine-associated cues and cocaine seeking. Similarly, Guillem and colleagues (2014) demonstrated an increase in the number of NAc core neurons that encoded cocaine-seeking behaviors following prolonged abstinence, and the degree of this increase was correlated with the magnitude of incubation of cocaine-seeking behaviors (Guillem, Ahmed, & Peoples, 2014). Collectively, these results indicate that the NAc and mPFC are more strongly activated by cocaine-associated stimuli after ~3-4 weeks of forced abstinence and this increased activation is involved in the expression of incubated cocaine craving in rats (Wolf, 2016). These preclinical findings are notable given the ability of cocaine cues to activate similar regions of the human brain (Garavan et al., 2000; Goldstein & Volkow, 2002, 2011; Jasinska, Stein, Kaiser, Naumer, & Yalachkov, 2014; Wolf, 2016) and elicit intense drug craving in individuals with CUD (Childress et al., 1993; Childress, McLellan, Ehrman, & O'Brien, 1988; Volkow et al., 2010), both of which are associated with SUD severity and risk of relapse (Childress et al., 1988; Jasinska et al., 2014).

Evidence for Incubation of Cocaine Craving in Individuals with CUD

More recently, electrophysiological indices of incubation of cocaine craving were examined in humans with CUD (Parvaz et al., 2016). In this cross-sectional study performed over five years from 2007-2012, electroencephalography (EEG) was used to record brain activity in response to cocaine-associated cues in individuals with CUD with varying durations of abstinence. The “late positive potential” component of the EEG was used as an objective marker of incubation

of cue-induced cocaine craving given its established role in tracking attention to salient stimuli (Hajcak, MacNamara, Foti, Ferri, & Keil, 2013), correlation with subjective (i.e., self-reported) cue-induced cocaine craving and simulated drug-seeking behavior (Franken et al., 2008; Moeller et al., 2012), and modulation by cocaine abstinence (Dunning et al., 2011). Participants also self-reported their levels of baseline craving before cocaine cue presentations as well as feelings of 'liking' and 'wanting' of cocaine following cocaine cues. Remarkably, LPP amplitudes among the study participants with varying durations of cocaine abstinence showed a parabolic trajectory strikingly similar to that observed in preclinical models of incubation of craving (L. Lu et al., 2004; Pickens et al., 2011) although here LPP responses to cocaine-related stimuli were highest in individuals with 1-6 months of abstinence compared to lower LPP amplitudes in individuals with shorter (2 days) or longer (1 year) abstinence durations (Parvaz et al., 2016). Interestingly, self-reported baseline (pre-cue) craving and cue-induced liking and wanting of cocaine showed a linear decline from early (2 days) to prolonged (1 year) abstinence, suggesting that individuals with CUD may be most vulnerable to relapse between 1-6 months when neural indices of cue-induced cocaine craving are highest but cognizance of craving is reduced (Parvaz et al., 2016). Indeed, some perspectives on SUDs postulate that continuation of substance use despite negative consequences reflects impaired awareness of disease severity (Goldstein & Volkow, 2002, 2011), and some preclinical studies detect drug-induced neuroadaptations following prolonged cocaine abstinence in the absence of behavioral indices of incubated craving (Chen et al., 2008; Jasinska, Chen, Bonci, & Stein, 2015).

Prefrontal Hypoactivity in CUD

Collectively, these studies indicate persistent changes in corticolimbic circuitry which render the brain more reactive to cocaine-associated stimuli and promote relapse. However, SUDs are also characterized by long-lasting cognitive impairments and reduced function of frontal cortical regions associated with executive control (Aharonovich et al., 2006; Garavan & Hester, 2007; Goldstein et al., 2004; Goldstein & Volkow, 2002, 2011; Hanlon et al., 2013; Jentsch & Taylor, 1999), and these are linked with negative outcomes of SUD including greater risk of relapse (Goldstein & Volkow, 2011). Neuroimaging studies show reduced gray matter volume (Franklin et al., 2002) and brain glucose metabolism in the PFC in individuals with CUD compared with matched control subjects which are detectable even after several weeks to months of cocaine abstinence (Matochik, London, Eldreth, Cadet, & Bolla, 2003; Volkow et al., 1992). Given the role of the PFC in behavioral inhibition, motivation, and decision making (Goldstein & Volkow, 2011), this persistent PFC hypoactivity likely contributes to hallmark features of SUD including reduced sensitivity to non-drug reinforcers and impaired top-down inhibition of disadvantageous behaviors (Goldstein & Volkow, 2002, 2011). In support, decreased resting cerebral blood flow in the dorsolateral PFC (DLPFC) of subjects with cocaine use disorder was associated with impaired decision making (Adinoff et al., 2003). Critically, impairments in PFC-dependent cognitive tasks in individuals with CUD may threaten sustained abstinence (Garavan & Hester, 2007) or increase attrition from SUD treatment (Aharonovich et al., 2006).

One limitation of clinical studies in SUD patients is the inability to determine whether biomarkers of SUDs such as PFC hypoactivity precede or follow substance use. Indeed, PFC dysfunction may in some instances predate substance use and confer individual vulnerability for developing SUDs (Goldstein & Volkow, 2011). This is supported by the established role of the

PFC in top-down executive control and behavioral inhibition (Goldstein & Volkow, 2002, 2011), together with the interpretation of SUD development as a transition from recreational to chronic substance use driven by a shift in PFC-mediated impulsive to compulsive behavior (Everitt & Robbins, 2005; Goldstein & Volkow, 2011; Koob & Volkow, 2010). Nevertheless, preclinical research has provided additional information on the effects of chronic cocaine self-administration on neural activity and subsequent behaviors. For example, our lab has demonstrated cocaine-induced deficits in higher-order associative learning and cognitive flexibility linked with reduced or altered activity in PrL and NAc neurons (Saddoris & Carelli, 2014; West et al., 2021). Likewise, studies from other labs implicate a role of PFC activity in 'compulsive' (i.e., resistant to punishment-induced suppression of drug seeking, 'punishment-resistant') cocaine seeking. In one study (Chen et al., 2013), rats underwent extended access cocaine self-administration under conditions which allow the measurement of drug seeking and taking in the presence of operant punishment. Briefly, in this model an extensive period of cocaine self-administration is followed by a second phase wherein the operant response that was reinforced by cocaine is now intermittently followed by footshock (Ahmed, 2012). Introduction of the footshock suppresses cocaine seeking and taking in most rats, while some are resistant to punishment-induced suppression of cocaine seeking and exhibit other behaviors that resemble features of SUDs (Deroche-Gamonet, Belin, & Piazza, 2004). Chen and colleagues (2013) reported punishment-resistant cocaine seeking was associated with decreased excitability of deep-layer pyramidal PrL neurons, the degree of which correlated with cocaine intake under shock. Further, optogenetic stimulation of PrL neurons reduced cocaine seeking in punishment-resistant rats, demonstrating a causal role of cocaine-induced PFC hypoactivity in compulsive cocaine seeking (Chen et al.,

2013). These results provided strong support for targeted stimulation of the PFC as a potential treatment option for individuals with SUDs.

Noninvasive Brain Stimulation for SUDs

Despite our understanding of the neurobiological changes underlying maladaptive behaviors observed in individuals with SUD, it has proven extremely challenging to develop effective SUD treatments and to date, there are no FDA-approved pharmacotherapies for CUD. However, one novel approach used for other psychiatric disorders such as depression (Perera et al., 2016) that holds great promise for the treatment of SUDs is noninvasive brain stimulation. Indeed, recent data indicate that one form of noninvasive brain stimulation, transcranial magnetic stimulation (TMS) targeting the PFC may modulate cortical excitability and promote sustained abstinence in individuals with SUD (Hone-Blanchet, Ciraulo, Pascual-Leone, & Fecteau, 2015; Pripfl, Tomova, Riecansky, & Lamm, 2014; Terraneo et al., 2016). Promising results from this growing area of clinical research suggest repetitive TMS (rTMS) delivered to PFC regions may reduce drug craving in individuals with SUDs (Bellamoli et al., 2014; Feil & Zangen, 2010; Gorelick, Zangen, & George, 2014; Hanlon et al., 2015; Hanlon, Dowdle, & Henderson, 2018). Thus far, two particularly viable approaches for using TMS to treat SUD are increasing activity in the DLPFC (homologous to the PrL in rodents) to putatively fortify top-down executive control, or decreasing corticolimbic activity in the presence of drug-associated cues to dampen cue reactivity (Hanlon et al., 2018).

In addition to TMS, transcranial direct current stimulation (tDCS) delivered over the PFC in individuals with SUDs shows promise for reducing drug craving, although findings from these studies should be interpreted with caution due to limited sample sizes, different stimulation

protocols or study durations, and lack of proper controls and blinding (Lupi et al., 2017). However, one recent randomized, double-blind sham-controlled tDCS study in individuals with CUD undergoing inpatient treatment reported a trend toward significantly reduced self-reported craving as well as improvements in daytime sleepiness and readiness to change drug use following tDCS versus sham treatment, although craving and sleepiness effects were transient, i.e., not observed at the one-month follow-up session (Gaudreault et al., 2021).

Limitations of TMS and tDCS for SUD Treatment

Interaction between cue reactivity and executive control in PFC-NAc circuitry: As described above, SUDs are associated with numerous drug-induced alterations in reward circuitry. One prominent perspective posits that SUDs are driven by impaired PFC control over hyperactive subcortical brain regions (Bechara, 2005; Goldstein & Volkow, 2002, 2011; Jasinska et al., 2015; Jentsch & Taylor, 1999). Said another way, chronic drug use augments sensitivity to drug-associated cues due to increased activation of subcortical structures like the NAc (Conrad et al., 2008; Loweth et al., 2014; Martin et al., 2006; Roberts-Wolfe, Bobadilla, Heinsbroek, Neuhofer, & Kalivas, 2018) and VTA (Chen et al., 2008; Wolf & Tseng, 2012), while generalized PFC dysfunction (Goldstein & Volkow, 2002, 2011) impairs top-down inhibition of cue-evoked drug craving and seeking, ultimately leading to compulsive drug use and vulnerability to relapse (Jasinska et al., 2015). This view is well-supported and has guided progress toward developing novel treatments for SUDs including noninvasive brain stimulation targeting PFC regions, described above (Hanlon et al., 2018; Mahoney, Hanlon, Marshalek, Rezai, & Krinke, 2020; Terraneo et al., 2016).

However, this view does not completely address the fact that the same brain regions which show baseline (i.e., resting) hypoactivity during top-down cognitive control processes and protracted abstinence – namely the PFC and NAc (Beveridge, Gill, Hanlon, & Porrino, 2008; Hammer, Pires, Markou, & Koob, 1993; Porrino, Smith, Nader, & Beveridge, 2007; Sun & Rebec, 2006) – also show *heightened* activation by cocaine cues following a similar period of cocaine abstinence (Guillem et al., 2014; Hollander & Carelli, 2005, 2007; Shin et al., 2016; West et al., 2014). Considering these findings, it is probable that the PFC-NAc circuit differentially guides (i.e., promotes or suppresses) behavior depending on the environmental conditions that are recruiting those brain regions (Jasinska et al., 2015). That is, for example, when PFC activity is recruited by inhibitory control processes (e.g., when operant behavior previously reinforced by cocaine is paired with footshock), stimulation of the PFC reduces cocaine seeking while PFC inactivation augments drug seeking (Chen et al., 2013; Jasinska et al., 2015). In contrast, in the presence of cocaine-associated cues, activation of the PFC (Kalivas, 2008; McFarland, Lapish, & Kalivas, 2003; Peters, Kalivas, & Quirk, 2009) and NAc (Cornish & Kalivas, 2000; McFarland et al., 2003) *promote* cocaine seeking. Ultimately, it appears that compulsive drug seeking and persistent vulnerability to relapse involve a *dynamic interplay between executive control and cue reactivity processes mediated by PFC-NAc signaling.* In support, rats that have undergone incubation of cocaine craving following prolonged abstinence are less sensitive to punishment-induced suppression of cocaine taking (Gancarz-Kausch, Adank, & Dietz, 2014).

Altered neurocircuitry and functional connectivity in SUD: Critically, the PFC and NAc do not function in isolation but rather are embedded within a larger neural circuit which guides behavior. For example, the amygdala is activated by drug cues in humans with SUDs (Childress et al., 1999), and in rats the basolateral amygdala (BLA) sends dense projections to the NAc

(Kelley, Domesick, & Nauta, 1982) and influences discrete aspects of NAc activity during goal-directed behaviors (Carelli, Williams, & Hollander, 2003; Jones, Day, Wheeler, & Carelli, 2010). In the rat, the mPFC consists of the PrL and IL which primarily project to the NAc core and shell, respectively (Krettek & Price, 1977; Pinto & Sesack, 2000; Sesack et al., 1989). Recent data indicate that synaptic plasticity within BLA- and IL-NAc shell and PrL-NAc core projections play circuit-specific causal roles in the incubation of cocaine craving following prolonged abstinence in rats (Dong, Taylor, Wolf, & Shaham, 2017; Lee et al., 2013; Ma et al., 2014). Briefly, optogenetic induction of LTD in the BLA-NAc shell (Lee et al., 2013) and PrL-NAc core (Ma et al., 2014) pathways reduced cue-elicited cocaine seeking following prolonged abstinence, while LTD in the IL-NAc shell projection augmented cocaine seeking (Ma et al., 2014). These results are consistent with prior work showing differential roles of mPFC (West et al., 2014), NAc (Hollander & Carelli, 2005, 2007), and amygdala subregions (L. Lu, Dempsey, Shaham, & Hope, 2005; L. Lu, Hope, et al., 2005) in incubated cocaine seeking, and extend those findings to suggest that the behavioral expression of heightened cocaine craving stems from converging input from cortical and limbic regions onto the NAc.

The growing consideration of circuit-level dysfunction in preclinical models of SUD is one result of recent advances in neuroscience technologies and provides new and important insight into the neurocircuitry of SUD (Dong et al., 2017; Luscher, 2016). Developments in neuroimaging technologies – which can be used in both preclinical and clinical research – also reveal large scale network-level disruptions in corticolimbic circuitry associated with specific behavioral aspects of SUD (Gu et al., 2010; H. Lu & Stein, 2014). In humans and animals, functional magnetic resonance imaging (fMRI) can be used to assess resting state functional connectivity (rsFC) between brain regions in functionally relevant networks (H. Lu & Stein, 2014). Such examinations have revealed

reduced rsFC between the PFC and midbrain (Tomasi et al., 2010), and PFC and striatum (Gu et al., 2010; Hu, Salmeron, Gu, Stein, & Yang, 2015) in individuals with CUD compared to matched non-substance using controls. In rats, prolonged abstinence from extended access cocaine self-administration led to reduced rsFC between the NAc and mPFC compared to rats that self-administered sucrose and naïve controls (H. Lu et al., 2014). These findings may reflect difficulty engaging reward, motivational, and emotional circuitry (Sutherland, McHugh, Pariyadath, & Stein, 2012), consistent with an impaired ability to attend to non-drug reinforcers (Goldstein & Volkow, 2002, 2011) and the emergence of negative affect during withdrawal and protracted abstinence in people with SUDs (Koob & Le Moal, 1997, 2005; Koob & Volkow, 2010). Interestingly, when neuroimaging was completed after a cue-induced craving test (wherein cocaine-associated stimuli were shown), rsFC between the ventral striatum (which includes the NAc) and PFC was *increased* in currently abstaining individuals with CUD compared with matched controls (Wilcox, Teshiba, Merideth, Ling, & Mayer, 2011). Together, these data reveal context-dependent, bidirectional shifts in network connectivity in individuals with SUD. Such findings are consistent with the view that *compulsive drug seeking and sustained vulnerability to relapse are driven by interactions between executive control and cue reactivity processes mediated by PFC-NAc signaling dynamics*. Finally, these results signify the necessity of a targeted, systems-level approach for SUD treatment.

Using *In Vivo* Electrophysiology to Assess PFC-NAc Functional Connectivity

An alternative approach for studying neurocircuit and network-level processing both within and between brain regions in awake, behaving rodents lies in *in vivo* local field potential (LFP) recordings. Here, electrophysiological recordings at lower frequencies (≤ 300 Hz) reflect

slower membrane potential changes in a larger population of neurons compared to that measured with single unit recordings detected at higher frequencies (van der Meer et al., 2010). The presence of rhythmic oscillations in the LFP signal reflects some degree of systematic, coordinated activity which can be influenced by external stimuli, behavior, spiking activity or LFPs in other brain regions or frequency bands (van der Meer et al., 2010). Common metrics used in the analysis of LFPs include power, which reflects the strength (i.e., amplitude) of a given frequency component, and coherence, which is a measure of the degree to which LFP power in two signals (i.e., raw traces from the PrL and NAc) co-occurs (van der Meer et al., 2010). This dissertation will focus on top-down (PrL-NAc core) coherence given the proposed critical role of neuronal coherence in network interactions that guide behavior and cognition (Fries, 2005).

Notably, LFP oscillations have been functionally linked with specific types of behavior (that vary slightly in frequency range between human EEG and animal LFP signals). In rats, previous work has defined functionally relevant frequency bands within the following ranges which informed our LFP analyses in this dissertation: 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). In humans with CUD, delta power in the frontal cortex increased during guided imagery of cocaine cues relative to neutral cues (Reid et al., 2003), suggesting a role of frontal cortex delta oscillations in cue-induced cocaine craving. Other reports indicate involvement of frontal theta and beta oscillations in top-down cognitive control in humans (Cavanagh & Frank, 2014; Cooper et al., 2019; Engel & Fries, 2010; Zavala et al., 2018), while subcortical theta and beta oscillations are linked with compulsive (i.e., punishment-resistant) cocaine seeking in rats (Degoulet, Tiran-Cappello, Combrisson, Baunez, & Pelloux, 2021). Gamma oscillations have been quantified in the rat NAc, where they have been separated into two functional bands (Gamma-50 and Gamma-80)

that have distinct behavioral correlates, related for example, to reward receipt and decision making (Berke, 2009; van der Meer & Redish, 2009). Interactions between specific frequency bands appear to be functionally relevant as well, including for example, beta-gamma interactions in stimulus processing (Richter, Thompson, Bosman, & Fries, 2017), and causal links between delta-beta and theta-gamma cross-frequency coupling in distinct components of cognitive control (Riddle, McFerren, & Frohlich, 2021). However, precisely how LFP oscillatory dynamics at specific frequencies in the PrL-NAc core circuit are linked to cocaine seeking following prolonged abstinence is unknown and a key goal of this dissertation.

Goals of Dissertation

As reviewed above, prolonged abstinence from cocaine leads to profound changes in PFC and NAc activity that contribute to relapse vulnerability. Neuroimaging studies in humans, non-human primates, and rats reveal a generalized (resting state) dampening of PFC and NAc activity which persists after extended periods of abstinence and is linked with impaired executive control (Beveridge et al., 2008; Goldstein & Volkow, 2002, 2011; Hanlon et al., 2013). Interestingly, however, these same brain regions also show heightened activation to cocaine-related stimuli following prolonged abstinence concomitant with increased drug seeking (Cameron & Carelli, 2012; Guillem et al., 2014; Hollander & Carelli, 2005, 2007; West et al., 2014). Opposing context-dependent shifts in neural signaling following prolonged cocaine abstinence are also observed at the neurocircuit and network level (Dong et al., 2017; Gu et al., 2010; Hu et al., 2015; Lee et al., 2013; Ma et al., 2014; Wilcox et al., 2011), and abstinence-related synaptic plasticity in the PrL-NAc core circuit is causally linked with incubation of cocaine craving (Ma et al., 2014).

Given these findings, an emerging strategy for treating SUDs involves using noninvasive brain stimulation to normalize drug-induced disruptions in brain activity and improve SUD treatment outcomes. While TMS and tDCS delivered to the PFC may reduce drug craving (Hanlon et al., 2018; Hone-Blanchet et al., 2015; Mahoney et al., 2020; Terraneo et al., 2016), we believe that combining our established *in vivo* electrophysiology methods with tACS represents a stronger mechanism-driven approach for the development of novel treatments for SUDs. As described briefly above, the cortex consists of synchronized neuronal activity that generates weak electric fields which can be measured via EEG in humans and LFPs in animals. Active cortical networks are susceptible to weak disruptions of the membrane voltage of a large number of neurons by electric fields and as such, the application of an exogenous alternating electrical current (such as that produced by tACS) can be used to modulate cortical oscillations (Frohlich, 2014, 2015; Frohlich, Sellers, & Cordle, 2015). The conceptual advantage of using tACS over other noninvasive brain stimulation approaches is that stimulation frequencies can be tailored to directly modulate specific circuit-level neuronal activity patterns that our electrophysiology studies show are disrupted following prolonged cocaine abstinence. Critically, Daughters and colleagues (2020) recently demonstrated recruitment, retention, and administration feasibility of tACS in individuals with SUDs in a community-based substance use treatment program (87% retention rate), and beneficial effects of tACS on inhibitory control in these patients (Daughters, Yi, Phillips, Carelli, & Frohlich, 2020). These findings indicate tACS is well tolerated in our target clinical population.

In collaboration with Dr. Flavio Fröhlich, the Carelli lab developed a rodent model of tACS and recently published data showing the efficacy of high frequency (80 Hz) tACS administered over the PrL for reversing cocaine-induced deficits in PrL-NAc signaling and restoring behavioral flexibility (West et al., 2021). Our tACS approach is relatively noninvasive (i.e., stimulation is

applied to screws dental cemented to the outer layer of the skull, not in brain) and as such holds great translational value. While noninvasive brain stimulation has shown some success in reducing drug craving in individuals with SUDs, effects are variable and the ability to perform a detailed parametric analysis of neurostimulation in humans is limited. As such, it is important to examine frequency-specific effects of tACS on PrL-NAc oscillatory dynamics and drug seeking in rats in order to assess clinical implications for its use in humans. Importantly, we can use our preclinical model of tACS in tandem with our established *in vivo* electrophysiology methods to probe the neural mechanisms underlying tACS effects on cocaine seeking and quantify target engagement of PrL-NAc core signaling.

Three specific aims were completed to investigate the role of the PrL-NAc core circuit in cocaine-seeking behaviors following short or prolonged abstinence and the feasibility of tACS to modulate PrL-NAc oscillatory dynamics and incubated cocaine seeking.

Specific Aims

1. **To determine the effects of high frequency (80 Hz tACS) on PrL-NAc coherence and cocaine-seeking behaviors in cocaine-abstinent rats.**

Abstinence from cocaine is associated with decreases in baseline (resting state) PFC and NAc activity linked with cognitive impairments and persistent vulnerability to relapse (Beveridge et al., 2008; Goldstein & Volkow, 2002, 2011; Hanlon et al., 2013; Porrino et al., 2007). Consistent with these findings, recent work from our lab demonstrated reduced PrL-NAc coherence in the high gamma (Gamma-80) frequency range and diminished behavioral flexibility following prolonged abstinence from cocaine versus saline self-administration (West et al., 2021). Remarkably, 80 Hz tACS over the PrL reversed cocaine-induced deficits in PrL-NAc signaling and restored behavioral flexibility (West et al., 2021). Here, we examined the effects of 80 Hz

tACS or sham directed at the PrL following one-month abstinence from cocaine or saline self-administration on rest PrL-NAc coherence and subsequent cocaine-seeking behaviors during a drug seeking and self-administration test. While 80 Hz tACS increased PrL-NAc LFP signaling dynamics across all frequency bands, an unexpected finding was that 80 Hz tACS treatment actually increased cocaine seeking in our task.

2. To determine the effects of beta frequency (16 Hz) tACS on PrL-NAc coherence and cocaine-seeking behaviors in cocaine-abstinent rats.

Given our unexpected finding that 80 Hz tACS increased cocaine-seeking behaviors in Aim 1, we reexamined our electrophysiological data to determine whether another stimulation frequency may be effective in reducing cocaine seeking. We next focused on the beta frequency, given the established link between frontal beta oscillations and cognitive control (Engel & Fries, 2010; Riddle et al., 2021; Zavala et al., 2018). Here, we examined the effects of 16 Hz tACS or sham following one-month abstinence from cocaine or saline on rest PrL-NAc coherence and cocaine seeking. Our results show that 16 Hz tACS selectively restored cocaine-induced deficits in PrL-NAc LFP signaling at this lower frequency band and revealed a trend toward a reduction in cocaine-seeking behavior.

3. To characterize resting versus event-related PrL-NAc coherence following short versus prolonged cocaine abstinence and their relationships with cocaine seeking.

While the results from Aim 2 were promising, it became clear that we needed to better characterize PrL-NAc oscillatory dynamics and their relationship to cocaine seeking to set the foundation for future studies that can more effectively use tACS as a treatment strategy for CUD. This is particularly important with respect to understanding the balance between *increased*

activation of the PFC-NAc pathway in cue-elicited cocaine craving (Hollander & Carelli, 2007; Ma et al., 2014; West et al., 2014), and *reduced* baseline activity in this circuit following protracted abstinence (Hu et al., 2015; H. Lu et al., 2014). Critically, the respective role of each of these opposing shifts in PFC-NAc dynamics in incubated cocaine seeking has not yet been directly assessed. Here, we used *in vivo* electrophysiology to examine rest and event-related PrL-NAc coherence following short (one-day) or prolonged (one-month) experimenter-imposed cocaine abstinence and their relationships with cocaine-seeking behaviors during a post-abstinence test. We found context-dependent bidirectional shifts in PrL-NAc coherence which differentially predicted cocaine-seeking behaviors. Interestingly, and consistent with Aim 2, we report that the beta frequency may be uniquely important for incubated cocaine seeking following prolonged (versus short) abstinence. Future directions include analysis of cross-frequency coupling linked with cocaine-seeking behaviors.

CHAPTER 2

HIGH FREQUENCY (80 HZ) tACS RESTORES PrL-NAc COHERENCE BUT INCREASES COCAINE SEEKING

Introduction

SUD is a chronically relapsing disease characterized by compulsion to seek and take drug, reduced control over intake, and negative emotional states that emerge during periods of abstinence from substance use (Koob & Le Moal, 1997; Koob & Volkow, 2010). Neuroimaging studies in individuals with CUD and non-human primates reveal long-lasting structural and functional abnormalities in prefrontal cortical and limbic circuitry associated with impaired behavioral inhibition, emotion regulation and decision-making, as well as heightened vulnerability to relapse (Goldstein & Volkow, 2002, 2011; Hanlon et al., 2013). Sustained vulnerability to relapse in individuals with CUD is also due in part to the ability of cocaine-associated stimuli to elicit intense drug craving (Childress et al., 1993; Childress et al., 1988), which progressively intensifies throughout protracted abstinence in humans with SUDs and animal models (Gawin & Kleber, 1986; Grimm et al., 2001; Li et al., 2016; Parvaz et al., 2016).

Our lab has previously shown that ~one-month abstinence from cocaine versus saline self-administration leads to altered neuronal activity in the NAc core (Saddoris & Carelli, 2014) and PrL (West et al., 2021) and reduced PrL-NAc LFP signaling dynamics (West et al., 2021) linked with impaired higher-order associative learning and cognitive flexibility. Likewise, studies from other labs implicate a causal role of cocaine-induced PFC hypoactivity in compulsive (i.e., punishment-resistant) cocaine seeking in rats (Chen et al., 2013), providing support for targeted

stimulation of the PFC as a potential treatment option for individuals with SUDs. Indeed, recent data indicate that noninvasive brain stimulation targeting the PFC may modulate cortical excitability, dampen drug craving and promote sustained abstinence in individuals with SUDs (Gaudreault et al., 2021; Hanlon et al., 2018; Hone-Blanchet et al., 2015; Pripfl et al., 2014; Terraneo et al., 2016).

In collaboration with Fröhlich and colleagues, our lab developed a rodent model of one form of noninvasive brain stimulation, tACS, and recently published data showing the efficacy of high frequency (80 Hz) tACS to reverse cocaine-induced deficits in PrL-NAc oscillatory signaling and restore flexible behavior (West et al., 2021). Our tACS approach is relatively noninvasive (i.e., stimulation is applied to screws dental cemented to the outer layer of the skull, not in brain) and therefore holds great translational value. Importantly, we can use our preclinical tACS model in tandem with our established *in vivo* electrophysiology methods to probe the neural mechanisms underlying tACS effects on cocaine seeking and quantify target engagement of PrL-NAc core LFP signaling dynamics.

Here, we examined the effects of 80 Hz tACS or sham on resting state ('rest') PrL-NAc coherence and cocaine-seeking behaviors following prolonged (one-month) abstinence from cocaine self-administration. Our lab has previously shown increased cue-evoked cocaine seeking following one-month (versus one-day) abstinence from short access (2 h/day) cocaine self-administration, concomitant with altered neuronal activity in the PrL and NAc core (Hollander & Carelli, 2007; West et al., 2014). Given these findings and our recent work showing 80 Hz tACS restored PrL-NAc coherence and rescued behavioral flexibility in cocaine-exposed rats (West et al., 2021), we predicted 80 Hz tACS would enhance PrL-NAc signaling and reduce cocaine-seeking behaviors following prolonged abstinence in the current study.

Methods

Animals

Adult male Sprague Dawley rats (Charles River, n=30 total), aged 90-120 days (300-350 grams) at the beginning of the study were used. 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 90% of their free-fed body weight by access to 20-25 g of standard rat chow (Purina RMH3000) per day (*ad libitum* water). During 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 houselight and speaker were centrally located on the opposite wall of the chamber. Masking 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 recoding cable, as well as insertion of the i.v. infusion line. Drug and water delivery were 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 intramuscular injection of a ketamine hydrochloride (100 mg/kg) and xylazine hydrochloride (10 mg/kg) mixture and implanted with custom-made intrajugular catheters (Access Technologies, Skokie, IL, USA) for i.v. cocaine or saline self-administration using established procedures (Haake, West, Wang, & Carelli, 2019; Moschak, Terry, Daughters, & Carelli, 2018; West et al., 2021; West et al., 2014). Two stainless steel screws (Fine Science Tools, Foster City, CA, USA) were dental cemented in direct contact with, but not penetrating the outer surface of the skull for tACS or sham, using established methods (West et al., 2021). Screws were positioned 2mm apart, at midline at the level of the PrL (bregma +2mm and +4mm). In a subset of animals (n=18), 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, using established procedures (Haake et al., 2019; Sackett, Moschak, & Carelli, 2019; West & Carelli, 2016; West et al., 2021; West et al., 2014). Figure 2.1A shows a schematic of surgical preparation for tACS and *in vivo* electrophysiology, with stimulation leads "S" in direct contact with the outer layer of the skull and microwires targeting the PrL and ipsilateral NAc core. Animals were allowed 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 food receptacle) then underwent surgery and recovery followed by reestablishment of lever pressing and habituation to the LFP recording chamber, using established procedures (Haake et al., 2019; Wheeler et al., 2008).

Figure 2.1B shows a timeline of self-administration training, LFP recordings, tACS or sham treatment, and behavioral testing. On self-administration days 1-14, water-restricted rats were placed in an operant chamber and a lever was extended into the chamber with the cue light above it illuminated. During self-administration sessions (2 h each), lever depression resulted in i.v. delivery of cocaine (6 s infusion, 0.33 mg/infusion, ~1 mg/kg) or 0.9% physiological saline (infusion duration and volume matched), paired with termination of the cue light and simultaneous onset of a tone (67 dB, 1 kHz)/houselight conditioned stimulus (CS) for 20 s, as described previously (Haake et al., 2019; Hollander & Carelli, 2005, 2007; West et al., 2014). During saline self-administration, i.v. saline infusion occurred simultaneously with delivery of water into the receptacle to control for operant experience, described previously (Saddoris & Carelli, 2014; Saddoris, Wang, Sugam, & Carelli, 2016; West et al., 2021). Next, rats underwent one month of experimenter-imposed abstinence from cocaine (n=26) or saline (n=4) self-administration, during which animals remained in their home cages (30 ml water/day). On day 27 of abstinence from cocaine or saline, rats were placed in the LFP recording chamber (contextually distinct from the self-administration and testing chamber) and connected to the electrophysiological recording

system. Simultaneous LFPs in the PrL and NAc core were recorded for 15 min while animals were not engaged in a behavioral task ('rest' LFP). Rats that were not outfitted with microwires (n=12) were placed in the LFP recording chamber for 15 min to control for experience. Immediately following the first rest LFP recording (day 27 of abstinence), rats that self-administered cocaine were split into two groups, one that received 80 Hz tACS (n=13) and one that received sham (attached to tACS system but no current delivered; n=13) administered over 3-5 consecutive days in a distinct "treatment" chamber, using established procedures (West et al., 2021). A subset of rats (n=2 for cocaine-tACS, n=3 for cocaine-sham) received rest LFP recordings and tACS or sham on days 13-15 of abstinence (all rats underwent behavioral testing on abstinence days 30-32). Critically, timing of rest LFP recordings and tACS or sham treatment (i.e., following 2 or 4 weeks of abstinence) had no significant effect on behavior or change in LFPs from pre- to post-treatment (See **Results**). Next, following prolonged abstinence from cocaine and 80 Hz tACS or sham treatment, rats received a post-treatment rest LFP recording to assess target engagement of tACS, followed immediately by the behavioral test session. Rats that self-administered saline were not treated with tACS or sham and did not undergo testing.

Shown in Figure 2.1C, the test session consisted of one to three phases: (1) CS Probes, (2) Extinction, and (3) Cocaine Self-Administration, described previously (Hollander & Carelli, 2007; West et al., 2014). All cocaine-tACS and cocaine-sham rats underwent Phase 1 of the test (n=13/group), and subsets of rats in each group (n=9 for cocaine-tACS, n=8 for cocaine-sham) underwent Phases 2 and 3. In Phase 1 of the test (CS Probes), the cue light above the lever was illuminated but the lever was not extended. Ten pseudorandom presentations of the tone/houselight CS (5s each) were given over 15 min. The cue light above the lever remained on throughout Phase 1. Behavior during Phase 1 was video recorded and analyzed for time spent in the quadrant of the

chamber where the cocaine-associated lever was extended during self-administration training, and approaches toward that quadrant during each 5s CS presentation. Next, Phase 2 (Extinction) was initiated wherein the lever previously associated with cocaine during self-administration was extended into the chamber. Here, lever depression resulted in termination of the cue light and presentation of the tone/houselight CS (20 s), but no drug delivery. After 2 h, Phase 3 (Cocaine Self-Administration) was initiated by administration of 0-3 priming infusions of cocaine (6 s; 0.33 mg/infusion) paired with the tone/houselight CS (20 s). Each subsequent lever press resulted in a cocaine infusion (6 s; 0.33 mg/infusion) paired with the CS (20 s) as in self-administration training sessions. The test session was completed 2 h after Phase 3 initiation.

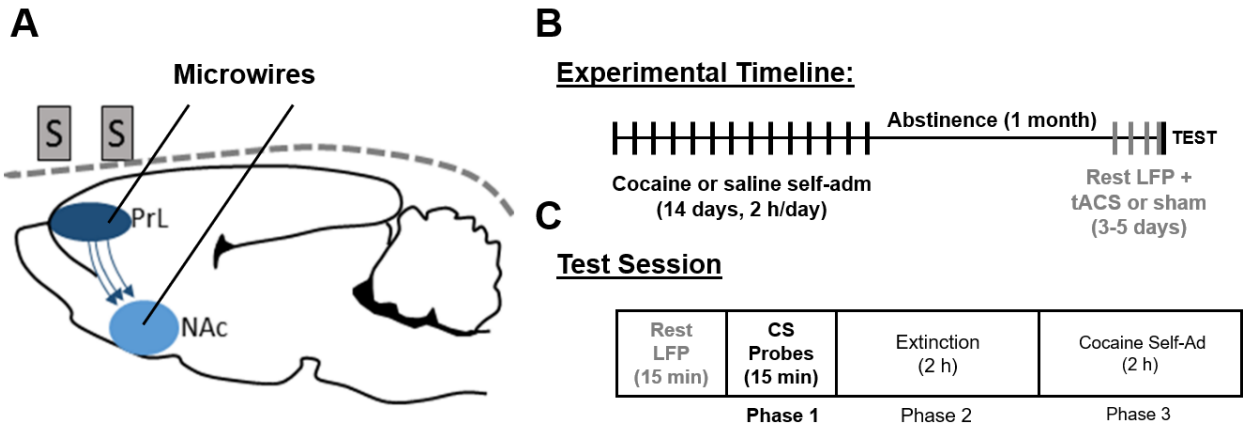

Figure 2.1 Surgical procedures for tACS and *in vivo* electrophysiology and experimental design. **A.** Schematic of stimulation lead ("S") placement for tACS and microwire implant into the PrL and ipsilateral NAc core. **B.** Experimental timeline for cocaine or saline self-administration, one-month abstinence, rest LFP recordings, 80 Hz tACS or sham treatment, and behavioral testing. **C.** Post-treatment rest LFP recording and behavioral testing procedures for cocaine-tACS and cocaine-sham rats. All cocaine-tACS and cocaine-sham rats underwent Phase 1 of the test (bolded), and subsets of rats in each group underwent Phases 2 and 3 as well.

tACS Procedures

tACS or sham were delivered using established methods (West et al., 2021). During tACS or sham sessions, rats were placed in the treatment chamber and a Linear Stimulus Isolator (World Precision Instruments; Sarasota, FL, USA) was attached to the two stimulation leads via insulated wires inserted through the commutator. A computer-generated sinewave 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 allowed free movement within the chamber during stimulation periods, which lasted ~15 minutes/day. Stimulations consisted of 40 cycles of 10s on then 10s off for three consecutive days. Sham rats received identical headcaps and treatments over 3-5 consecutive days, but no current was delivered.

Electrophysiology

Electrophysiological procedures have been described in detail previously (Carelli, 2000; Hollander & Carelli, 2005). 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; Plexon, Inc., Dallas, TX, USA), described previously (Haake et al., 2019; Moschak, Wang, & Carelli, 2018; 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 and 60 Hz noise was removed using a notch filter in a subset of recordings. LFPs were recorded and analyzed in Neuroexplorer (Plexon, Inc).

Histology

Histological reconstruction of electrode positions was accomplished using established procedures (Haake et al., 2019; West et al., 2021). Briefly, upon completion of the experiment, rats were deeply anesthetized with an intraperitoneal injection of a ketamine hydrochloride and xylazine hydrochloride mixture (100 and 10 mg/kg, respectively). A 13.5-μA current was passed through each microwire electrode for 5s to mark the placement of electrode tips. Transcardial perfusions were then performed using 0.9% saline and 3% potassium ferrocyanide in 10% formalin, and brains were removed. After post-fixing and freezing, 40-μm coronal brain sections were mounted. The addition of potassium ferrocyanide allowed for a blue reaction corresponding to the location of the electrode tip which was viewed under a 1X microscope lens. Placement of an electrode tip was determined by examining the relative position of observable reaction product to visual landmarks and anatomical organization of the PrL and NAc core represented in a stereotaxic atlas (Paxinos & Watson, 2005).

Data Analysis

Behavior: The number of lever presses during self-administration training were compared for the three groups (saline vs cocaine-sham vs cocaine tACS) using a two-way repeated-measures ANOVA with session (1-14) and group as factors.

For rats that underwent cocaine self-administration and 80 Hz tACS treatment or cocaine self-administration and sham treatment, behavioral data during the three distinct phases of the test session were also analyzed. In Phase 1 (CS Probes), the amount of time each rat spent in the quadrant where the cocaine-associated lever had previously been extended during training, and the number of approaches toward this quadrant during each 5s CS presentation were recorded.

Approaches toward and time in the quadrant were defined by at least one-half of a body length into and orientation of the rat toward the quadrant, as described previously (Hollander & Carelli, 2007; West et al., 2014). Due to technical issues with video recording, behavioral data during Phase 1 of the test was not recorded for one animal in the cocaine-sham group. Unpaired *t*-tests were used to compare the two groups (cocaine-tACS versus cocaine-sham) for all behavioral measures during the test, i.e., amount of time (seconds) in and number of approaches toward the cocaine lever-associated quadrant during Phase 1, the number of lever presses during Phase 2 (Extinction), and the number of cocaine infusions during Phase 3 (Cocaine Self-Administration). Due to catheter failure during one-month abstinence or differences in testing procedures (i.e., rats underwent either Phase 1 only, or multiple phases of the test), a subset of animals in the cocaine-sham (n=6) and cocaine-tACS (n=7) groups were not included in analyses of Phase 2 and Phase 3 behavioral data.

Electrophysiology: LFPs were used to assess network activity across brain regions, described previously (West et al., 2021). For coherence data (PrL-NAc), two-way ANOVAs were run with group (e.g., saline, cocaine-sham, cocaine-tACS) and frequency (0-100 Hz [58-62Hz excluded]; 2 Hz/bin) as factors (Fig. 2.2B and 2.3B). Additionally, peak delta (0.5-4 Hz), theta (6-10 Hz), beta (12-30 Hz), low gamma (32-56 Hz), and average high gamma (64-100 Hz) coherence values were calculated for each animal. For the first rest LFP recording (following ~1-month abstinence from saline or cocaine and prior to tACS or sham treatment for rats that self-administered cocaine), unpaired *t*-tests were used to compare coherence at individual frequency bands (e.g., peak delta, average high gamma) for the saline versus cocaine groups (Fig. 2.2C-G). One-way ANOVAs were used to compare coherence at individual frequency bands following treatment for the cocaine-sham and cocaine-tACS groups with 'pre-treatment' coherence for the

saline group (Fig. 2.3C-G). Due to technical issues with electrophysiological recordings, two rats (n=1/cocaine-tACS and cocaine-sham group) were excluded from analyses of the second rest LFP recording. Pearson's correlations were calculated between behaviors during the test (e.g., approach during Phase 1, extinction presses during Phase 2) and post-treatment coherence values at each individual frequency bin (0-100 Hz [58-62Hz excluded]; 2Hz/bin) across cocaine-tACS and cocaine-sham rats (Fig. 2.5B, 2.6B, and 2.7B). Simple linear regression was used to examine relationships between coherence at individual frequency bands (e.g., peak delta, average high gamma) and behaviors during the test session for cocaine-tACS and cocaine-sham animals (Fig. 2.5C-G, 2.6C-G, and 2.7C-G).

LFP coherence values were exported from NeuroExplorer. *Post hoc* comparisons (e.g., Tukey's multiple comparisons, Sidak's multiple comparisons) were used where appropriate. All statistical analyses were completed using GraphPad Prism 8 (GraphPad Software, Inc., La Jolla, CA, USA).

Results

Self-Administration Training

Rats that self-administered saline and rats that were later divided into cocaine-tACS or cocaine-sham groups did not significantly differ in self-administration behavior during training (prior to abstinence and treatment). A two-way repeated measures ANOVA of cocaine infusions earned with session (1-14) and group (saline vs cocaine-tACS vs cocaine-sham) as factors revealed a main effect of session ($F_{5.893, 159.1} = 11.17$, $p < 0.0001$) but no main effect of group ($F_{2, 27} = 1.812$, $p = 0.1827$) nor a group x session interaction ($F_{26, 351} = 1.420$, $p = 0.0867$), showing all rats acquired self-administration behavior.

No Significant Effects of Timing of tACS or Sham on Behavior or PrL-NAc Activity

A subset of rats received rest LFP recordings and tACS or sham on days 13-15 of abstinence while the remaining rats underwent rest LFP recordings and tACS or sham beginning on day 27 of abstinence (all rats underwent behavioral testing on abstinence days 30-32). Timing of rest LFP recordings and tACS or sham treatment (i.e., following 2 or 4 weeks of abstinence) had no significant effect on behaviors during the test session or change in LFP activity (pre- to post-treatment), as described below.

Specifically, for cocaine-tACS rats, unpaired *t*-tests revealed no significant differences between rats that received tACS following 2 weeks versus 4 weeks of abstinence in time spent ($t_{11} = 1.706$, $p = 0.1160$) or approaches toward the quadrant ($t_{11} = 2.135$, $p = 0.0561$) during Phase 1, extinction presses during Phase 2 ($t_6 = 0.6203$, $p = 0.5579$), or cocaine infusions during Phase 3 of the test ($t_4 = 0.5064$, $p = 0.6392$). For cocaine-sham rats, unpaired *t*-tests revealed no significant differences between rats that received sham following 2 weeks versus 4 weeks of abstinence in

time spent (t_{10} = 1.684, p = 0.1231) or approaches toward the quadrant (t_{10} = 0.2635, p = 0.7975) during Phase 1, extinction presses during Phase 2 (t_5 = 0.8777, p = 0.4203), or cocaine infusions during Phase 3 of the test (t_5 = 1.698, p = 0.1502).

Two-way ANOVAs with timing of tACS or sham (following 2 versus 4 weeks of abstinence) and frequency (0-100 Hz [58-62Hz excluded]; 2 Hz/bin) as factors were used to compare the change in PrL-NAc core coherence (post-treatment minus pre-treatment) for cocaine-tACS and cocaine-sham rats treated in the middle or at the end of abstinence. For cocaine-tACS rats, there was no main effect of timing of tACS ($F_{1, 196}$ = 2.680, p = 0.1032), frequency ($F_{48, 196}$ = 1.192, p = 0.2040), nor a timing x frequency interaction ($F_{48, 196}$ = 0.1810, p > 0.9999). Similarly, for cocaine-sham rats, there was no main effect of timing of sham ($F_{1, 245}$ = 2.648, p = 0.1050), frequency ($F_{48, 245}$ = 0.9079, p = 0.6470), nor a timing x frequency interaction ($F_{48, 245}$ = 0.5440, p = 0.9936).

As such, cocaine-tACS and cocaine-sham rats that received treatment following 2 weeks of abstinence were combined with rats that received treatment following one month of abstinence for all subsequent behavioral and LFP analyses.

Rest PrL-NAc Core Coherence is Reduced Following One-Month Abstinence from Cocaine versus Saline

Following one-month abstinence from cocaine or saline self-administration (prior to tACS or sham treatment), rats were placed in a contextually distinct chamber for rest LFP recordings (n=4 for saline, n=14 for cocaine outfitted with microwires). Figure 2.2A shows the experimental timeline with an arrow indicating the timepoint of this rest LFP recording. Shown in Figure 2.2B, PrL-NAc core coherence at rest was reduced across the frequency spectrum following prolonged abstinence from cocaine compared with saline controls. A two-way ANOVA of PrL-NAc

coherence with group (saline vs cocaine) and frequency (0-100 Hz [58-62Hz excluded]; 2 Hz/bin) revealed a main effect of group ($F_{1, 784} = 440.6$, $p < 0.0001$) and frequency ($F_{48, 784} = 4.5597$, $p < 0.0001$), but no significant group x frequency interaction ($F_{48, 784} = 1.158$, $p = 0.2199$). To further examine the effects of prolonged abstinence from cocaine versus saline on PrL-NAc core signaling, we compared peak coherence values in the delta (0.5-4 Hz), theta (6-10 Hz), beta (12-30 Hz), and low gamma (32-56) ranges, and average coherence values in the high gamma (64-100 Hz) range for the saline versus cocaine group (Fig. 2.2C-G). Unpaired *t*-tests revealed a significant decrease in rest PrL-NAc core coherence for the cocaine versus saline group at delta ($t_{16} = 2.956$, $p = 0.0093$; Fig. 2.2C), beta ($t_{16} = 2.374$, $p = 0.0304$; Fig. 2.2E), low gamma ($t_{16} = 2.512$, $p = 0.0231$; Fig. 2.2F), and high gamma ($t_{16} = 4.284$, $p = 0.0006$; Fig. 2.2G) frequencies. There was no significant difference in PrL-NAc core peak theta coherence between the saline and cocaine groups ($t_{16} = 0.9701$, $p = 0.3464$; Fig. 2.2D). Critically, rats that were later divided into cocaine-tACS and cocaine-sham groups did not show significant differences in PrL-NAc core coherence at delta ($t_{12} = 0.04526$, $p = 0.9646$), theta ($t_{12} = 0.3929$, $p = 0.7013$), beta ($t_{12} = 1.372$, $p = 0.1953$), low gamma ($t_{12} = 1.581$, $p = 0.1398$), or high gamma ($t_{12} = 1.350$, $p = 0.2019$) frequencies prior to treatment (not shown). These findings reveal that cocaine self-administration followed by prolonged abstinence leads to broadband decreases in PrL-NAc core coherence at rest compared with saline controls. Further, the robust dampening of PrL-NAc coherence in the high gamma range for the cocaine versus saline group provided additional support for our hypothesis that 80 Hz tACS would reduce cocaine seeking.

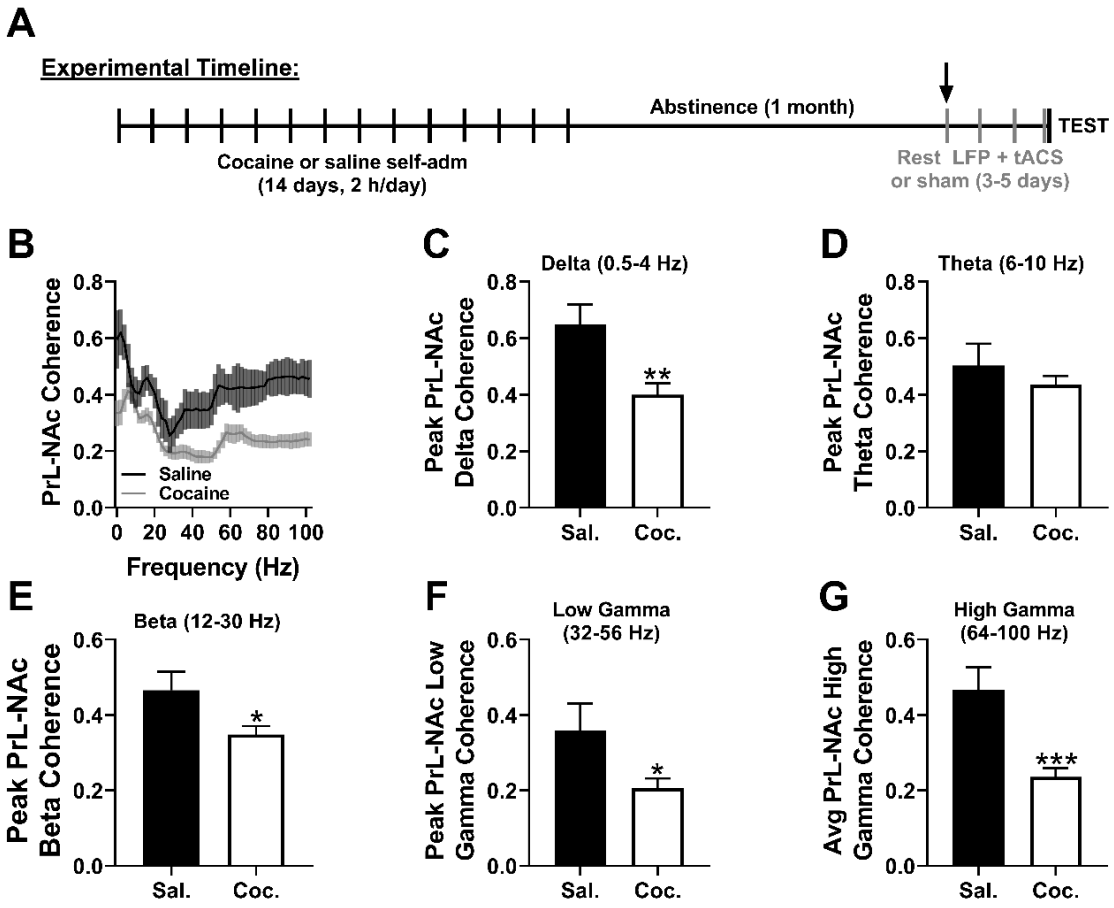

Figure 2.2 PrL-NAc coherence following abstinence from cocaine or saline self-administration. **A.** Experimental timeline with arrow indicating timepoint of rest LFP recording. **B.** PrL-NAc core coherence across the frequency spectrum (0-100 Hz; 2Hz/bin), showing a broadband reduction in PrL-NAc core coherence for the cocaine versus saline group. Error bars represent mean ± SEM. **C.** Peak PrL-NAc core coherence value in the delta range for the saline versus cocaine group. ***p*<0.01 for saline vs cocaine. **D.** Peak PrL-NAc core theta coherence for the saline versus cocaine group. **E.** Peak PrL-NAc core beta coherence. **p*<0.05 for saline vs cocaine. **F.** Peak PrL-NAc core low gamma coherence. **p*<0.05 for saline vs cocaine. **G.** Average PrL-NAc core low gamma coherence. ****p*<0.001 for saline vs cocaine. Error bars represent mean + SEM in **C-G**.

80 Hz tACS Increases Rest PrL-NAc Core Coherence Across Frequencies

Following the first rest LFP recording, rats that self-administered cocaine were placed into one of two treatment groups: 80 Hz tACS or sham (n=6/group outfitted with microwires). 80 Hz

tACS or sham treatment was administered over 3-5 consecutive days in a separate and distinct 'treatment' chamber. Following tACS or sham treatment and immediately prior to the test session, cocaine-tACS and cocaine-sham rats were returned to the LFP recording chamber for a post-treatment rest LFP recording. Figure 2.3 shows the experimental timeline with an arrow indicating the timepoint of the post-treatment rest LFP recording for cocaine-tACS and cocaine-sham groups (Fig. 2.3A), and PrL-NAc core coherence for saline, cocaine-tACS, and cocaine-sham groups (note: since saline rats did not undergo tACS or sham treatment, coherence data from the first LFP recording is reused for the saline group here). Shown in Fig. 2.3B, 80 Hz tACS led to broadband increases in PrL-NAc core coherence for the cocaine-tACS group compared to the cocaine-sham group, and restored coherence in this circuit to saline control levels at multiple frequency bands. A two-way ANOVA of PrL-NAc coherence with group (saline vs cocaine-tACS vs cocaine-sham) and frequency (0-100 Hz [58-62Hz excluded]; 2 Hz/bin) as factors revealed a main effect of group ($F_{2, 637} = 256.3$, $p < 0.0001$) and frequency ($F_{48, 637} = 6.679$, $p < 0.0001$), but no group x frequency interaction ($F_{96, 637} = 1.141$, $p = 0.1833$).

To further examine the effects of 80 Hz tACS or sham on PrL-NAc core oscillatory dynamics, we compared peak coherence values in the delta (0.5-4 Hz), theta (6-10 Hz), beta (12-30 Hz), and low gamma (32-56) ranges, and average coherence values in the high gamma (64-100 Hz) range for the saline versus cocaine-tACS versus cocaine-sham groups using one-way ANOVAs (Fig. 2.3C-G). Specifically, a one-way ANOVA of peak delta coherence for saline vs cocaine-tACS vs cocaine-sham groups revealed a significant effect of group ($F_{2, 13} = 4.994$, $p = 0.0246$). Shown in Fig. 2.3C, Tukey's multiple comparisons revealed a significant increase in peak delta coherence for the cocaine-tACS versus cocaine-sham group ($p = 0.0264$), but no significant difference between the saline and cocaine-sham groups ($p = 0.0985$), nor between the saline and

cocaine-tACS groups (p = 0.9096). Similarly, a one-way ANOVA of peak theta coherence revealed a significant effect of group ($F_{2, 13}$ = 8.844, p = 0.0038), and shown in Fig. 2.3D, Tukey's multiple comparisons revealed a significant increase in peak theta coherence for the cocaine-tACS versus cocaine-sham group (p = 0.0030), but no significant difference between the saline and cocaine-sham groups (p = 0.4176), nor between the saline and cocaine-tACS groups (p = 0.0746). Additionally, a one-way ANOVA of peak beta coherence revealed a significant effect of group ($F_{2, 13}$ = 8.110, p = 0.0052), and shown in Fig. 2.3E, Tukey's multiple comparisons revealed a significant increase in peak beta coherence for the cocaine-tACS versus cocaine-sham group (p = 0.0046), but no significant difference between the saline and cocaine-sham groups (p = 0.0575), nor between the saline and cocaine-tACS groups (p = 0.6212). We also compared peak low gamma and average high gamma coherence values for saline versus cocaine-tACS versus cocaine-sham groups. A one-way ANOVA of peak low gamma coherence for saline vs cocaine-tACS vs cocaine-sham groups revealed a significant effect of group ($F_{2, 13}$ = 5.343, p = 0.0203). Shown in Fig. 2.3F, Tukey's multiple comparisons revealed a significant decrease in low gamma coherence for the cocaine-sham versus saline group (p = 0.0467), and a significant increase in low gamma coherence for the cocaine-tACS versus cocaine-sham group (p = 0.0326), but no significant difference between the saline and cocaine-tACS groups (p = 0.9937). Similarly, a one-way ANOVA of average high gamma coherence revealed a significant effect of group ($F_{2, 13}$ = 8.582, p = 0.0042), and Tukey's multiple comparisons revealed a significant decrease in average high gamma coherence for the cocaine-sham versus saline group (p = 0.0039) and a significant increase in average high gamma coherence for the cocaine-tACS versus cocaine-sham group (p = 0.0434), but no significant difference between saline and cocaine-tACS groups (p = 0.2886; Fig. 2.3G).

Collectively, these findings suggest that high-frequency 80 Hz tACS increases PrL-NAc core coherence in a non-specific manner (i.e., across all frequency bands in addition to the specific stimulation frequency; Fig. 2.3B-G) and may reverse cocaine-induced deficits in PrL-NAc core coherence at low and high gamma frequencies (Fig. 2.3F, 2.3G).

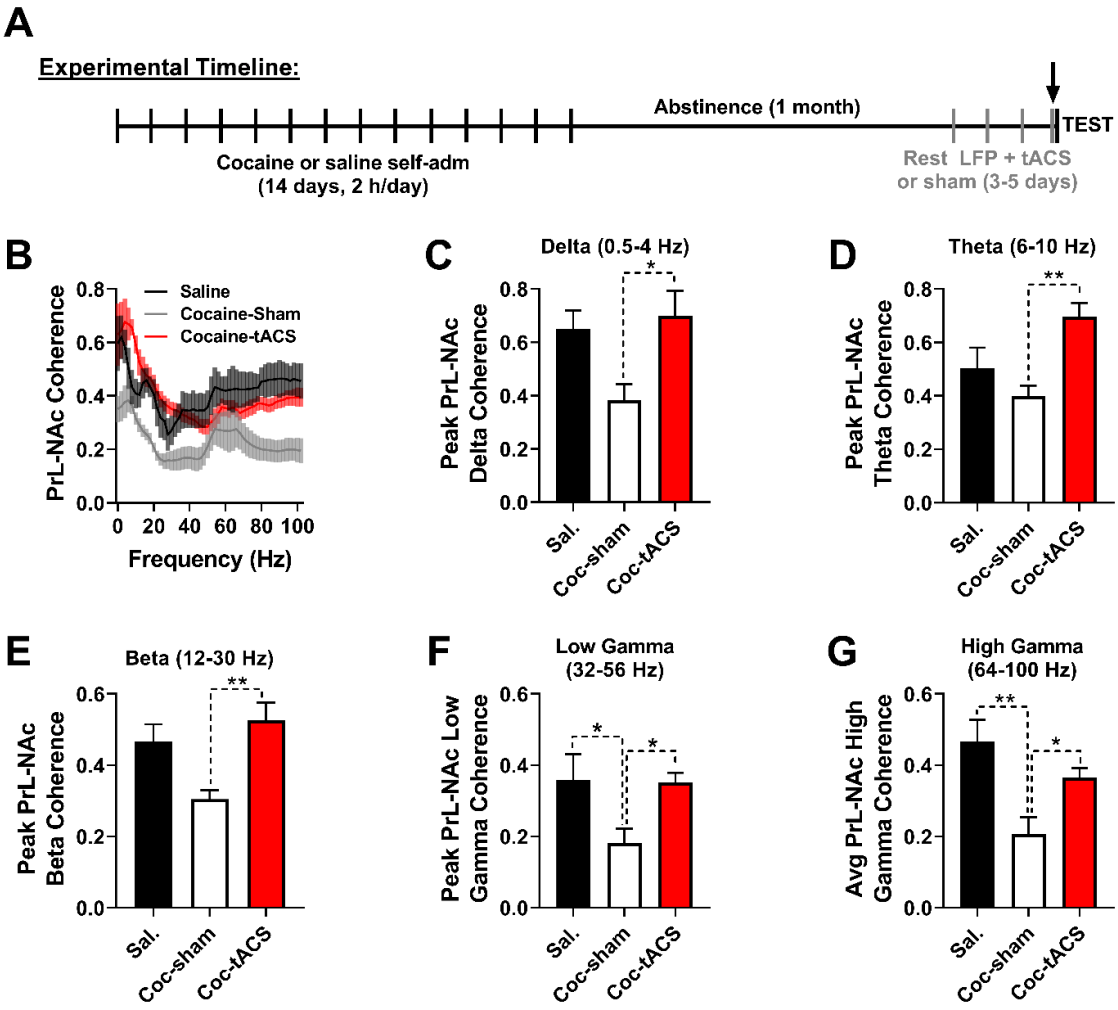

Figure 2.3 Effects of 80 Hz tACS on rest PrL-NAc core coherence. **A.** Experimental timeline with arrow indicating timepoint of rest LFP recording. **B.** Rest PrL-NAc core coherence across frequencies (0-100 Hz; 2Hz/bin) for saline, cocaine-tACS, and cocaine-sham groups, showing increased PrL-NAc core coherence for the cocaine-tACS versus cocaine-sham group across the frequency spectrum. Error bars represent mean ± SEM. **C.** Peak PrL-NAc core coherence value in the delta range for saline versus cocaine-tACS versus cocaine-sham groups. *p<0.05 for cocaine-

tACS vs cocaine-sham. **D.** Peak PrL-NAc core theta coherence. $^{**}p<0.01$ for cocaine-tACS vs cocaine-sham. **E.** Peak PrL-NAc core beta coherence. $^{**}p<0.01$ for cocaine-tACS vs cocaine-sham. **F.** Peak PrL-NAc core low gamma coherence. $^{*}p<0.05$ for saline vs cocaine-sham; $^{*}p<0.05$ for cocaine-sham vs cocaine-tACS. **G.** Average PrL-NAc core high gamma coherence. $^{**}p<0.01$ for saline vs cocaine-sham; $^{*}p<0.05$ for cocaine-sham vs cocaine-tACS. Error bars represent mean + SEM for **C-G**.

80 Hz tACS May Increase Cocaine-Seeking Behavior

Given these findings, we hypothesized that 80 Hz tACS applied after one-month abstinence from cocaine self-administration may decrease cocaine-seeking behaviors during the test session in our task. Here, following 80 Hz tACS or sham treatment, cocaine-tACS and cocaine-sham rats (n=13/group) were given a behavioral test session consisting of 1-3 phases: (1) CS Probes, (2) Extinction, and (3) Cocaine Self-Administration. All cocaine-tACS and cocaine-sham rats underwent Phase 1 of the test, and subsets of rats in each group underwent Phases 2 and 3 as well. Figure 2.4 shows a schematic of the test session (Fig. 2.4A) and cocaine-seeking behaviors for cocaine-sham versus cocaine-tACS groups during the three distinct phases of the test. During Phase 1 of the test (CS Probes; Fig. 2.4B-C), there was no significant difference in time spent in the cocaine lever-associated quadrant of the chamber between cocaine-sham and cocaine-tACS groups ($t_{23} = 0.6974$, $p = 0.4925$), but there was a trend toward a significant increase in approaches toward the quadrant for the cocaine-tACS versus cocaine-sham group ($t_{23} = 1.995$, $p = 0.0580$). Shown in Fig. 2.4D-E, there were no significant differences between cocaine-sham and cocaine-tACS groups in extinction presses during Phase 2 ($t_{13} = 1.231$, $p = 0.2401$) or reinforced lever presses (cocaine infusions) during Phase 3 ($t_{12} = 0.1800$, $p = 0.8601$) of the test. These results show that 80 Hz tACS, which increases PrL-NAc core coherence across multiple behaviorally relevant frequency bands, is insufficient to reduce cocaine seeking and may, under certain circumstances, actually increase drug craving and seeking.

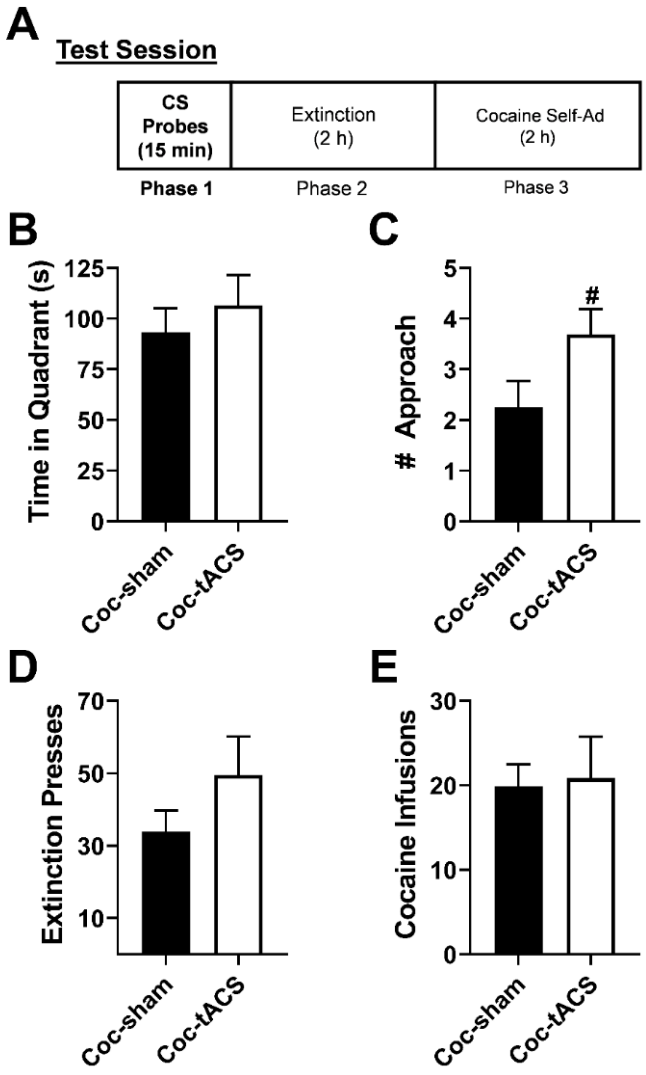

Figure 2.4 Effects of 80 Hz tACS on cocaine-seeking behaviors during the test. **A.** Schematic of the three-phase behavioral test session. All cocaine-tACS and cocaine-sham rats underwent Phase 1 of the test (bolded) and a subset of rats in each group subsequently underwent Phases 2 and 3. **B.** Time spent (seconds) in the quadrant where the cocaine-associated lever was previously extended for the cocaine-sham versus cocaine-tACS groups (Phase 1 of the test). **C.** Approaches toward the quadrant during Phase 1 of the test. #$p < 0.10$ for cocaine-sham vs cocaine-tACS. **D.** Extinction presses during Phase 2 of the test. **E.** Cocaine infusions during Phase 3 of the test. Error bars represent mean + SEM for **B**-**E**.

Rest PrL-NAc Core Coherence Following Treatment Predicts Approach Toward, but not Time Spent in the Cocaine-Associated Quadrant (Phase 1 of Test)

We next examined the *relationship* between rest PrL-NAc core coherence following 80 Hz tACS or sham treatment and subsequent cocaine-seeking behaviors during the test session. Specifically, Pearson's correlations were calculated between behaviors during the test (e.g., time spent, and approaches toward, the cocaine-associated quadrant during Phase 1) and post-treatment rest PrL-NAc core coherence values at each individual frequency (0-100 Hz; 2 Hz/bin) across cocaine-tACS and cocaine-sham rats. Further, simple linear regression was used to examine relationships between rest PrL-NAc coherence at individual frequency bands (e.g., peak coherence value in the delta range, average coherence value in the high gamma range) and behaviors during the test session for cocaine-tACS and cocaine-sham rats.

Figure 2.5 shows the relationship between rest PrL-NAc core coherence following treatment and time spent in the quadrant during Phase 1 of the test (Fig. 2.5A, arrows indicating timepoint of rest LFP recording and phase of test session in which behavior was measured). Time spent in the quadrant was not significantly correlated with PrL-NAc core coherence at any individual frequency bin across the spectrum (reflected in purple in Fig. 2.5B), and linear regression revealed that time spent in the quadrant was not significantly predicted by post-treatment PrL-NAc core peak delta (R^2 = 0.00009303, p = 0.9775; Fig. 2.5C), peak theta (R^2 = 0.000007633, p = 0.9936; Fig. 2.5D), peak beta (R^2 = 0.1374, p = 0.2617; Fig. 2.5E), peak low gamma (R^2 = 0.004267, p = 0.8487; Fig. 2.5F), or average high gamma (R^2 = 0.009626, p = 0.7741; Fig. 2.5G) coherence.

Figure 2.6 shows the relationship between rest PrL-NAc core coherence following treatment and approaches toward the quadrant during Phase 1 of the test (Fig. 2.6A, arrows indicating timepoint of rest LFP recording and test phase in which behavior was measured).

Approaches toward the quadrant were significantly positively correlated with PrL-NAc core coherence at the following frequencies ($R^2 \geq 0.3992$, $p < 0.05$ for all; highlighted in red in Fig. 2.6B): 0-4 Hz (delta), 6-10 Hz (theta), 12-18 and 28 Hz (beta), and 68-100 Hz (high gamma). Linear regression revealed that the number of approaches toward the quadrant were significantly predicted by post-treatment PrL-NAc core peak delta ($R^2 = 0.6753$, $p = 0.0019$; Fig. 2.6C), peak theta ($R^2 = 0.6289$, $p = 0.0036$; Fig. 2.6D), peak beta ($R^2 = 0.4244$, $p = 0.0299$; Fig. 2.6E), peak low gamma ($R^2 = 0.3681$, $p = 0.0478$; Fig. 2.6F), and average high gamma ($R^2 = 0.6875$, $p = 0.0016$; Fig. 2.6G) coherence. These findings show that increased rest PrL-NAc core coherence at multiple frequency bands predicted increased approach behavior during Phase 1 of the test, consistent with increased approach behavior observed for 80 Hz tACS-treated compared to sham-treated rats (Fig. 2.4C).

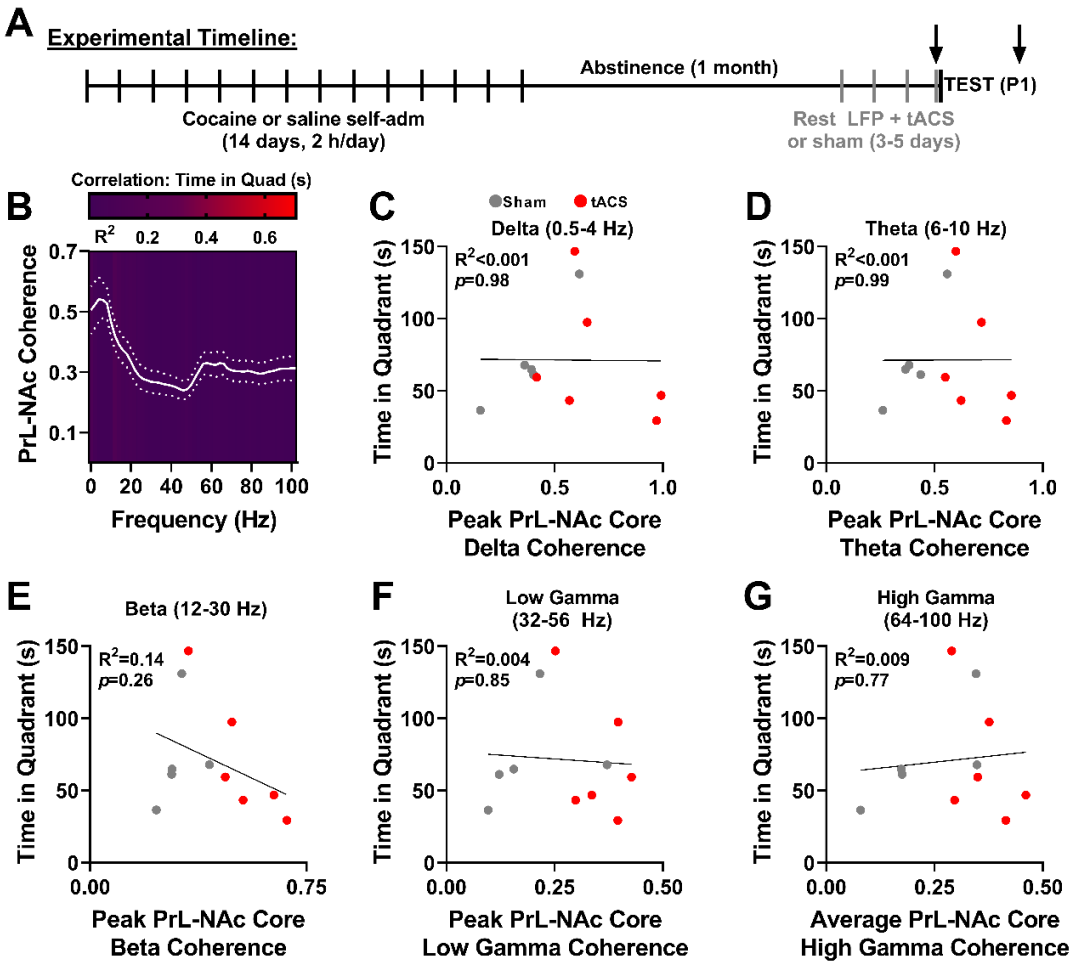

Figure 2.5 No relationship between rest PrL-NAc core coherence following 80 Hz tACS or sham treatment and time spent in the quadrant during Phase 1 of the test. **A.** Experimental timeline with arrows indicating time point of rest LFP recording and test phase during which behavior was measured. **B.** PrL-NAc coherence across frequencies following tACS or sham treatment (white line). Error bars (dotted white line) represent mean ± SEM. Coherence values at each frequency were correlated with individual rats' time spent (s) in the quadrant (n=11) and the R^2 value for each of these correlations (0-100 Hz; 2 Hz/bin) are represented in the color overlay (R^2 values ≥ 0.40 are significant, $p < 0.05$). **C.** Linear regression of peak PrL-NAc core delta coherence and time in quadrant (s). **D.** Linear regression of peak theta coherence and time in quadrant. **E.** Linear regression of peak beta coherence and time in quadrant. **F.** Linear regression of peak low gamma coherence and time in quadrant. **G.** Linear regression of average high gamma coherence and time in quadrant. Gray and red reflect sham and 80 Hz tACS treated rats, respectively, in **C-G**.

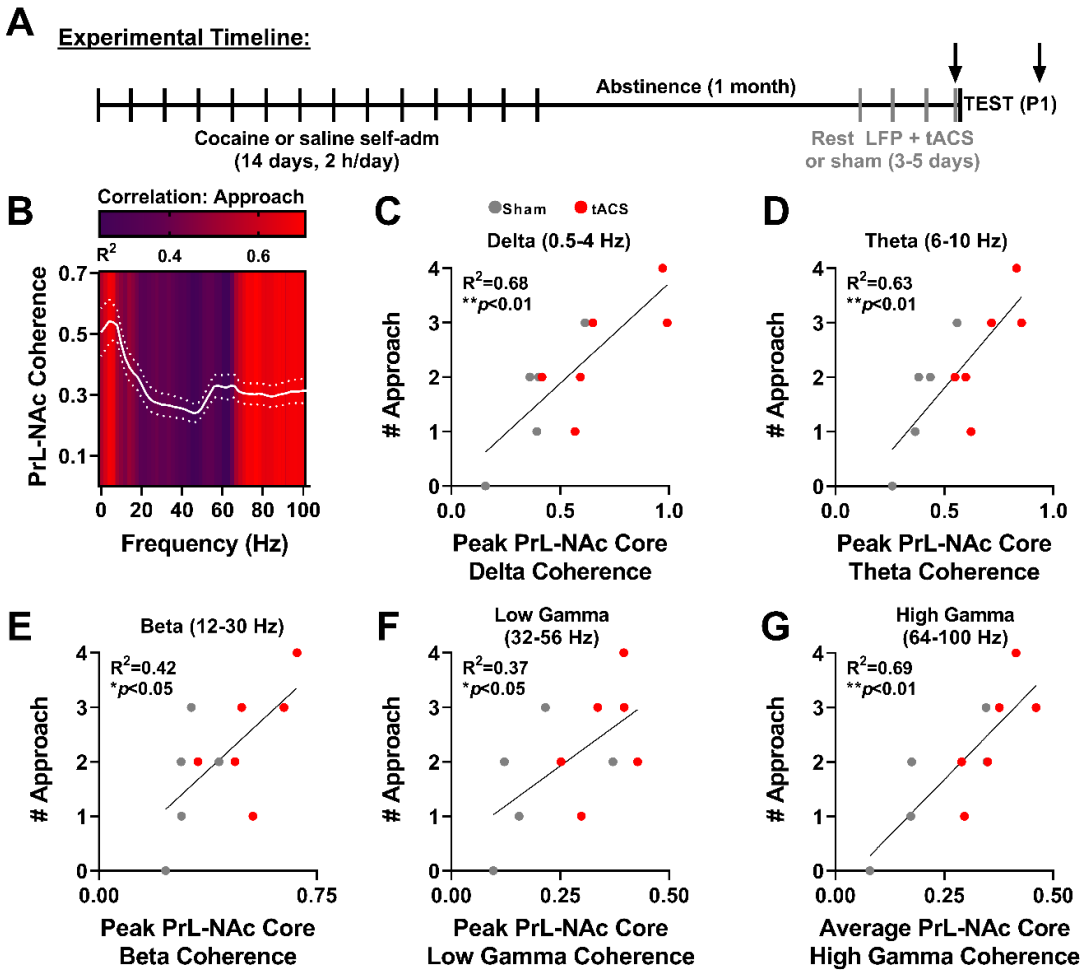

Figure 2.6 Increased rest PrL-NAc core coherence following treatment predicts increased number of approaches toward the cocaine-associated quadrant during Phase 1 of the test. **A.** Experimental timeline with arrows indicating time point of rest LFP recording and test phase. **B.** PrL-NAc coherence across frequencies following tACS or sham treatment (white line). Error bars (dotted white line) represent mean ± SEM. Coherence values at each frequency were correlated with individual rats' number of approaches toward the quadrant (n=11) and the R^2 value for each of these correlations (0-100 Hz; 2 Hz/bin) are represented in the color overlay (R^2 values ≥ 0.40 are significant, $p < 0.05$, represented as red in the overlay). **C.** Linear regression of peak PrL-NAc core delta coherence and approach. **D.** Linear regression of peak theta coherence and approach. **E.** Linear regression of peak beta coherence and approach. **F.** Linear regression of peak low gamma coherence and approach. **G.** Linear regression of average high gamma coherence and approach. *$p < 0.05$; **$p < 0.01$ for **C-G**. Gray and red reflect sham and 80 Hz tACS treated rats, respectively, in **C-G**.

Increased Rest PrL-NAc Core Coherence at Lower Frequencies May be Associated with Reduced Cocaine Seeking Under Extinction (Phase 2 of Test)

Figure 2.7 shows the relationship between rest PrL-NAc core coherence following treatment and extinction presses during Phase 2 of the test (Fig. 2.7A, arrows indicating timepoint of rest LFP recording and phase 2 of the test session). There was a trend toward a negative correlation between number of extinction presses and PrL-NAc core coherence at 0-4 Hz ($R^2 \geq 0.4660$, $p < 0.10$). However, linear regression revealed that the number of extinction presses during Phase 2 was not significantly predicted by PrL-NAc core peak delta ($R^2 = 0.5008$, $p = 0.1157$; Fig. 2.7C), peak theta ($R^2 = 0.3013$, $p = 0.2593$; Fig. 2.7D), peak beta ($R^2 = 0.2732$, $p = 0.2874$; Fig. 2.7E), low gamma ($R^2 = 0.0004381$, $p = 0.9686$; Fig. 2.7F), or average high gamma ($R^2 = 0.07159$, $p = 0.6082$; Fig. 2.7G) coherence, although relationships between PrL-NAc coherence and extinction presses appeared to be negative for lower frequencies (Fig. 2.7C-E).

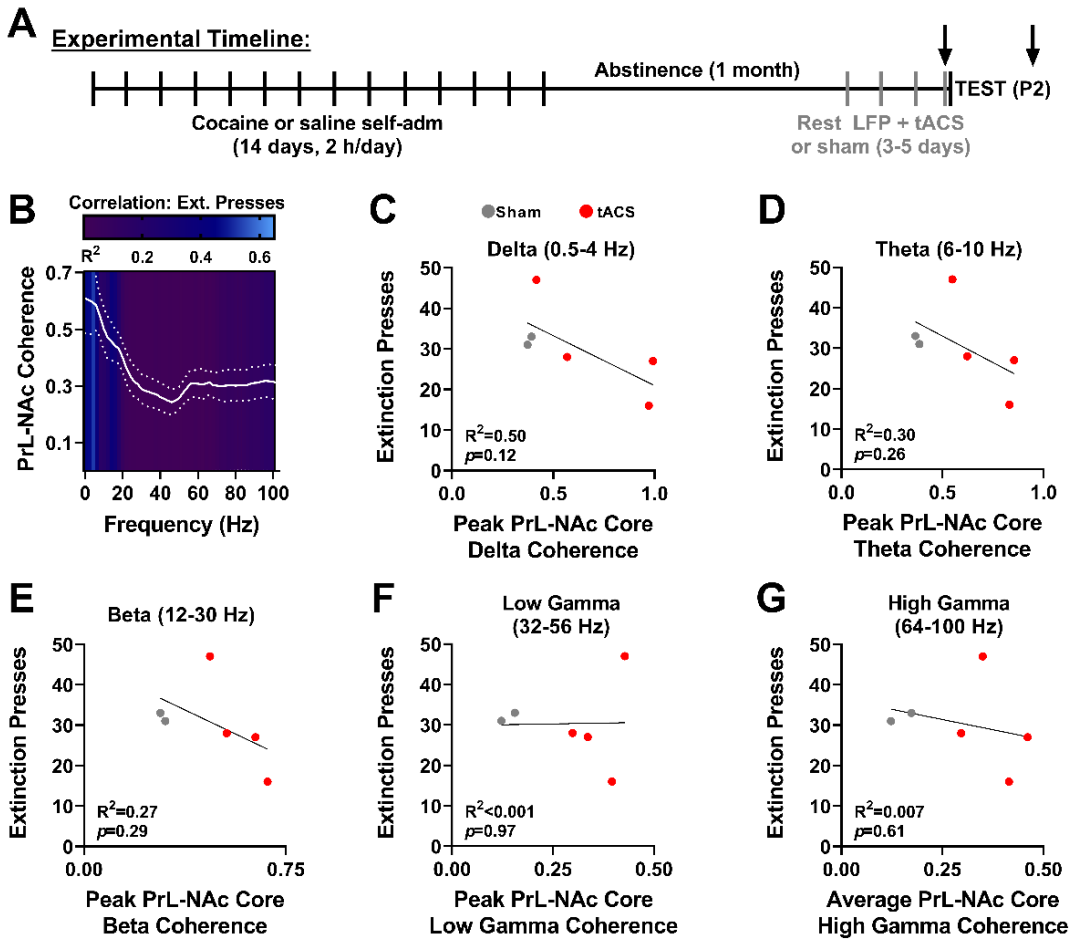

Figure 2.7 Increased rest PrL-NAc core coherence at low frequencies following treatment may be associated with reduced cocaine seeking under extinction (Phase 2 of test). **A.** Experimental timeline with arrows indicating time point of rest LFP recording and test phase. **B.** PrL-NAc coherence across frequencies following tACS or sham treatment (white line). Error bars (dotted white line) represent mean ± SEM. Coherence values at each frequency were correlated with individual rats' extinction presses (n=6) and the R^2 value for each of these correlations (0-100 Hz; 2 Hz/bin) are represented in the color overlay (R^2 values ≥ 0.47 are trending, $p < 0.10$, represented as blue in the overlay). **C.** Linear regression of peak PrL-NAc core delta coherence and extinction presses. **D.** Linear regression of peak theta coherence and extinction presses. **E.** Linear regression of peak beta coherence and extinction presses. **F.** Linear regression of peak low gamma coherence and extinction presses. **G.** Linear regression of average high gamma coherence and extinction presses. Gray and red reflect sham and 80 Hz tACS treated rats, respectively, in **C-G**.

Due to differences in testing procedures (i.e., rats underwent either Phase 1 only or all three phases of the test), number of animals with microwire implants per group, and loss of catheter

patency during prolonged abstinence, we were not able to examine relationships between rest PrL-NAc core coherence following treatment and cocaine infusions earned during Phase 3 of the test. These relationships will be examined in Aim 2.

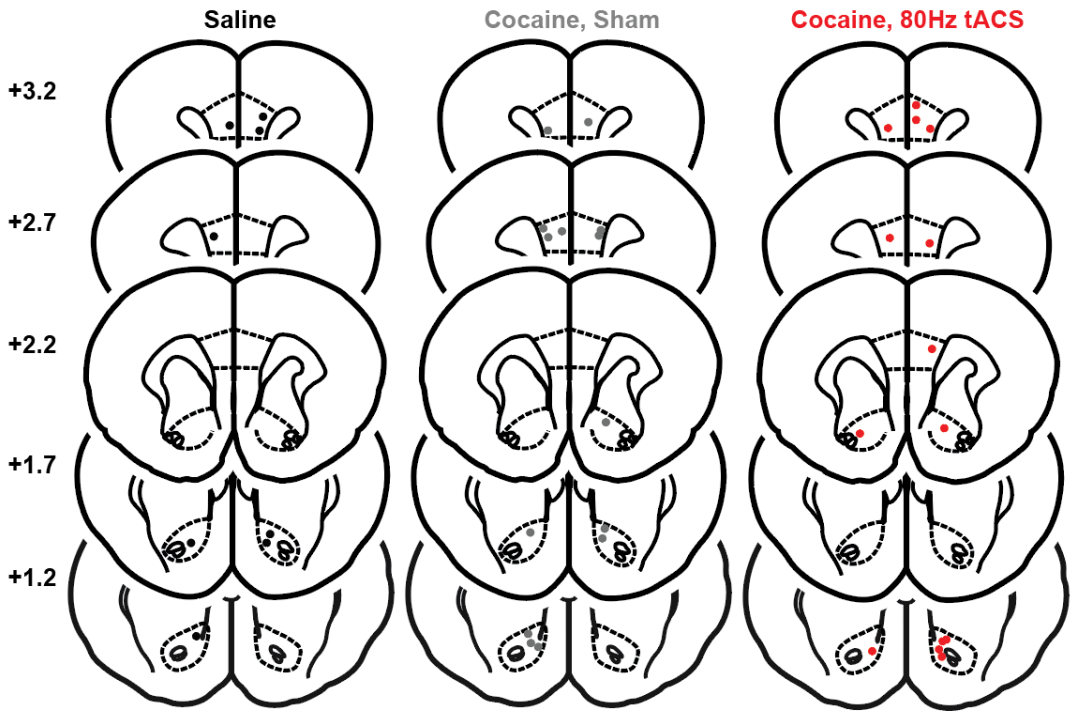

Figure 2.8 Histology. Electrode tip placements in the PrL and NAc core for saline (black), cocaine-sham (gray), and cocaine-tACS animals (red).

Discussion

The current study examined the effects of 80 Hz tACS administered over the PrL on resting state PrL-NAc coherence and cocaine-seeking behaviors. Here, we used *in vivo* electrophysiology to record simultaneous LFPs in the PrL and NAc core at rest following one-month abstinence from cocaine or saline self-administration. We then applied tACS (or sham) targeting specific neuronal activity patterns that were disrupted following one-month abstinence from cocaine (here, PrL-NAc coherence at high gamma, 80 Hz), and examined the effects of 80 Hz tACS or sham treatment on PrL-NAc core oscillatory dynamics and cocaine seeking. PrL-NAc coherence was reduced at multiple frequency bands following one-month abstinence from cocaine compared with saline controls, consistent with recent work from our lab (West et al., 2021). This dampening of PrL-NAc coherence was most robust in the high gamma frequency range. Given these findings, and our recent data showing 80 Hz tACS restores cocaine-induced deficits in PrL-NAc coherence and behavioral flexibility (West et al., 2021), we hypothesized that 80 Hz tACS would *reduce* cocaine seeking in this task. While 80 Hz tACS (versus sham) increased PrL-NAc coherence across the frequency spectrum, it actually *increased* and was positively correlated with cocaine seeking during the test. These results indicate that tACS targeting high gamma frequencies is insufficient to reduce cocaine seeking and may actually augment cue-elicited cocaine craving.

Our lab has previously shown that one-month abstinence from cocaine versus saline self-administration leads to aberrant signaling in the NAc core (Saddoris & Carelli, 2014) and PrL (West et al., 2021) and reduced coherence in the PrL-NAc circuit (West et al., 2021), linked with impaired associative learning and behavioral flexibility. These findings are consistent with numerous studies in individuals with CUD and animal models showing reduced baseline PFC and NAc activity (Beveridge et al., 2008; Hanlon et al., 2013; Porrino et al., 2007; Volkow et al., 1992)

and rsFC between the striatum and PFC (Gu et al., 2010; Hu et al., 2015; H. Lu et al., 2014) following prolonged abstinence. Likewise, our findings are in line with theories suggesting that dysfunction of the PFC and its related circuitry drives prominent features of SUD including impaired cognitive flexibility (Goldstein & Volkow, 2002, 2011; West et al., 2021). Here, we replicate our recent work and show reduced PrL-NAc core coherence at rest across frequencies following one-month abstinence from cocaine versus saline. While dampened PrL-NAc coherence was observed across frequencies, this decrease was most robust in the high gamma frequency range. Given the established link between high gamma oscillations (Gamma-80) in the NAc, which originate from the frontal cortex (Berke, 2009), and reward-motivated behavior in rats (van der Meer & Redish, 2009), we hypothesized that reduced PrL-NAc gamma coherence may be an important therapeutic target for reducing disadvantageous behaviors following prolonged cocaine abstinence.

As such, and given our recent work showing 80 Hz tACS restores PrL-NAc core signaling and behavioral flexibility following prolonged cocaine abstinence, we next examined the effects of 80 Hz tACS or sham on PrL-NAc oscillatory dynamics and *cocaine-seeking* behaviors (saline control rats did not undergo tACS/sham or testing). We found that 80 Hz tACS increased rest PrL-NAc coherence across frequencies relative to sham-treated rats and restored PrL-NAc coherence to saline control levels, consistent with our recent work (West et al., 2021). We believe the broad-band increase in PrL-NAc core coherence following 80 Hz tACS reflects a general effect of this high frequency on cells in the PrL-NAc projection rather than a frequency-specific entrainment of oscillatory signaling in this circuit. In support, we previously showed that 83 Hz photostimulation of cell bodies in the PrL that project to the NAc core induced phasic neuronal activity in the core

time-locked to stimulation (West et al., 2021), perhaps suggesting this high frequency is sufficient to induce a general increase in activity across the PrL-NAc circuit.

Although 80 Hz tACS increased PrL-NAc coherence relative to sham-treated rats, it actually *increased* cocaine seeking during the test. While unexpected at first, these results are consistent with the notion that activation of PFC-NAc circuitry differentially modulates behavior depending on the experimental conditions recruiting those regions (see **General Introduction**). That is, in the presence of cocaine-associated cues here, strengthening PrL-NAc signaling with 80 Hz tACS promoted cocaine seeking, whereas when this neural circuit was recruited by executive control processes, strengthening PrL-NAc signaling with 80 Hz tACS restored behavioral flexibility (West et al., 2021). Similarly, when operant behavior previously reinforced by cocaine was paired with footshock, stimulation of the PFC suppressed cocaine seeking (Chen et al., 2013), but in the presence of cocaine cues activation of the PFC (Kalivas, 2008) and NAc (Cornish & Kalivas, 2000; McFarland et al., 2003) promotes cocaine seeking. Further, increased cue-evoked cocaine seeking following prolonged abstinence requires abstinence-related synaptic potentiation in the PrL-NAc core projection (Conrad et al., 2008; Loweth et al., 2014; Ma et al., 2014). Collectively these findings demonstrate the importance of context in designing tACS methods for modulating behaviors associated with CUD and support the need to undergo a multifaceted approach in treating SUDs. Future studies need to extend the circuit-level mechanisms underlying the interplay between cue-induced drug craving and diminished executive function in individuals with SUDs.

We also examined relationships between rest PrL-NAc coherence following 80 Hz tACS or sham treatment and subsequent cocaine-seeking behaviors during the test. We found significant positive correlations between approaches toward, but not time spent in the cocaine-associated

quadrant (Phase 1 of test) and rest PrL-NAc coherence across frequencies (Figs. 2.5, 2.6), suggesting a link between 80 Hz tACS-induced increases in PrL-NAc coherence and approach behavior in our task. However, it is difficult to speculate on the presence or absence of significant relationships between PrL-NAc coherence and distinct cocaine-seeking behaviors (i.e., approach versus time), since LFPs and behavior were measured after animals underwent either tACS (putatively modulating LFP signaling and behavior) or sham treatment and examined *across groups*. Nevertheless, previous studies have demonstrated links between prefrontal delta oscillations and cue-induced cocaine craving in humans with CUD (Reid et al., 2003), and NAc high gamma oscillations with reward anticipation in rats (van der Meer & Redish, 2009), and the present results are consistent with these findings. Interestingly, higher PrL-NAc coherence at lower frequencies (e.g., beta) following treatment showed non-significant negative relationships with extinction presses during Phase 2 of the test, suggesting tACS targeting lower frequencies may be sufficient to reduce cocaine seeking under extinction, which we examined in Aim 2. Future studies should also determine PrL-NAc LFP signaling and its relationship to cocaine seeking before and following prolonged cocaine abstinence to better assess the role of distinct oscillatory frequency bands in incubation of cocaine craving. Aim 3 begins to address these concerns.

While administration of 80 Hz tACS over the PrL increased coherent activity in the PrL-NAc circuit, this stimulation method is less specific than other techniques (e.g., optogenetics) and may have additional effects on other downstream regions via alternative projections (e.g., PrL-BLA). Given the complex neurocircuitry underlying cocaine seeking and relapse (Dong et al., 2017; Kalivas, 2008; Koob & Volkow, 2010; McFarland & Kalivas, 2001; Wolf, 2016), future studies are needed to determine how oscillatory dynamics in these additional regions are linked to cocaine seeking following prolonged abstinence, and how tACS affects the broader neurocircuitry

underlying this behavior. Finally, although 80 Hz tACS increased cocaine-seeking behaviors in the current study, if and when we identify a stimulation frequency that is sufficient to reduce cocaine seeking, it will be important to also examine the effect of tACS versus sham on motivated behavior for non-drug reinforcers (e.g., water/saline self-administration).

CHAPTER 3

BETA FREQUENCY (16 HZ) tACS RESTORES PrL-NAc BETA COHERENCE AND MAY REDUCE COCAINE SEEKING

Introduction

SUD is associated with profound disruptions in multiple brain regions and their associated circuitry. One prominent perspective posits that SUDs are driven by impaired PFC control over hyperactive subcortical regions (Bechara, 2005; Goldstein & Volkow, 2002, 2011; Jasinska et al., 2015; Jentsch & Taylor, 1999). That is, chronic substance use augments sensitivity to drug-associated cues due to increased activation of subcortical structures (Conrad et al., 2008; Loweth et al., 2014; Martin et al., 2006; Roberts-Wolfe et al., 2018), while generalized PFC dysfunction (Goldstein & Volkow, 2002, 2011) impairs top-down inhibition of cue-evoked drug craving and seeking, ultimately leading to compulsive drug use and vulnerability to relapse (Jasinska et al., 2015). This view has guided progress toward developing novel treatments for SUDs including noninvasive brain stimulation targeting PFC regions, which shows promise for dampening drug craving and promoting sustained abstinence in people with SUDs (Hanlon et al., 2018; Mahoney et al., 2020; Terraneo et al., 2016).

In collaboration with Fröhlich and colleagues, our lab developed a rodent model of one form of noninvasive brain stimulation, tACS, and recently published data showing the efficacy of 80 Hz tACS to reverse cocaine-induced deficits in PrL-NAc oscillatory signaling and restore flexible behavior (West et al., 2021). Our tACS approach is relatively noninvasive (i.e., stimulation

is applied to screws dental cemented to the outer layer of the skull, not in brain) and therefore holds great translational value. Importantly, we can use our preclinical tACS model with our established *in vivo* electrophysiology methods to probe the neural mechanisms underlying tACS effects on cocaine seeking.

In Aim 1, we found reduced PrL-NAc coherence following one-month abstinence from cocaine versus saline self-administration, consistent with our recent work (West et al., 2021). Interestingly, 80 Hz tACS increased PrL-NAc coherence across frequencies compared to sham-treated animals, perhaps suggesting this high frequency is sufficient to induce a general increase in activity across the PrL-NAc circuit rather than a frequency-specific entrainment of oscillatory signaling. In support, we showed that 83 Hz photostimulation of cell bodies in the PrL that project to the NAc core induced phasic neuronal activity in the core time-locked to stimulation (West et al., 2021). In Aim 1, we also found that this strengthening of PrL-NAc LFP signaling following 80 Hz tACS was accompanied by an *increase* in cocaine seeking, consistent with studies demonstrating that synaptic potentiation in the PrL-NAc core projection drives the behavioral expression of incubated cocaine craving following prolonged abstinence (Dong et al., 2017; Ma et al., 2014).

Given these findings, in the current study we aimed to improve the frequency-specificity of our tACS effect on PrL-NAc core oscillatory dynamics and target a different frequency that may reduce cocaine seeking. As such, our tACS stimulation frequency for this Aim (beta, 16 Hz) was chosen in collaboration with Fröhlich and colleagues by closer examination of our electrophysiology data which revealed an endogenous beta peak at 16 Hz in rats that self-administered saline that was reduced in rats that self-administered cocaine (Fig. 3.2B). Further, emerging evidence indicates that beta oscillations are elevated during top-down cognitive control

processes (Engel & Fries, 2010), and rats that have undergone incubation of craving following prolonged cocaine abstinence are less sensitive to punishment-induced suppression of cocaine taking, i.e., show less PrL-dependent (Chen et al., 2013) top-down executive control over 'compulsive' cocaine seeking (Gancarz-Kausch et al., 2014). Given these findings, we hypothesized that elevating PrL-NAc beta coherence with 16 Hz tACS may reduce cocaine seeking following one-month abstinence.

Methods

Animals

Adult male Sprague Dawley rats (Charles River, n=27 total), 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 07:00 AM). During preoperative behavioral training, rats were restricted to no less than 90% of their free-fed body weight by access to 20-25 g of standard rat chow (Purina RMH3000) per day (*ad libitum* water). During 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, described in Aim 1 (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 houselight and speaker were centrally located on the opposite wall of the chamber. Masking 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 recoding cable, as well as insertion of the i.v. infusion line. Drug and water delivery were 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

Surgical procedures used here were the same as in Aim 1. Briefly, rats were anesthetized with an intramuscular injection of a ketamine hydrochloride (100 mg/kg) and xylazine hydrochloride (10 mg/kg) mixture and implanted with custom-made intrajugular catheters (Access Technologies, Skokie, IL, USA) for i.v. cocaine or saline self-administration using established procedures (Haake et al., 2019). Two stainless steel screws (Fine Science Tools, Foster City, CA, USA) were dental cemented in direct contact with, but not penetrating the outer surface of the skull for tACS or sham, described previously (West et al., 2021) and in Aim 1. Screws were positioned 2mm apart, at midline at the level of the PrL (bregma +2mm and +4mm). In a subset of animals (n=21), 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, using established procedures (Haake et al., 2019; West et al., 2021). As in Aim 1, Figure 3.1A shows a schematic of surgical preparation for tACS and *in vivo* electrophysiology, with stimulation leads "S" in direct contact with the outer layer of the skull and microwires targeting the PrL and ipsilateral NAc core. Animals were allowed 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 and habituation to the LFP recording chamber, as described in Aim 1.

Figure 3.1B shows a timeline of self-administration training, LFP recordings, tACS or sham treatment, and behavioral testing, and is similar to that described for Aim 1. On self-administration days 1-14, water-restricted rats were placed in an operant chamber and a lever was extended into the chamber with the cue light above it illuminated. During self-administration sessions (2 h each), lever depression resulted in i.v. delivery of cocaine (6 s infusion, 0.33 mg/infusion, ~1 mg/kg) or 0.9% physiological saline (infusion duration and volume matched), paired with termination of the cue light and simultaneous onset of a tone (67 dB, 1 kHz)/houselight conditioned stimulus (CS) for 20 s, as described in Aim 1. During saline self-administration, i.v. saline infusion occurred simultaneously with delivery of water into the receptacle to control for operant experience (Saddoris & Carelli, 2014; Saddoris et al., 2016; West et al., 2021). Next, rats underwent one month of experimenter-imposed abstinence from cocaine (n=23) or saline (n=4; the same saline self-administration rats were used for both Aims 1 and 2), during which animals remained in their home cages (30 ml water/day). On day 27 of abstinence from cocaine or saline, rats were placed in the LFP recording chamber (contextually distinct from the self-administration and testing chamber) and connected to the electrophysiological recording system. As in Aim 1,

simultaneous LFPs in the PrL and NAc core were recorded for 15 min while animals were not engaged in a behavioral task ('rest' LFP). Rats that were not outfitted with microwires (n=6) were placed in the LFP recording chamber for 15 min to control for experience. Immediately following the first rest LFP recording (day 27 of abstinence), rats that self-administered cocaine were split into two groups, one that received 16 Hz tACS (n=10) and one that received sham (n=13; the same cocaine-sham rats were used for both Aims 1 and 2) administered over 3-5 consecutive days in a distinct "treatment" chamber, using established procedures described in Aim 1 (West et al., 2021). Rats that self-administered saline (n=4) and rats that self-administered cocaine then received sham treatment (n=13) were used as control groups for Aims 1 and 2 (i.e., the additional subjects in Aim 2 are the cocaine-tACS rats, n=10). Next, following prolonged abstinence from cocaine self-administration, and 16 Hz tACS or sham treatment, rats received a post-treatment rest LFP recording (day 30-32 of abstinence) to assess target engagement of tACS, followed immediately by the behavioral test session. As in Aim 1, rats that self-administered saline were not treated with tACS or sham and did not undergo testing.

As described for Aim 1 and shown in Figure 3.1C, the test session consisted of one to three phases: (1) CS Probes, (2) Extinction, and (3) Cocaine Self-Administration. All cocaine-tACS and cocaine-sham rats underwent Phase 1 of the test (n=10 for cocaine-tACS; n=13 for cocaine-sham), and a subset of rats (n=10 for cocaine-tACS; n=8 for cocaine-sham) subsequently underwent Phases 2 and 3. In Phase 1 of the test (CS Probes), the cue light above the lever was illuminated but the lever was not extended. Ten pseudorandom presentations of the tone/houselight CS (5s each) were given over 15 min. The cue light above the lever remained on throughout Phase 1. As in Aim 1, behavior during Phase 1 of the test was recorded and analyzed for time spent in the lever-associated quadrant of the chamber and approaches toward that quadrant during each 5s CS

presentation. Next, Phase 2 (Extinction) was initiated wherein the lever previously associated with cocaine during self-administration was extended into the chamber. Here, lever depression resulted in termination of the cue light and presentation of the tone/houselight CS (20 s), but no drug delivery. After 2 h, Phase 3 (Cocaine Self-Administration) was initiated by administration of 0-3 priming infusions of cocaine (6 s; 0.33 mg/infusion) paired with the tone/houselight CS (20 s). Each subsequent lever press resulted in a cocaine infusion (6 s; 0.33 mg/infusion) paired with the CS (20 s) as in self-administration training sessions. The test session was completed 2 h after initiation of Phase 3.

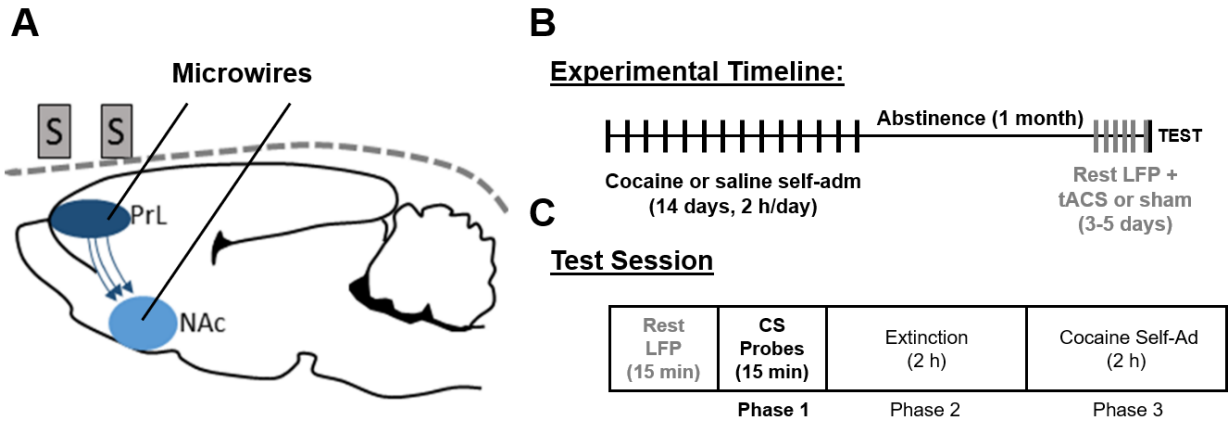

Figure 3.1 Surgical procedures for tACS and *in vivo* electrophysiology and experimental design. **A.** Schematic of stimulation lead ("S") placement for tACS and microwire implant into the PrL and ipsilateral NAc core. **B.** Experimental timeline for cocaine or saline self-administration, one-month abstinence, rest LFP recordings, 16 Hz tACS or sham treatment, and behavioral testing. **C.** Post-treatment rest LFP recording and behavioral testing procedures for cocaine-tACS and cocaine-sham rats. All cocaine-tACS and cocaine-sham rats underwent Phase 1 of the test (bolded), and a subset of rats underwent Phases 2 and 3 as well. Note: since the same experimental design was used in Aim 1 (except frequency and amount of tACS applied), this figure is similar to **Fig. 2.1.**

tACS Procedures

tACS or sham were delivered using established methods (West et al., 2021) described in Aim 1. During tACS or sham sessions, rats were placed in the treatment chamber and a Linear Stimulus Isolator (World Precision Instruments; Sarasota, FL, USA) was attached to the two stimulation leads via insulated wires inserted through the commutator. A computer-generated sinewave was input into the stimulator at the desired frequency (*16 Hz*) such that the stimulation oscillated between a +18 µA and -18 µA current across the screws. An electric swivel allowed free movement within the chamber during stimulation periods, which lasted ~15 minutes/day. Stimulations consisted of 40 cycles of 10s on then 10s off for five consecutive days. Sham rats received identical headcaps and treatments over 3-5 consecutive days, but no current was delivered.

Electrophysiology

The same electrophysiology methods used in Aim 1 were applied here and have been described in detail previously (Carelli, 2000; Hollander & Carelli, 2005). 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; Plexon, Inc., Dallas, TX, USA), described previously (Haake et al., 2019; Moschak, Wang, et al., 2018; 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 and 60 Hz noise was removed using a notch filter in a subset of recordings. LFPs were recorded and analyzed in Neuroexplorer (Plexon, Inc).

Histology

Histological reconstruction of electrode positions was accomplished using established procedures (Haake et al., 2019; West et al., 2021) described in Aim 1. Briefly, upon completion of the experiment, rats were deeply anesthetized with an i.p. injection of a ketamine hydrochloride and xylazine hydrochloride mixture (100 and 10 mg/kg, respectively). A 13.5-μA current was passed through each microwire electrode for 5s to mark the placement of electrode tips. Transcardial perfusions were then performed using 0.9% saline and 3% potassium ferrocyanide in 10% formalin, and brains were removed. After post-fixing and freezing, 40-μm coronal brain sections were mounted. The addition of potassium ferrocyanide allowed for a blue reaction corresponding to the location of the electrode tip which was viewed under a 1X microscope lens. Placement of an electrode tip was determined by examining the relative position of observable reaction product to visual landmarks and anatomical organization of the PrL and NAc core represented in a stereotaxic atlas (Paxinos & Watson, 2005).

Data Analysis

Behavior: As in Aim 1, the number of lever presses during self-administration training were compared for the three groups (saline vs cocaine-sham vs cocaine-tACS) using a two-way repeated-measures ANOVA with session (1-14) and group as factors. For rats that underwent cocaine self-administration and 16 Hz tACS treatment or cocaine self-administration and sham treatment, behavioral data during the three distinct phases of the test session were also analyzed. In Phase 1 (CS Probes), the amount of time each rat spent in the quadrant where the cocaine-associated lever had previously been extended during training, and the number of approaches toward this quadrant during each 5s CS presentation were recorded. Approaches toward and time

in the quadrant were defined by at least one-half of a body length into and orientation of the rat toward the quadrant, as described previously (Hollander & Carelli, 2007; West et al., 2014) and in Aim 1. Due to technical issues with video recording, behavioral data during Phase 1 of the test was not recorded for five animals in the cocaine-tACS group and one animal in the cocaine-sham group. Unpaired *t*-tests were used to compare the two groups (cocaine-tACS versus cocaine-sham) for all behavioral measures during the test, i.e., amount of time (seconds) in and number of approaches toward the cocaine lever-associated quadrant during Phase 1, the number of lever presses during Phase 2 (Extinction), and the number of cocaine infusions during Phase 3 (Cocaine Self-Administration). Due to catheter failure or differences in testing procedures (i.e., rats underwent either Phase 1 only, or multiple phases of the test), a subset of animals in the cocaine-sham (n=6) and cocaine-tACS (n=1) groups were not included in analyses of Phases 2 and 3 behavioral data.

Electrophysiology: The same electrophysiology analyses used in Aim 1 were also used here. For coherence data (PrL-NAc), two-way ANOVAs were run with group (e.g., saline, cocaine-sham, cocaine-tACS) and frequency (0-100 Hz [58-62Hz excluded]; 2 Hz/bin) as factors (Fig. 3.2B and 3.3B). Additionally, peak delta (0.5-4 Hz), theta (6-10 Hz), beta (12-30 Hz), low gamma (32-56 Hz), and average high gamma (64-100 Hz) coherence values were calculated for each animal. For the first rest LFP recording (following ~1-month abstinence from saline or cocaine and prior to tACS or sham treatment for rats that self-administered cocaine), unpaired *t*-tests were used to compare coherence at individual frequency bands (e.g., peak delta, average high gamma) for the saline versus cocaine groups (Fig. 3.2C-G). One-way ANOVAs were used to compare coherence at individual frequency bands following treatment for the cocaine-sham and cocaine-tACS groups with 'pre-treatment' coherence for the saline group (Fig. 3.3C-G). Due to

technical issues with electrophysiological recordings, one rat in the cocaine-sham group was excluded from analyses of the second rest LFP recording. Pearson's correlations were calculated between behaviors during the test and post-treatment coherence values at each individual frequency bin (0-100 Hz [58-62Hz excluded]; 2Hz/bin) across cocaine-tACS and cocaine-sham rats (Fig. 3.5B, 3.6B, and 3.7B). Finally, simple linear regression was used to examine relationships between coherence at individual frequency bands (e.g., peak delta coherence, average high gamma coherence), or change in coherence within these frequency bands (post-treatment minus pre-treatment), and behaviors during the test session for cocaine-tACS and cocaine-sham animals (Fig. 3.5C-G, 3.6C-G, 3.7C-G, and 3.8).

LFP coherence values were exported from NeuroExplorer. *Post hoc* comparisons (e.g., Tukey's multiple comparisons, Sidak's multiple comparisons) were used where appropriate. All statistical analyses were completed using GraphPad Prism 8 (GraphPad Software, Inc., La Jolla, CA, USA).

Results

Self-Administration Training

Rats that self-administered saline and rats that were later divided into cocaine-tACS or cocaine-sham groups did not significantly differ in self-administration behavior during training (prior to abstinence and treatment). A two-way repeated measures ANOVA of cocaine infusions earned with session (1-14) and group (saline vs cocaine-tACS vs cocaine-sham) as factors revealed a main effect of session ($F_{5.118, 122.8} = 10.38$, $p < 0.0001$) but no main effect of group ($F_{2, 24} = 1.527$, $p = 0.2376$) nor a group x session interaction ($F_{26, 312} = 1.202$, $p = 0.2320$), showing all rats acquired self-administration behavior.

Rest PrL-NAc Core Coherence is Reduced Following One-Month Abstinence from Cocaine versus Saline

Following one-month abstinence from cocaine or saline self-administration (prior to tACS or sham treatment), rats were placed in a contextually distinct chamber for rest LFP recordings (n=4 for saline, n=17 for cocaine outfitted with microwires). Figure 3.2A shows the experimental timeline with an arrow indicating the timepoint of this rest LFP recording. Shown in Figure 3.2B, rest PrL-NAc core coherence was reduced across the frequency spectrum following prolonged abstinence from cocaine compared with saline controls, replicating findings from Aim 1 (Fig. 2.2). A two-way ANOVA of PrL-NAc coherence with group (saline vs cocaine) and frequency (0-100 Hz [58-62Hz excluded]; 2 Hz/bin) revealed a main effect of group ($F_{1, 931} = 573.5$, $p < 0.0001$) and frequency ($F_{48, 931} = 5.436$, $p < 0.0001$), but no group x frequency interaction ($F_{48, 931} = 1.358$, $p = 0.0557$). To further examine the effects of prolonged abstinence from cocaine versus saline on PrL-NAc core signaling, we compared peak coherence values in the delta (0.5-4 Hz), theta (6-10

Hz), beta (12-30 Hz), and low gamma (32-56) ranges and average coherence values in the high gamma (64-100 Hz) range for the saline versus cocaine groups (Fig. 3.2C-G). Unpaired *t*-tests revealed a significant decrease in rest PrL-NAc core coherence for the cocaine versus saline group at delta ($t_{19} = 2.394$, $p = 0.0272$; Fig. 3.2C), beta ($t_{19} = 2.230$, $p = 0.0380$; Fig. 3.2E), low gamma ($t_{19} = 3.643$, $p = 0.0017$; Fig. 3.2F), and high gamma ($t_{19} = 4.551$, $p = 0.0002$; Fig. 3.2G) frequencies. There was no significant difference in PrL-NAc core peak theta coherence between the saline and cocaine groups ($t_{19} = 0.7728$, $p = 0.4492$; Fig. 3.2D). Critically, rats that were later divided into cocaine-tACS and cocaine-sham groups did not show significant differences in PrL-NAc core coherence at delta ($t_{15} = 0.8953$, $p = 0.3848$), theta ($t_{15} = 0.2089$, $p = 0.8373$), beta ($t_{15} = 1.134$, $p = 0.2744$), low gamma ($t_{15} = 0.3508$, $p = 0.7306$), or high gamma ($t_{15} = 0.2244$, $p = 0.8255$) frequencies prior to treatment (not shown). As in Aim 1, these findings reveal that cocaine self-administration followed by one-month abstinence leads to broadband decreases in PrL-NAc core coherence at rest compared with saline controls.

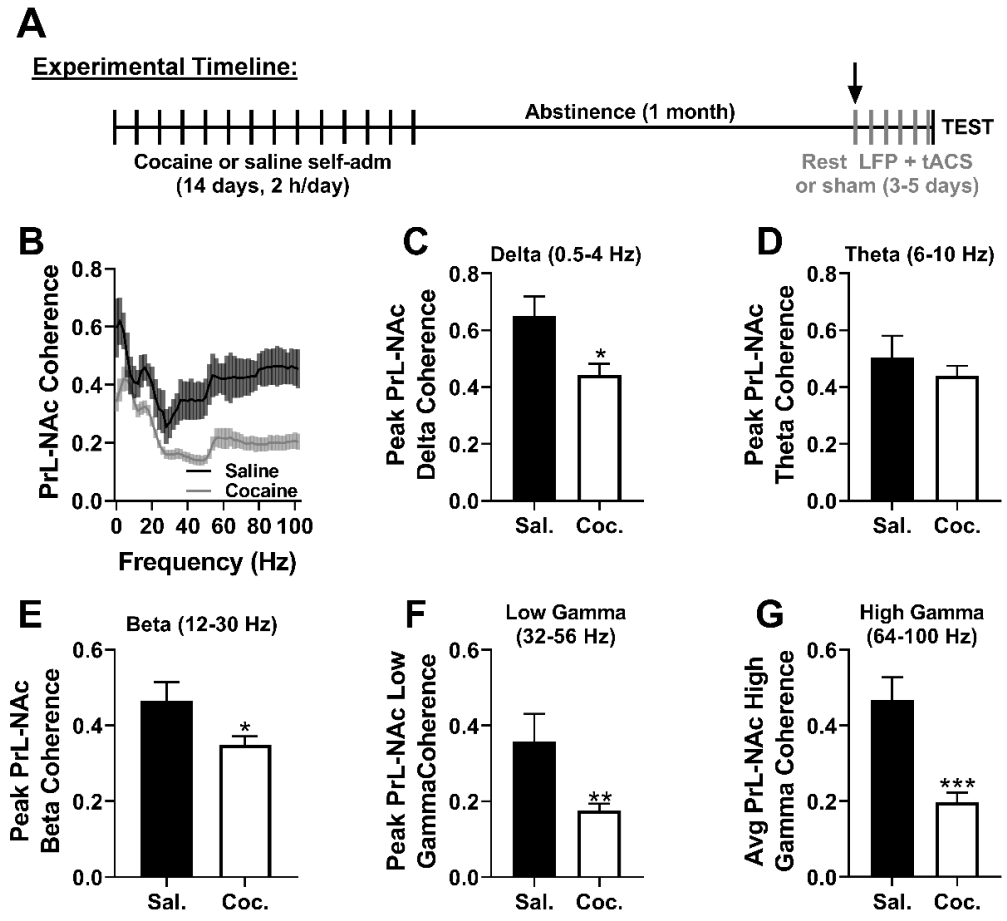

Figure 3.2 Broadband dampening of rest PrL-NAc coherence following one-month abstinence from cocaine versus saline self-administration, replicating findings from Aim 1. **A.** Experimental timeline with arrow indicating timepoint of rest LFP recording. **B.** PrL-NAc core coherence across the frequency spectrum (0-100 Hz; 2Hz/bin), showing a broadband reduction in PrL-NAc core coherence for the cocaine versus saline group. Error bars represent mean ± SEM. **C.** Peak PrL-NAc core coherence value in the delta range for the saline versus cocaine group. *p<0.05 for saline vs cocaine. **D.** Peak PrL-NAc core theta coherence for the saline versus cocaine group. **E.** Peak PrL-NAc core beta coherence. *p<0.05 for saline vs cocaine. **F.** Peak PrL-NAc core low gamma coherence. **p<0.01 for saline vs cocaine. **G.** Average PrL-NAc core high gamma coherence. ***p<0.001 for saline vs cocaine. Error bars represent mean + SEM in **C-G**.

16 Hz tACS Increases PrL-NAc Core Coherence at Lower Frequencies

Following the first rest LFP recording, rats that self-administered cocaine were placed into one of two treatment groups: 16 Hz tACS (n=10) or sham (n=6 outfitted with microwires). Similar

to Aim 1 in which 80 Hz tACS was applied, here, 16 Hz tACS or sham treatment was administered over 3-5 consecutive days in a separate and distinct 'treatment' chamber. Following tACS or sham treatment and immediately prior to the test session, cocaine-tACS and cocaine-sham rats were returned to the LFP recording chamber for a post-treatment rest LFP recording. Figure 3.3 shows the experimental timeline with an arrow indicating the timepoint of the post-treatment rest LFP recording for cocaine-tACS and cocaine-sham groups (Fig. 3.3A, similar to Fig. 2.3A), and PrL-NAc core coherence for saline, cocaine-tACS, and cocaine-sham groups (note: since saline rats did not undergo tACS or sham treatment, coherence data from the first LFP recording shown in Fig. 3.2 is reused for the saline group here). Shown in Fig. 3.3B, 16 Hz tACS increased rest PrL-NAc coherence at lower frequencies for the cocaine-tACS group compared to the cocaine-sham group, and appeared to restore coherence in this circuit to saline control levels at these lower frequency bands. A two-way ANOVA of PrL-NAc core coherence with group (saline vs cocaine-tACS vs cocaine-sham) and frequency (0-100 Hz [58-62Hz noise excluded]; 2 Hz/bin) as factors revealed a main effect of group ($F_{2, 833} = 190.1$, $p < 0.0001$) and frequency ($F_{48, 833} = 7.105$, $p < 0.0001$), but no group x frequency interaction ($F_{96, 833} = 0.9913$, $p = 0.5068$).

To further examine the effects of 16 Hz tACS or sham on rest PrL-NAc core oscillatory dynamics, we compared peak coherence values in the delta (0.5-4 Hz), theta (6-10 Hz), beta (12-30 Hz), and low gamma (32-56) ranges, and average coherence values in the high gamma (64-100 Hz) range for the saline versus cocaine-tACS versus cocaine-sham groups using one-way ANOVAs (Fig. 3.3C-G). Specifically, a one-way ANOVA of peak PrL-NAc core delta coherence for saline vs cocaine-tACS vs cocaine-sham groups revealed no significant effect of group ($F_{2, 17} = 3.073$, $p = 0.0726$; Fig. 3.3C). Similarly, a one-way ANOVA of peak PrL-NAc theta coherence revealed no significant effect of group ($F_{2, 17} = 2.320$, $p = 0.1285$; Fig. 3.3D). At low gamma, a

one-way ANOVA revealed a significant effect of group ($F_{2, 17} = 3.798$, $p = 0.0433$), and shown in Fig. 3.3F, Tukey's multiple comparisons revealed a significant decrease in peak PrL-NAc low gamma coherence for the cocaine-sham versus saline group ($p = 0.0343$), but no significant difference between the saline and cocaine-tACS groups ($p = 0.1833$), nor between the cocaine-sham and cocaine-tACS groups ($p = 0.4022$). At high gamma, a one-way ANOVA revealed a significant effect of group ($F_{2, 17} = 7.984$, $p = 0.0036$), and shown in Fig. 3.3G, Tukey's multiple comparisons revealed a significant decrease in PrL-NAc high gamma coherence for the cocaine-sham versus saline group ($p = 0.0038$) and for the cocaine-tACS versus saline group ($p = 0.0088$), but no significant difference between the cocaine-sham and cocaine-tACS groups ($p = 0.6819$).

Interestingly, a one-way ANOVA of peak PrL-NAc beta coherence for saline versus cocaine-tACS versus cocaine-sham groups revealed a significant effect of group ($F_{2, 17} = 6.802$, $p = 0.0068$), and shown in Fig. 3.3E, Tukey's multiple comparisons revealed a significant decrease in peak PrL-NAc beta coherence for the cocaine-sham versus saline group ($p = 0.0067$), and a significant increase in peak PrL-NAc beta coherence for the cocaine-tACS versus cocaine-sham group ($p = 0.0409$), but no significant difference between the saline and cocaine-tACS groups ($p = 0.3004$). Collectively, these findings suggest that 16 Hz (beta frequency) tACS selectively increases PrL-NAc core coherence at lower frequencies and reverses cocaine-induced deficits in PrL-NAc core coherence in the beta frequency band (Fig. 3.3B, E).

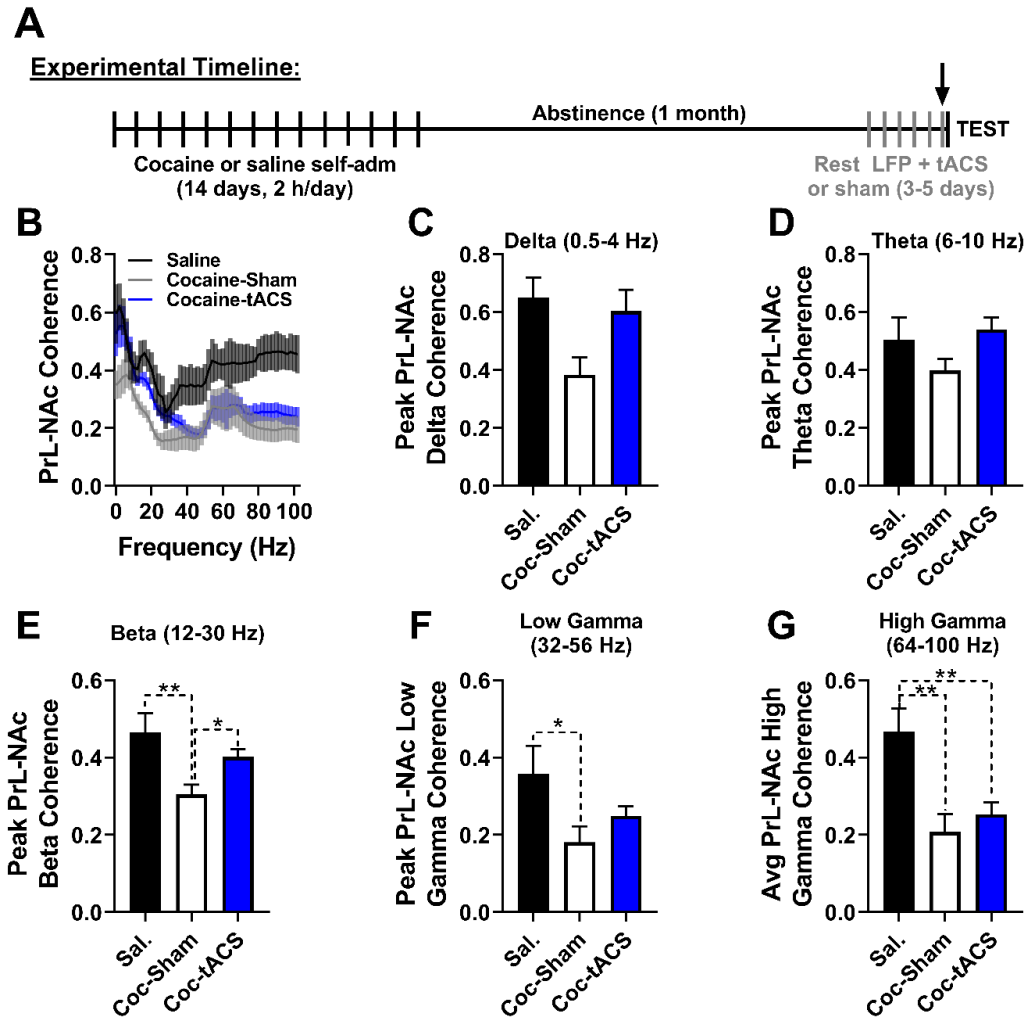

Figure 3.3 Effects of 16 Hz tACS on rest PrL-NAc core coherence. **A.** Experimental timeline with arrow indicating timepoint of rest LFP recording. **B.** Rest PrL-NAc core coherence across the frequency spectrum (0-100 Hz; 2Hz/bin) for saline, cocaine-tACS, and cocaine-sham groups, showing increased PrL-NAc core coherence for the cocaine-tACS versus cocaine-sham group at low frequencies (delta and beta) only. Error bars represent mean ± SEM. **C.** Peak PrL-NAc core coherence value in the delta range for saline versus cocaine-tACS versus cocaine-sham groups. **D.** Peak PrL-NAc core theta coherence for saline versus cocaine-tACS versus cocaine-sham groups. **E.** Peak PrL-NAc core beta coherence. $^{**}p<0.01$ for saline vs cocaine-sham; $^{*}p<0.05$ for cocaine-sham vs cocaine-tACS. **F.** Peak PrL-NAc core low gamma coherence. $^{*}p<0.05$ for saline vs cocaine-sham. **G.** Average PrL-NAc core high gamma coherence. $^{**}p<0.01$ for saline vs cocaine-sham; saline vs cocaine-tACS. Error bars represent mean + SEM for **C**-**G**.

Effects of 16 Hz tACS on Cocaine-Seeking Behavior

Given the specificity of effects of 16 Hz tACS on rest PrL-NAc core coherence, we hypothesized that 16 Hz tACS applied after one-month abstinence from cocaine self-administration may decrease cocaine-seeking behaviors during the test session relative to sham-treated animals. Here, following 16 Hz tACS or sham treatment, cocaine-tACS (n=10) and cocaine-sham rats (n=13) were given a behavioral test session consisting of 1-3 phases: (1) CS Probes, (2) Extinction, and (3) Cocaine Self-Administration, as described for Aim 1. All cocaine-tACS and cocaine-sham rats underwent Phase 1 of the test, and a subset of rats underwent Phases 2 and 3 as well. Figure 3.4 shows a schematic of the test session (Fig. 3.4A) and cocaine-seeking behaviors for cocaine-sham versus cocaine-tACS groups during the three distinct phases of the test. During Phase 1 of the test (CS Probes; Fig. 3.4B-C), there were no significant differences in time spent in the cocaine lever-associated quadrant of the chamber ($t_{16} = 1.441$, $p = 0.1690$) or approaches toward the quadrant ($t_{16} = 0.9020$, $p = 0.3804$) between cocaine-sham and cocaine-tACS rats. Shown in Fig. 3.4D-E, there were also no significant differences between cocaine-sham and cocaine-tACS groups in number of extinction presses during Phase 2 ($t_{15} = 0.9521$, $p = 0.3561$) or reinforced lever presses (cocaine infusions) during Phase 3 ($t_{14} = 0.4761$, $p = 0.6414$) of the test.

These results show that 16 Hz tACS, which appeared to increase rest PrL-NAc coherence at delta and beta frequencies and reversed cocaine-induced deficits in PrL-NAc beta coherence, is insufficient to reduce cocaine seeking for tACS-treated rats compared to sham treatment. Interestingly, however, while behavioral responses to the cocaine-paired CS during Phase 1 of the test appear to be slightly heightened in the cocaine-tACS compared to cocaine-sham groups, extinction presses during Phase 2 appear to be reduced.

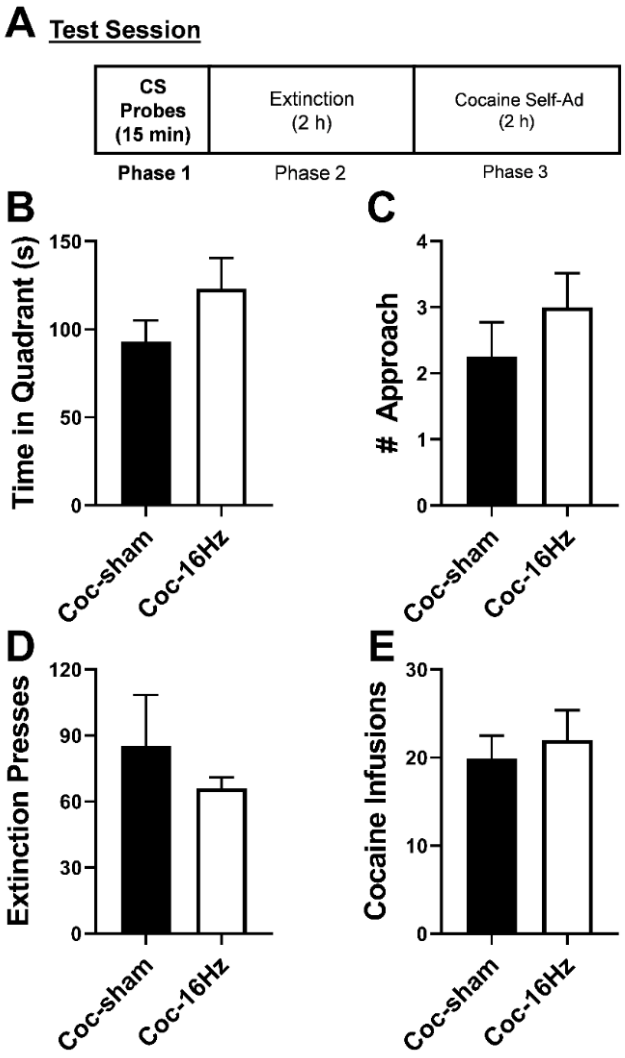

Figure 3.4 Effects of 16 Hz tACS on cocaine-seeking behaviors during the test. **A.** Schematic of the three-phase behavioral test session. All cocaine-tACS and cocaine-sham rats underwent Phase 1 of the test (bolded) and a subset of rats subsequently underwent Phases 2 and 3. **B.** Time spent (seconds) in the quadrant where the cocaine-associated lever was previously extended (Phase 1 of the test). **C.** Approaches toward the quadrant during Phase 1 of the test. **D.** Extinction presses during Phase 2 of the test. **E.** Cocaine infusions earned during Phase 3 of the test. Error bars represent mean + SEM in **B-E**.

Rest PrL-NAc Core Coherence Following Treatment Bidirectionally Predicts Cocaine-Seeking Behaviors During the Test

We next examined relationships between rest PrL-NAc core coherence following 16 Hz tACS or sham treatment and subsequent cocaine-seeking behaviors during the test session. Specifically, Pearson's correlations were calculated between behaviors during the test and post-treatment coherence values at each individual frequency (0-100 Hz; 2 Hz/bin) across cocaine-tACS and cocaine-sham rats. Further, simple linear regression was used to examine relationships between coherence at individual frequency bands (e.g., peak delta coherence, average high gamma coherence) and behaviors during the test for cocaine-tACS and cocaine-sham rats.

Figure 3.5 shows the relationship between rest PrL-NAc coherence following treatment and time spent in the quadrant during Phase 1 of the test (Fig. 3.5A, arrows indicating timepoint of rest LFP recording and phase of test session in which behavior was measured). Time spent in the quadrant was significantly positively correlated with PrL-NAc core coherence at the following frequencies ($R^2 \geq 0.4197$, $p < 0.05$ for all; highlighted in red in Fig. 3.5B): 0-4 Hz (delta), 6-10 Hz (theta), and 82-88 Hz (high gamma). Additionally, linear regression revealed that time spent in the quadrant was significantly predicted by post-treatment PrL-NAc core peak delta ($R^2 = 0.6603$, $p = 0.0024$; Fig. 3.5C) and peak theta ($R^2 = 0.6864$, $p = 0.0016$; Fig. 3.5D) coherence, and there was a trend toward a significant predictive relationship between post-treatment PrL-NAc core average high gamma coherence and time spent in the quadrant ($R^2 = 0.3451$, $p = 0.0574$; Fig. 3.5G). Time spent in the quadrant was not significantly predicted by post-treatment PrL-NAc core peak beta ($R^2 = 0.1777$, $p = 0.1965$), or low gamma ($R^2 = 0.1033$, $p = 0.3352$) coherence (Fig. 3.5E-F).

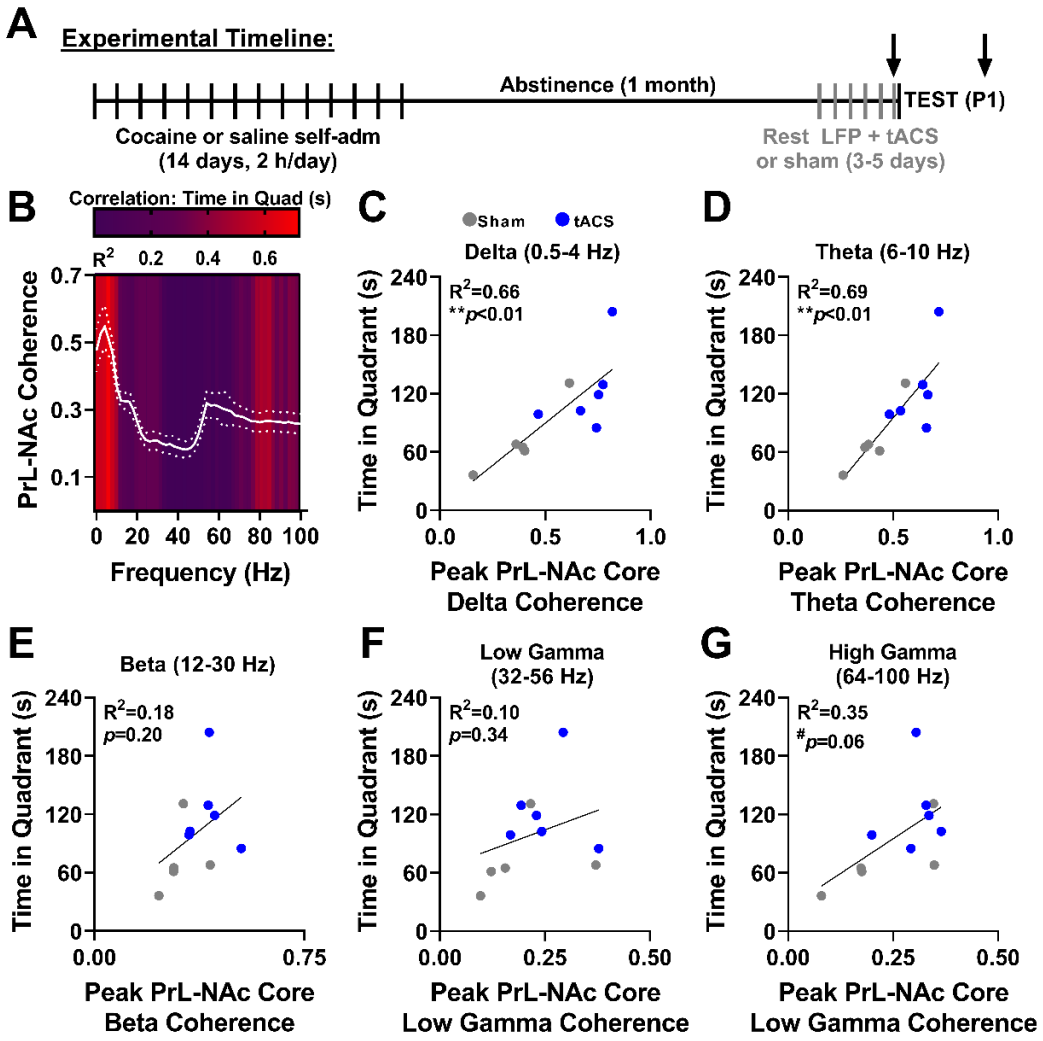

Figure 3.5 Relationship between rest PrL-NAc core coherence following 16 Hz tACS or sham and time spent in the quadrant during Phase 1 of the test. **A.** Experimental timeline with arrows indicating time point of rest LFP recording after tACS or sham treatment and test phase during which behavior was measured. **B.** PrL-NAc coherence across frequencies following tACS or sham treatment (white line). Error bars (dotted white line) represent mean ± SEM. Coherence values at each frequency were correlated with individual rats' time spent in the quadrant (n=11) and the R^2 value for each of these correlations (0-100 Hz; 2 Hz/bin) are represented in the color overlay (R^2 values ≥ 0.42 are significant, $p < 0.05$, represented as red in the overlay). **C.** Linear regression of peak PrL-NAc core delta coherence and time in quadrant (s). $^{**}p<0.01$. **D.** Linear regression of peak theta coherence and time in quadrant. $^{**}p<0.01$. **E.** Linear regression of peak beta coherence and time in quadrant. **F.** Linear regression of peak low gamma coherence and time in quadrant. **G.** Linear regression of average high gamma coherence and time in quadrant. $^{\#}p<0.10$. Gray and blue reflect sham and 16 Hz tACS treated rats, respectively, in **C-G**.

Figure 3.6 shows the relationship between rest PrL-NAc core coherence following treatment and approaches toward the quadrant during Phase 1 of the test (Fig. 3.6A, arrows indicating timepoint of rest LFP recording and test phase in which behavior was measured). Pearson's correlations revealed that approaches toward the quadrant were significantly ($R^2 \geq 0.4064$, $p < 0.05$) or near-significantly ($R^2 \geq 0.3450$, $p < 0.10$) positively correlated with PrL-NAc core coherence at the following frequencies: 4 Hz (delta; dark red strip in Fig. 3.6B; $p<0.10$), and 82-90 Hz (high gamma; highlighted in red in Fig. 3.6B; $p<0.05$). Additionally, linear regression revealed that the number of approaches toward the quadrant were not significantly predicted by post-treatment PrL-NAc core peak theta ($R^2 = 0.2700$, $p = 0.1014$; Fig. 3.6D), peak beta ($R^2 = 0.1230$, $p = 0.2903$; Fig. 3.6E), or low gamma ($R^2 = 0.1567$, $p = 0.2281$; Fig. 3.6F) coherence. However, there was a trend toward a significant predictive relationship between PrL-NAc peak delta coherence and approach ($R^2 = 0.3468$, $p = 0.0566$; Fig. 3.6C), and a significant relationship between PrL-NAc core high gamma coherence and approach ($R^2 = 0.4092$, $p = 0.0341$; Fig. 3.6G).

These findings show that PrL-NAc core coherence in distinct frequency bands predicted behavioral responses to the cocaine-paired CS during Phase 1 of the test, with delta, theta, and high gamma frequencies showing predictive relationships with *time spent in the quadrant*, and delta and high gamma frequencies with *approaches* toward the quadrant. The latter relationship (high gamma and approach) is consistent with findings from Aim 1, supporting the conclusion that tACS targeting high gamma frequencies (80 Hz tACS) may increase behavioral responsiveness to cocaine-associated stimuli. Interestingly, here, 16 Hz tACS selectively reversed cocaine-induced deficits in PrL-NAc core coherence in the beta frequency band (Fig. 3.3E), but higher PrL-NAc core beta coherence following treatment did not predict increased behavioral responses to the cocaine-paired CS during Phase 1 of the test.

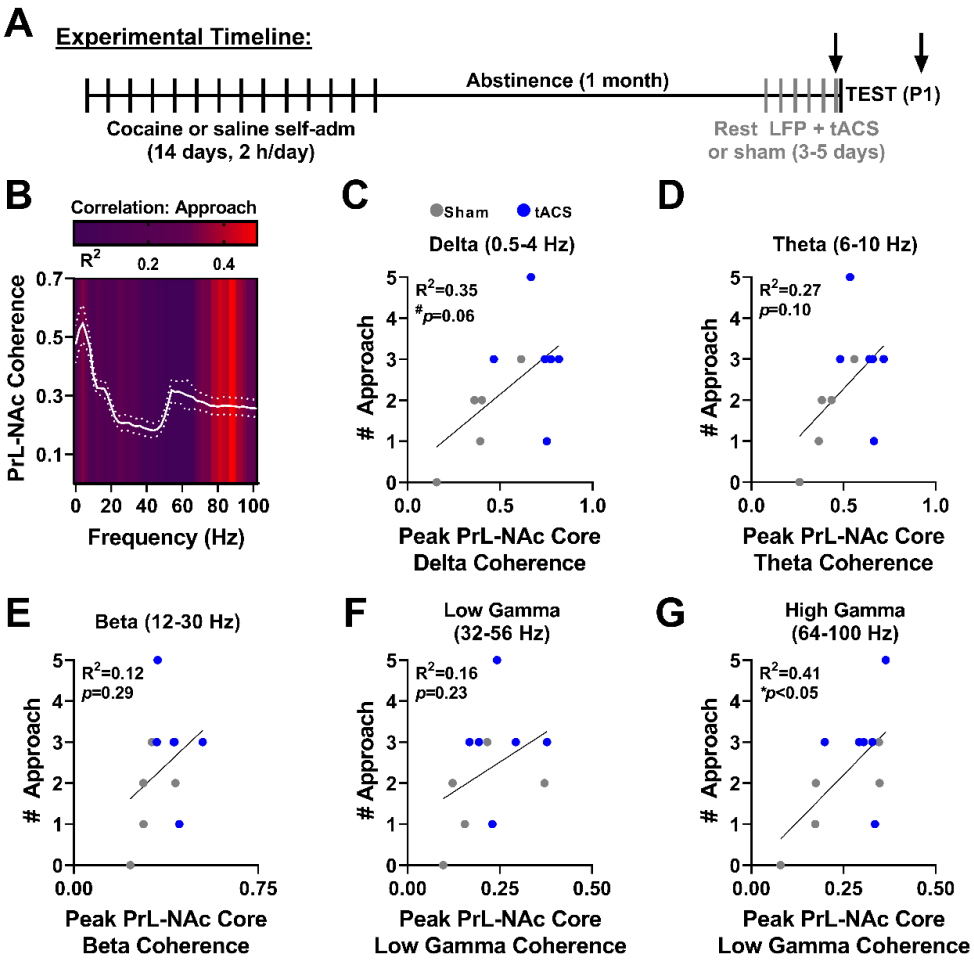

Figure 3.6 Relationship between rest PrL-NAc core coherence following 16 Hz tACS or sham and approaches toward the cocaine-associated quadrant during Phase 1 of the test. **A.** Experimental timeline with arrows indicating time point of rest LFP recording and test phase in which behavior was measured. **B.** PrL-NAc coherence across frequencies following tACS or sham treatment (white line). Error bars (dotted white line) represent mean ± SEM. Coherence values at each frequency were correlated with individual rats' number of approaches toward the quadrant (n=11) and the R^2 value for each of these correlations (0-100 Hz; 2 Hz/bin) are represented in the color overlay (R^2 values ≥ 0.41 are significant, $p < 0.05$, represented as red in the overlay). **C.** Linear regression of peak PrL-NAc core delta coherence and approach. $^{\#}p < 0.10$. **D.** Linear regression of peak theta coherence and approach. **E.** Linear regression of peak beta coherence and approach. **F.** Linear regression of peak low gamma coherence and approach. **G.** Linear regression of average high gamma coherence and approach. $^{*}p<0.05$. Gray and blue reflect sham and 16 Hz tACS treated rats, respectively, in **C-G**.

We also examined the relationship between rest PrL-NAc core coherence following treatment and extinction presses during Phase 2 of the test (Fig. 3.7). Given the slightly reduced number of extinction presses for the cocaine-tACS group compared to the cocaine-sham group shown in Fig. 3.4D, we predicted negative relationships between post-treatment rest PrL-NAc core coherence (particularly at lower frequencies, e.g., beta) and number of extinction presses. Such findings would suggest that tACS targeting lower frequencies may be more effective for reducing cocaine seeking under certain circumstances (i.e., lever presses under extinction conditions) compared with high frequency (80 Hz) tACS. Figure 3.7 shows the relationship between rest PrL-NAc core coherence following treatment and extinction presses during Phase 2 of the test (Fig. 3.7A, arrows indicating timepoint of rest LFP recording and Phase 2 of the test session). Pearson's correlations revealed that the number of extinction presses was significantly negatively correlated with PrL-NAc core coherence following treatment at the following frequencies ($R^2 \geq 0.3753$, $p < 0.05$ for all; highlighted in blue in Fig. 3.7B): 12 Hz and 20-30 Hz (beta), 32-34 Hz (low gamma). Linear regression revealed that the number of extinction presses during Phase 2 of the test were significantly predicted by peak PrL-NAc beta ($R^2 = 0.4312$, $p = 0.0204$; Fig. 3.7E) and low gamma coherence ($R^2 = 0.6069$, $p = 0.0028$; Fig. 3.7F), but not by peak delta ($R^2 = 0.0135$, $p = 0.7195$; Fig. 3.7C), theta ($R^2 = 0.0731$, $p = 0.3955$; Fig. 3.7D), or average high gamma coherence ($R^2 = 0.000083$, $p = 0.9776$; Fig. 3.7G). These findings suggest that increased rest PrL-NAc core coherence at beta and low gamma frequencies predicts reduced cocaine seeking following prolonged abstinence under certain circumstances (i.e., extinction presses).

Finally, Pearson's correlations revealed no significant correlations between cocaine infusions earned during Phase 3 of the test (Cocaine Self-Administration) and rest PrL-NAc core coherence following treatment at any frequency (0-100 Hz), and linear regression revealed that

infusions earned during Phase 3 of the test were not predicted by post-treatment PrL-NAc core peak delta (R^2 = 0.00060, p = 0.9430), theta (R^2 = 0.06369, p = 0.4541), beta (R^2 = 0.00016, p = 0.9701), low gamma (R^2 = 0.000021, p = 0.9894), or average high gamma (R^2 = 0.0275, p = 0.6264) coherence (not shown).

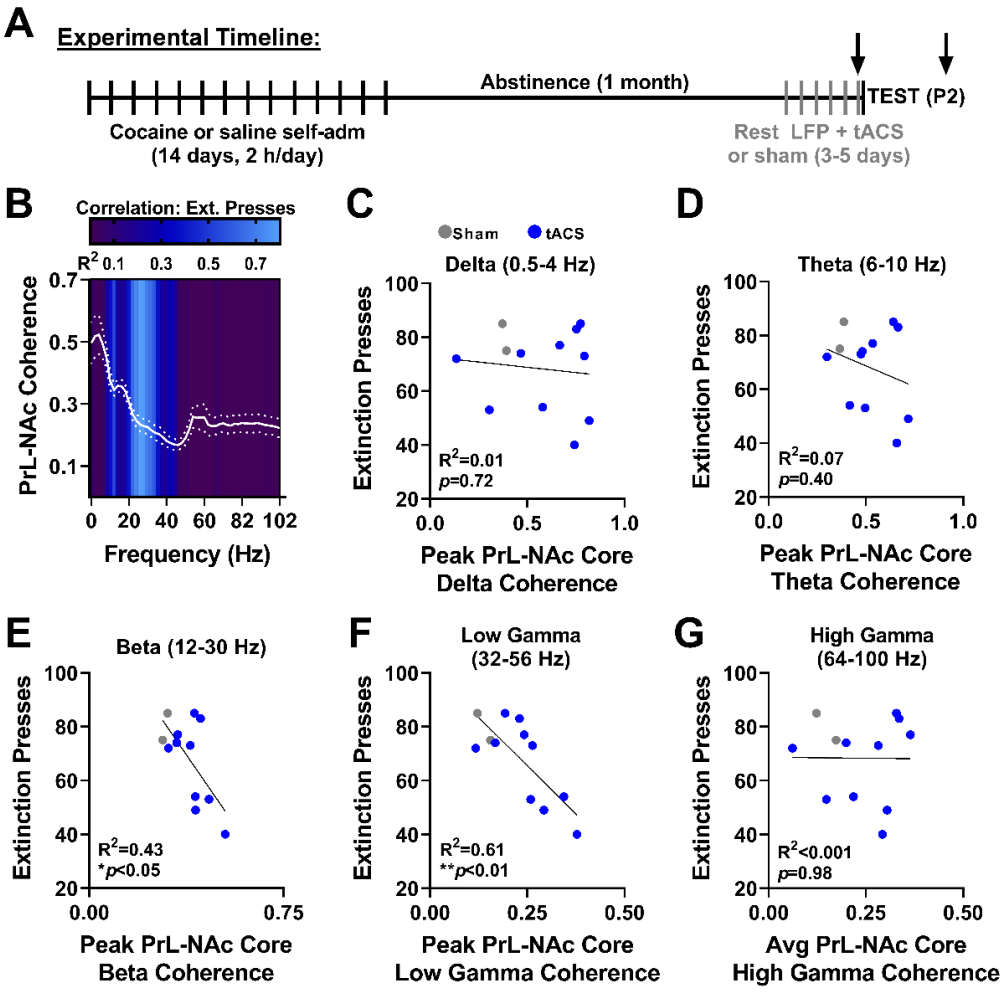

Figure 3.7 Increased rest PrL-NAc coherence at beta and low gamma frequencies following treatment predicts reduced cocaine seeking under extinction (Phase 2 of test). **A.** Experimental timeline with arrows indicating time point of rest LFP recording and test phase. **B.** PrL-NAc coherence across frequencies following tACS or sham treatment (white line). Error bars (dotted white line) represent mean ± SEM. Coherence values at each frequency were correlated with individual rats' extinction presses (n=12) and the R^2 value for each of these correlations (0-100

Hz; 2 Hz/bin) are represented in the color overlay (R^2 values ≥ 0.38 are significant, $p < 0.05$, represented as light blue in the overlay). **C.** Linear regression of peak PrL-NAc core delta coherence and extinction presses. **D.** Linear regression of peak theta coherence and extinction presses. **E.** Linear regression of peak beta coherence and extinction presses. *p<0.05. **F.** Linear regression of peak low gamma coherence and extinction presses. **p<0.01. **G.** Linear regression of average gamma coherence and extinction presses. Gray and blue reflect sham and 16 Hz tACS treated rats, respectively, in **C-G**.

We next sought to determine whether behaviors during Phase 2 of the test (extinction presses) could be predicted by behaviors during Phase 1 of the test (time and approach). A significant relationship might suggest that for increased PrL-NAc beta and low gamma coherence following tACS treatment to predict reduced cocaine-seeking behaviors during the second phase of the test (extinction presses), it must first increase behavioral responsiveness to the cocaine-paired CS in the absence of the lever during Phase 1. However, linear regressions revealed that extinction presses during Phase 2 were not significantly predicted by time spent ($R^2 = 0.0610$, $p = 0.4389$) or approaches ($R^2 = 0.1528$, $p = 0.2089$) toward the quadrant during Phase 1 (not shown). As such, we believe that the relationship between increased rest PrL-NAc core coherence at beta and low gamma frequencies with reduced extinction presses during Phase 2 reflects an independent circuit mechanism underlying cocaine seeking following prolonged drug abstinence.

Entrainment of Theta, Beta, and Low Gamma Oscillatory Dynamics in the PrL-NAc Core Pathway Predicts Reduced Cocaine Seeking

Next, we determined whether entrainment of low frequency oscillations (i.e., the change in coherence from before to following tACS or sham treatment) in the PrL-NAc core pathway predicted reduced cocaine seeking. Here, a difference score was calculated between pre-treatment (rest LFP on day 27 of abstinence) and post-treatment (rest LFP on day 30-32 of abstinence) coherence across the spectrum (0-100 Hz; 2 Hz/bin) for each animal. Fig. 3.8A shows the change

in rest PrL-NAc core coherence (post-treatment minus pre-treatment) for the cocaine-tACS versus cocaine-sham groups (0-100 Hz [58-62 Hz noise excluded]; 2 Hz/bin). Linear regression revealed that extinction presses were significantly predicted by change in peak theta (R^2 = 0.4835, p = 0.0120; Fig. 3.8B), beta (R^2 = 0.4807, p = 0.0124; Fig. 3.8C), and low gamma coherence (R^2 = 0.5028, p = 0.0098; Fig. 3.8D), but not by change in delta (R^2 = 0.2263, p = 0.1180) or high gamma (R^2 = 0.2196, p = 0.1244) coherence (delta and high gamma not shown). Significant predictive relationships between change in peak theta, beta, and low gamma coherence and extinction presses were also observed when tACS-treated rats were analyzed separately (not shown). These results may suggest that tACS protocols that optimally and selectively strengthen rest PrL-NAc oscillatory dynamics at theta, beta, or low gamma frequencies have the potential to reduce cocaine craving/seeking under certain circumstances. These possibilities are further explored in the **General Discussion**.

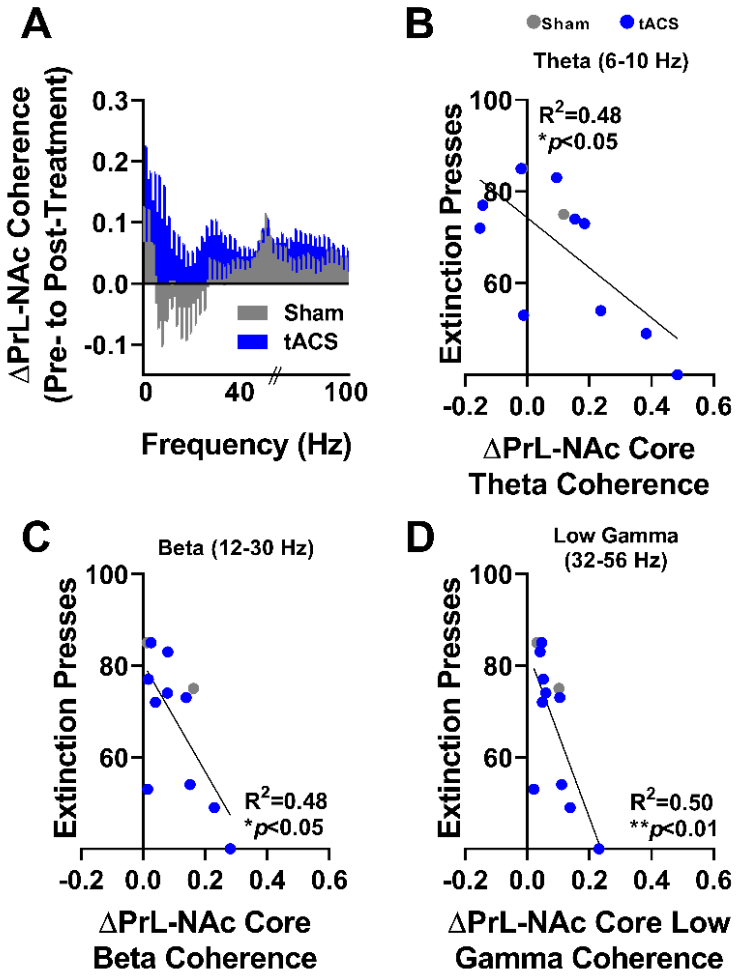

Figure 3.8 Entrainment of PrL-NAc core oscillatory dynamics by 16 Hz tACS predicts reduced cocaine seeking under extinction (Phase 2 of test). **A.** Change in rest PrL-NAc core coherence (post-treatment minus pre-treatment) across the frequency spectrum (0-100 Hz [58-62Hz excluded]; 2Hz/bin) for cocaine-tACS and cocaine-sham groups. Error bars represent mean + SEM. **B.** Linear regression of change in PrL-NAc theta coherence and extinction presses. **C.** Linear regression of change in PrL-NAc beta coherence and extinction presses. **D.** Linear regression of change in PrL-NAc low gamma coherence and extinction presses. *p<0.05; **p<0.01 for **B**-**D**. Gray/blue reflect sham/16 Hz tACS-treated rats respectively in **A**-**D**.

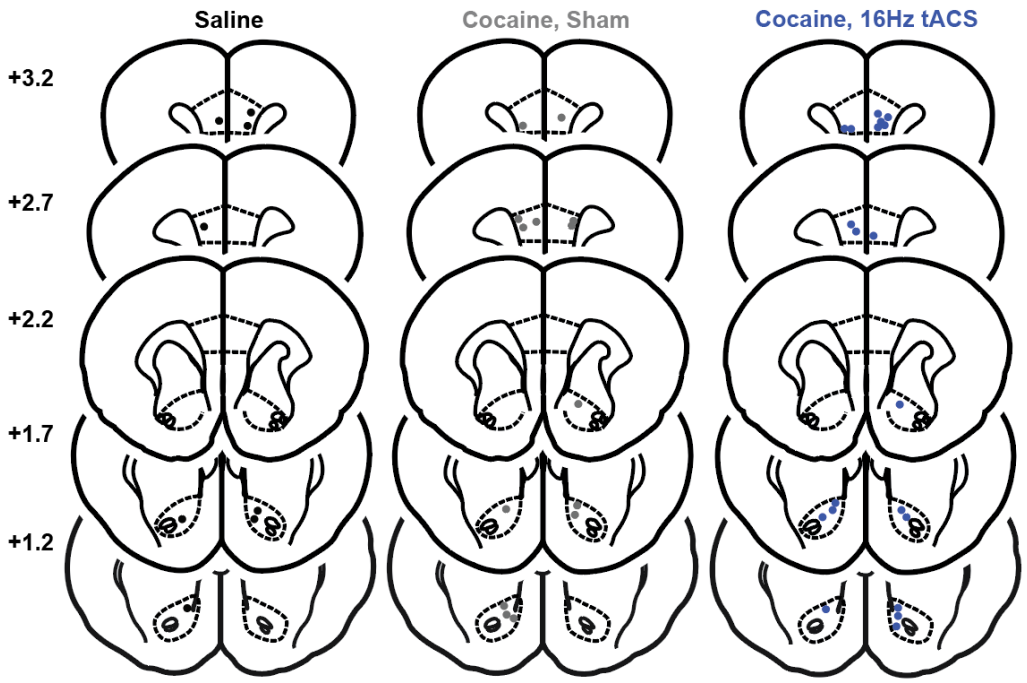

Figure 3.9 Histology. Electrode tip placements in the PrL and NAc core for saline (black), cocaine-sham (gray), and cocaine-tACS animals (blue).

Discussion

The current study examined the effects of 16 Hz tACS administered over the PrL on rest PrL-NAc coherence and cocaine seeking. As in Aim 1, we used *in vivo* electrophysiology to record simultaneous LFPs in the PrL and NAc core at rest following one-month abstinence from cocaine or saline self-administration. Here however, we applied 16 Hz tACS (or sham) to target a low frequency oscillation that was disrupted following one-month abstinence from cocaine and examined the effects of this treatment on oscillatory dynamics and cocaine seeking. PrL-NAc coherence was reduced at multiple frequency bands (including beta) following one-month abstinence from cocaine compared with saline controls, consistent with our previous work (West et al., 2021) and Aim 1. Given these findings and the putative role of beta oscillations in top-down cognitive control (Engel & Fries, 2010; Richter et al., 2017; Riddle et al., 2021; Zavala et al., 2018), we hypothesized that 16 Hz tACS would reduce cocaine seeking in this task. While 16 Hz tACS reversed cocaine-induced deficits in PrL-NAc beta coherence, it also appeared to increase delta and theta frequencies and did not significantly reduce cocaine-seeking behaviors compared to sham-treated rats. Similar to Aim 1, we observed positive correlations between PrL-NAc coherence following treatment and behavioral responses to the cocaine-paired CS (Phase 1 of test). Interestingly, however, higher PrL-NAc beta coherence and greater tACS-induced increases in this frequency predicted *less* cocaine seeking under extinction (Phase 2 of test). Collectively, these results indicate that tACS protocols which selectively and maximally entrain beta oscillations may reduce cocaine seeking following prolonged abstinence.

Consistent with Aim 1 and our recent work (West et al., 2021), we observed reduced PrL-NAc core coherence at rest across frequencies following one-month abstinence from cocaine versus saline self-administration. These results are in line with preclinical and clinical research

showing reduced baseline PFC and NAc activity and rsFC between the striatum and PFC following protracted cocaine abstinence (Beveridge et al., 2008; Hanlon et al., 2013; Hu et al., 2015; H. Lu et al., 2014; Porrino et al., 2007). In this Aim, however, our tACS stimulation frequency (beta, 16 Hz) was chosen in collaboration with Fröhlich and colleagues by closer examination of our electrophysiology data which revealed an endogenous beta peak at 16 Hz in rats that self-administered saline that was reduced in rats that self-administered cocaine (Fig. 3.2B). Further, beta oscillations are elevated during top-down cognitive control processes (Engel & Fries, 2010), and rats that have undergone incubation of cocaine craving are less sensitive to punishment-induced suppression of cocaine taking, i.e., show less top-down executive control over compulsive cocaine seeking (Gancarz-Kausch et al., 2014). As such, we hypothesized that elevating PrL-NAc beta coherence with 16 Hz tACS may reduce cocaine seeking following one-month abstinence.

We found that 16 Hz tACS (versus sham) significantly increased rest PrL-NAc beta coherence and reversed cocaine-induced deficits in circuit coherence at this frequency specifically (Fig. 3.3E), although it appeared to (non-significantly) increase PrL-NAc coherence at delta and theta frequencies as well (Fig. 3.3B-D). This frequency-specific effect of tACS on PrL-NAc coherence is promising and demonstrates target engagement in our rodent tACS model. However, future studies should determine whether target engagement of beta frequency tACS on PrL-NAc oscillatory dynamics is improved by rational design (Frohlich, 2014) of experimental parameters such as individualized frequency stimulation, number of stimulations per session (dose), and number of sessions (duration) to achieve optimal, durable, and frequency-specific modulation of PrL-NAc circuit dynamics (Ekhtiari et al., 2019; Herrmann, Rach, Neuling, & Struber, 2013). Although 16 Hz tACS selectively reversed cocaine-induced deficits in PrL-NAc coherence at the beta frequency, cocaine-seeking behaviors during the test were not significantly different between

the tACS and sham groups (Fig. 3.4). However, while behavioral responses to the cocaine-paired CS during Phase 1 (CS Probes) were slightly elevated, extinction lever presses during Phase 2 (Extinction) were slightly reduced for the tACS versus sham group. Critically, we show that reduced extinction lever presses were not correlated with number of approaches or time spent in the cocaine-associated quadrant, suggesting an independent effect of 16 Hz tACS on cocaine seeking under extinction (Phase 2 of test).

Similar to Aim 1, we observed positive correlations between behavioral responses (time and approach) to the cocaine-paired CS during Phase 1 of the test and PrL-NAc coherence at multiple frequencies following 16 Hz tACS or sham treatment (Figs. 3.5-6). As in Aim 1, the presence or absence of significant relationships between PrL-NAc coherence and distinct cocaine-seeking behaviors (e.g., time vs approach) may not imply a general role for distinct PFC-NAc oscillatory frequencies in cocaine seeking, since LFPs and behavior were measured after either tACS or sham and examined across treatment groups. Nevertheless, the robust positive correlation between PrL-NAc high gamma and approaches toward the cocaine-associated quadrant observed here (Fig. 3.6) are consistent with Aim 1 data showing 80 Hz tACS targeting high gamma frequencies increased approach behavior, and studies linking striatal gamma oscillations with reward anticipation in rats (van der Meer & Redish, 2009). Additionally, prefrontal delta oscillations have been implicated in cue-induced craving in individuals with CUD (Reid et al., 2003), and theta oscillations in the PFC and NAc have been linked with engaged attention in rats (Donnelly et al., 2014).

Interestingly, higher PrL-NAc beta and low gamma coherence following treatment, which did not correlate with behavioral responses to the cocaine-paired CS (Fig. 3.5-6), predicted less extinction presses during Phase 2 of the test (Fig. 3.7). To further probe the relationship between

rest PrL-NAc coherence following 16 Hz tACS or sham treatment and cue-evoked cocaine seeking under extinction (Phase 2 of test), we calculated the change in PrL-NAc coherence across frequencies from before to after treatment and examined its relationship with extinction presses. We found significant negative relationships between extinction presses and change in PrL-NAc coherence at theta, beta, and low gamma frequencies (Fig. 3.8), such that greater increases in PrL-NAc theta, beta, and low gamma coherence induced by tACS predicted less extinction presses. Given the significant *positive* correlation between PrL-NAc theta coherence and behavioral responses to the cocaine-paired CS here and in Aim 1, and the fact that change in PrL-NAc low gamma coherence from pre- to post-treatment was minimal (Fig. 3.8D x-axis), we propose that tACS protocols which *selectively and maximally entrain beta oscillations* may be sufficient to reduce cocaine seeking following prolonged abstinence. In Aim 3, we further examine PrL-NAc LFP signaling and its relationship to cocaine seeking following short or prolonged cocaine abstinence and provide support for a unique role of PrL-NAc beta in incubated cocaine seeking.

As described for Aim 1, tACS is less specific than other techniques (e.g., optogenetics) and may have additional effects on other downstream regions via alternative projections. Given the complex neural underpinnings of cocaine seeking and relapse (Dong et al., 2017; Kalivas, 2008; Koob & Volkow, 2010; McFarland & Kalivas, 2001; Wolf, 2016), future studies are needed to determine how oscillatory dynamics in these additional regions are linked to cocaine seeking following prolonged abstinence, and how tACS affects this behavior and its associated neurocircuitry. Further, although no significant group differences in cocaine-seeking behaviors during the test were observed for the tACS versus sham group (Fig. 3.4), our linear regression analyses indicate that the degree of target engagement of PrL-NAc core beta coherence by 16 Hz tACS significantly predicted reduced cocaine seeking (Fig. 3.8). Said another way, reduction of

putatively incubated cocaine craving was determined by the degree to which we effectively entrained coherent beta oscillatory activity in the PrL-NAc circuit with 16 Hz tACS. In addition to utilizing rational design of individualized experimental parameters (e.g., stimulation dose and duration) in future studies, it will also be important to examine the effects of beta frequency tACS on behavioral flexibility, decision making, motivated behavior for non-drug reinforcers, and other behavioral impairments associated with SUDs. Finally, our 16 Hz tACS stimulation frequency was chosen based on our electrophysiology data, the proposed role of beta oscillations in top-down executive control (Engel & Fries, 2010), and the link between incubation of craving and compulsive cocaine seeking in rats following prolonged cocaine abstinence. However, future studies should directly examine the interplay between cue-induced drug craving and diminished executive function in SUD patients or animal models to develop effective and multifaceted SUD treatment strategies.

CHAPTER 4

BIDRECTIONAL SHIFTS IN PrL-NAc CORE OSCILLATORY DYNAMICS DIFFERENTIALLY PREDICT INCUBATION OF COCAINE CRAVING

Introduction

In individuals with SUDs, sustained vulnerability to relapse even after prolonged periods of abstinence represents a major obstacle in treatment and recovery. This persistent risk for relapse is due in part to the ability of drug-related stimuli to elicit intense drug craving (Childress et al., 1988), which progressively intensifies throughout the initial stages of abstinence (Gawin & Kleber, 1986). In preclinical examinations of this phenomenon, termed incubation of craving, rats are typically trained to self-administer drug (e.g., cocaine) for ~2 weeks, then tested for cue-evoked cocaine seeking following varying durations of experimenter-imposed abstinence (Wolf, 2016). As the duration of forced abstinence increases, so does drug-seeking behavior (Grimm et al., 2001; L. Lu et al., 2004; Neisewander et al., 2000; Pickens et al., 2011; Wolf, 2016). Behavioral expression of incubated cocaine craving and underlying neurobiological changes are observed following prolonged abstinence from both short (2 h/day) and extended access (6 h/day) cocaine self-administration (Grimm et al., 2001; Hollander & Carelli, 2005, 2007; L. Lu et al., 2004; Ma et al., 2014; Pickens et al., 2011; West et al., 2014; Wolf, 2016). Preclinical studies have revealed cocaine-induced neuroadaptations in reward circuitry that persist following extended drug

abstinence and are associated with incubation of cocaine craving (Wolf, 2016). For example, extensive work from Wolf and colleagues has demonstrated synaptic changes in the NAc core following prolonged cocaine abstinence (Conrad et al., 2008; Loweth et al., 2014; Purgianto et al., 2013; Scheyer et al., 2014; Wolf, 2010, 2016), which are required for incubated cocaine-seeking behavior (Conrad et al., 2008; Loweth et al., 2014), and increase NAc responsiveness to incoming glutamate (Purgianto et al., 2013) such as that released in response to cocaine-associated stimuli, thereby increasing cue-evoked cocaine-seeking (Wolf, 2016). Similarly, our lab has shown neurons in the NAc core and PrL exhibit increased responsiveness to cocaine-associated stimuli following prolonged (one-month) versus short (one-day) cocaine abstinence concomitant with heightened cocaine seeking (Hollander & Carelli, 2005, 2007; West et al., 2014). In these studies, rats were trained to self-administer i.v. cocaine during 14 daily short access sessions, followed by either one day or one month of experimenter-imposed abstinence. Next, single unit activity in the NAc (Hollander & Carelli, 2005, 2007) and mPFC (West et al., 2014) were recorded during a cue-evoked cocaine seeking test. We reported significantly increased cocaine-seeking behaviors following prolonged versus short abstinence, as well as an increase in the proportion and strength of neurons in the NAc core (not shell) and PrL (not IL) that exhibited phasic responses (i.e., significant changes in firing rate within seconds of behavioral/task events) to cocaine-associated cues and cocaine seeking (Hollander & Carelli, 2005, 2007; West et al., 2014). These findings are notable given the ability of cocaine cues to activate similar regions of the human brain in individuals with CUD (Garavan et al., 2000; Goldstein & Volkow, 2002, 2011; Jasinska et al., 2014; Wolf, 2016).

Collectively, these studies reveal drug-induced changes in PFC-NAc circuitry which render the brain more responsive to cocaine-associated stimuli and promote relapse. However,

SUDs are also characterized by long-lasting cognitive impairments and reduced prefrontal cortical function, linked with compulsive drug taking and persistent vulnerability to relapse (Aharonovich et al., 2006; Garavan & Hester, 2007; Goldstein et al., 2004; Goldstein & Volkow, 2002, 2011; Hanlon et al., 2013; Jentsch & Taylor, 1999). Indeed, Chen and colleagues (2013) demonstrated a causal role of cocaine-induced PrL hypoactivity in 'compulsive' (i.e., resistant to punishment-induced suppression) cocaine seeking in rats (Chen et al., 2013). Additionally, our lab has demonstrated deficits in higher-order associative learning and cognitive flexibility linked with altered activity in PrL and NAc neurons (Saddoris & Carelli, 2014; West et al., 2021) and reduced PrL-NAc LFP coherence (West et al., 2021) following prolonged (one-month) abstinence from cocaine versus saline self-administration. Together these findings suggest that compulsive drug seeking and persistent vulnerability to relapse involve an interplay between executive control and cue reactivity processes mediated by PFC-NAc signaling, which may be dynamically modulated by abstinence from drug taking. Indeed, rats that have undergone incubation of craving are less sensitive to punishment-induced suppression of cocaine taking (Gancarz-Kausch et al., 2014).

Consistent with this hypothesis, recent work examining the efficacy of noninvasive brain stimulation for SUD treatment has revealed two leading approaches: *increasing* activity in the DLPFC (homologous to the PrL in rodents) to putatively fortify top-down executive control (Hanlon et al., 2018; Hone-Blanchet et al., 2015; Lupi et al., 2017), or *decreasing* corticolimbic activity in the presence of drug-associated cues to dampen cue reactivity (Hanlon et al., 2018). Theoretically, such approaches could be individually tailored based on various factors including the presence and severity of behavioral hallmarks of SUD including impulsivity, cognitive inflexibility, and reduced motivation for non-drug rewards (Bechara, 2005; Goldstein & Volkow, 2002, 2011; Hanlon et al., 2018; Koob & Volkow, 2010). Critically, our recent tACS study (West

et al., 2021) and results from Aims 1 and 2 also demonstrate the importance of context in rational design of tACS methods for modulating behavior and support the need to develop multifaceted and individualized approaches for treating SUDs. Indeed, while 80 Hz tACS reversed cocaine-induced deficits in PrL-NAc LFP coherence and restored behavioral flexibility (West et al., 2021), in Aim 1 this same stimulation frequency actually *increased* cocaine seeking.

Therefore, while results from Aim 2 were very promising, it became clear that we needed to better characterize relationships between PrL-NAc oscillatory dynamics and cocaine seeking in order to set the foundation for future studies that can more effectively use tACS to modulate maladaptive behaviors associated with SUD. More specifically, we were interested in examining shifts in *resting state versus event-related* (i.e., induced by cocaine-associated cues or cocaine seeking) PrL-NAc coherence following short or prolonged cocaine abstinence, and their relationships with cocaine-seeking behaviors. This goal was particularly important with respect to understanding the balance between *increased* activation of the PFC-NAc pathway in cue-elicited cocaine craving (Hollander & Carelli, 2007; Ma et al., 2014; West et al., 2014), and *reduced* baseline activity in this network during protracted abstinence (Hu et al., 2015; H. Lu et al., 2014). To our knowledge, this is the first study to examine the respective role of each of these opposing shifts in PFC-NAc dynamics in incubation of cocaine craving. We found context-dependent bidirectional shifts in PrL-NAc coherence which differentially predicted cocaine-seeking behaviors, and consistent with Aim 2 we report that the beta frequency may be uniquely important for heightened cocaine seeking following prolonged abstinence.

Methods

Animals

Adult male Sprague Dawley rats (Charles River, n=16 total), aged 90-120 days (300-350 grams) at the beginning of the study were used. 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 90% of their free-fed body weight by access to 20-25 g 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 the same chambers described in Aims 1 and 2. Briefly, 43 x 43 x 53 cm custom-made Plexiglas operant chambers housed within commercial sound-attenuating cubicles (Med. Associates, Inc., St Albans, VT, USA) were used. 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 houselight and speaker were centrally located on the opposite wall of the chamber. Masking 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 recoding 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 an intramuscular injection of a ketamine hydrochloride and xylazine hydrochloride (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, and microwire electrode arrays (8 microwires/array; 50 µm diameter; NB Labs, Denison TX, USA) 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 allowed 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, as described for Aims 1 and 2.

Figure 4.1A shows a schematic of cocaine self-administration, LFP recordings, and testing. One day prior to beginning 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. Simultaneous LFPs in the PrL and NAc core were recorded for 15 min while animals were not engaged in a behavioral task (pre-self-administration rest LFP; leftmost purple line in Fig. 4.1A). 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 cocaine self-administration sessions (2 h each), 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, as described for Aims 1 and 2. One day following the final cocaine self-administration session, rats were returned to the LFP recording chamber for a post-self-administration rest LFP recording (second purple line in Fig. 4.1A). Next, rats were divided into two groups: short (1-day) or prolonged (1-month/30-day) cocaine abstinence (n=8/group), with the short abstinence group undergoing testing procedures ≥2 h following the post-self-administration rest LFP recording (top-right black line in Fig. 4.1A). Rats in the prolonged abstinence group remained in their home cages for 30 days except during rest LFP recordings (15 min each) conducted on days 9, 20, and 27-28 of abstinence (these rest LFP data are not included in this dissertation but will be analyzed and reported in the future). Finally, rest LFP was recorded on day 29 of cocaine abstinence (bottom-right purple line in Fig. 4.1A) for the prolonged abstinence group, followed by the three-phase test session on day 30 (bottom-right black line in Fig. 4.1A).

Shown in Fig. 4.1B and described in Aims 1 and 2, the test session consisted of three phases: (1) CS probes, (2) Extinction, and (3) Cocaine Self-Administration (all rats underwent all phases of the test). In Phase 1 (CS Probes), the cue light above the lever was illuminated but the

lever was not extended. Ten pseudorandom presentations of the tone/houselight CS (5s each) were given over 15 min. The cue light above the lever remained on throughout Phase 1. As in Aims 1 and 2, behavior during Phase 1 was recorded and analyzed for time spent in the lever-associated quadrant of the chamber and approaches toward that quadrant during each 5s CS presentation. Next, Phase 2 (Extinction) was initiated wherein the lever previously associated with cocaine during self-administration was extended into the chamber. Here, lever depression resulted in termination of the cue light and presentation of the tone/houselight CS (20 s), but no drug delivery. After 2 h, Phase 3 (Cocaine Self-Administration) was initiated by administration of 0-3 priming infusions of cocaine (6 s; 0.33 mg/infusion) paired with the tone/houselight CS (20 s). Each subsequent lever press resulted in a cocaine infusion (6 s; 0.33 mg/infusion) paired with the CS (20 s) as in self-administration training sessions. The test session was completed 2 h after initiation of Phase 3. Event-related LFPs in the PrL and NAc core (e.g., LFP activity immediately before and following extinction press in Phase 2) were also recorded during Phases 1 and 2 of the test.

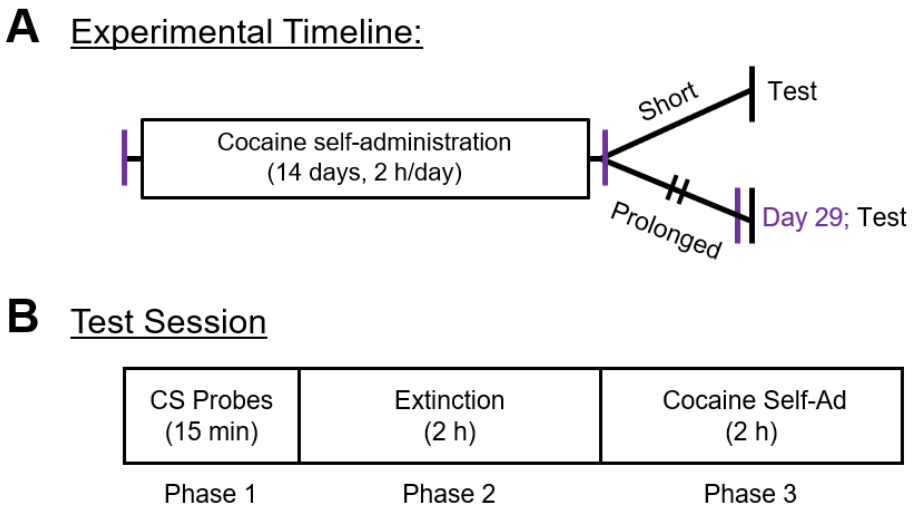

Figure 4.1 Experimental design. **A.** Timeline for cocaine self-administration, one-day (top right) or one-month (bottom right) abstinence, *rest* LFP recordings (purple lines), and testing. **B.**

Schematic of the three-phase test session including CS Probes, Extinction, and Cocaine-Self-Administration. *Event-related* LFPs were recorded during Phases 1 and 2 of the test.

Electrophysiology

The same electrophysiology procedures used in Aims 1 and 2 were used here and have been described in detail previously (Carelli, 2000; Hollander & Carelli, 2005). 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

Histological reconstruction of electrode positions was accomplished using established procedures described in Aims 1 and 2. Briefly, upon completion of the experiment, rats were deeply anesthetized with an i.p. injection of a ketamine hydrochloride and xylazine hydrochloride mixture (100 and 10 mg/kg, respectively). A 13.5-μA current was passed through each microwire electrode for 5s to mark the placement of electrode tips. Transcardial perfusions were then performed using 0.9% saline and 3% potassium ferrocyanide in 10% formalin, and brains were

removed. After post-fixing and freezing, 40-μm coronal brain sections were mounted. The addition of potassium ferrocyanide allowed for a blue reaction corresponding to the location of the electrode tip which was viewed under a 1X microscope lens. Placement of an electrode tip was determined by examining the relative position of observable reaction product to visual landmarks and anatomical organization of the PrL and NAc core represented in a stereotaxic atlas (Paxinos & Watson, 2005).

Data Analysis

Behavior: Analysis of behavioral data was accomplished using procedures described in Aims 1 and 2. Briefly, the number of lever presses during self-administration training were compared for the short and prolonged abstinence groups using a two-way repeated-measures ANOVA with session (1-14) and group as factors. Behavioral data during the three distinct phases of the test session were also analyzed. Specifically, in Phase 1 (CS Probes), the amount of time each rat spent in the quadrant where the cocaine-associated lever had previously been extended during training, and the number of approaches toward this quadrant during each 5s CS presentation were recorded. Approaches toward and time in the quadrant were defined by at least one-half of a body length into and orientation of the rat toward the quadrant, as described for Aims 1 and 2. Due to technical issues with video recording, behavioral data during Phase 1 of the test was not recorded for one animal in the short abstinence group. Unpaired *t*-tests were used to compare the short versus prolonged abstinence groups for all behavioral measures during the test, i.e., amount of time (seconds) in and number of approaches toward the cocaine lever-associated quadrant during Phase 1, the number of lever presses during Phase 2 (Extinction), and the number of cocaine infusions during Phase 3 (Cocaine Self-Administration).

Electrophysiology: Electrophysiology analyses were similar to those described for Aims 1 and 2. Briefly, raw electrical outputs from each region were low-pass filtered (≤200 Hz) from individual wires implanted within the PrL and NAc core. For coherence data (PrL-NAc), two-way ANOVAs were run with group (short vs prolonged abstinence) and frequency (0-100 Hz [54-68Hz excluded]; 2 Hz/bin) as factors. Because a majority of LFP recordings did not use a notch filter for 60 Hz noise, a larger frequency range was excluded in this Aim (54-68 Hz) than in Aims 1 and 2. Additionally, peak delta (0.5-4 Hz), theta (6-10 Hz), beta (12-30 Hz), low gamma (32-52 Hz), and average high gamma (70-100 Hz) coherence values were calculated for each animal. Unpaired *t*-tests were used to compare coherence at individual frequency bands for the short versus prolonged abstinence groups. To assess event-related network coherence, time interval filters were created around timestamps of behavioral/task events (e.g., CS onset during Phase 1, extinction lever press during Phase 2), and PrL-NAc core coherence values were calculated at each frequency (0-100 Hz; 2 Hz/bin) from raw LFP traces within those time intervals. Due to technical issues with electrophysiological recordings (e.g., noise artifact during lever press), the following were excluded from LFP analyses: n=1 for short, n=3 for prolonged abstinence groups during rest LFP; n=2 for short, n=1 for prolonged abstinence groups for event-related LFPs during Phase 1 of the test; n=3 for the short abstinence group for event-related LFPs during Phase 2 of test. To examine relationships between PrL-NAc network connectivity and cocaine-seeking behaviors, Pearson's correlations were calculated between behaviors during the test, and rest or event-related coherence values at each individual frequency (0-100 Hz [54-68Hz excluded]; 2Hz/bin) across short and prolonged abstinence groups. Finally, simple linear regression was used to examine relationships between coherence at individual frequency bands (e.g., peak delta coherence, average high gamma coherence) and behaviors during the test session for one-day and one-month abstinent rats.

LFP coherence values were exported from NeuroExplorer. *Post hoc* comparisons (e.g., Tukey's multiple comparisons, Sidak's multiple comparisons) were used where appropriate. All statistical analyses were completed using GraphPad Prism 8 (GraphPad Software, Inc., La Jolla, CA, USA).

Results

Behavior and PrL-NAc Coherence *Before* Abstinence

Rats that were later divided into one-day and one-month abstinence groups did not significantly differ in cocaine self-administration behavior during training or rest PrL-NAc coherence before or immediately following self-administration (prior to abstinence). A two-way repeated measures ANOVA of cocaine infusions during training, with session (1-14) and group (1-day versus 1-month abstinence) as factors revealed a main effect of session ($F_{3.339, 46.75} = 5.720$, $p = 0.0014$) but no main effect of group ($F_{1, 14} = 1.408$, $p = 0.2551$) nor a group x session interaction ($F_{13, 182} = 0.9346$, $p = 0.5185$), showing all rats acquired cocaine self-administration and cocaine intake did not differ between groups (not shown). Additionally, a two-way ANOVA of rest PrL-NAc coherence *before self-administration* (pre-self-administration rest LFP) with frequency (0-100 Hz; 54-68 Hz noise excluded; 2 Hz/bin) and group (1-day versus 1-month abstinence) as factors revealed a main effect of frequency ($F_{43, 352} = 1.756$, $p = 0.0034$), but no main effect of group ($F_{1, 352} = 3.372$, $p = 0.0672$), nor a group x frequency interaction ($F_{43, 352} = 0.1375$, $p > 0.9999$; not shown). Similarly, a two-way ANOVA of rest PrL-NAc coherence *immediately following self-administration* (post-self-administration rest LFP) with frequency and group as factors revealed a main effect of frequency ($F_{43, 440} = 11.86$, $p < 0.0001$), but no main effect of group ($F_{1, 440} = 1.483$, $p = 0.2240$), nor a group x frequency interaction ($F_{43, 440} = 0.8671$, $p = 0.7109$; not shown).

Rest PrL-NAc Core Coherence is Reduced Following One-Month vs One-Day Abstinence from Cocaine

Following cocaine self-administration training, all rats were returned to the LFP chamber for a post-self-administration rest LFP recording, then divided into two groups: one-day (short) and one-month (prolonged) abstinence (n=8/group). The short abstinence group was tested at least 2 h after the post-self-administration rest LFP recording, while the prolonged abstinence group remained in their home cages until an additional rest LFP recording on abstinence day 29, followed by the test on day 30. To assess the effects of prolonged versus short cocaine abstinence on rest PrL-NAc coherence, we compared LFPs during the post-self-administration recording for the short abstinence group with LFPs during the Day 29 recording for the prolonged abstinence group. Figure 4.2A shows the experimental timeline, with black and gray arrows indicating the time of rest LFP recordings for the short and prolonged abstinence groups, respectively. Shown in Figure 4.2B, rest PrL-NAc coherence was reduced following prolonged versus short cocaine abstinence. A two-way ANOVA of PrL-NAc coherence with group (short vs prolonged abstinence) and frequency (0-100 Hz; 54-68 Hz noise excluded; 2 Hz/bin) as factors revealed main effects of group ($F_{1, 440} = 12.38$, $p = 0.0005$) and frequency ($F_{43, 440} = 11.30$, $p < 0.0001$), but no group x frequency interaction ($F_{43, 440} = 0.9643$, $p = 0.5390$). To further examine the effects of prolonged versus short cocaine abstinence on rest PrL-NAc core signaling, we compared peak coherence values in the delta (0.5-4 Hz), theta (6-10 Hz), beta (12-30 Hz), and low gamma (32-52) ranges and average coherence values in the high gamma (70-100 Hz) range for the short versus prolonged abstinence groups (Fig. 4.2C-G). Unpaired *t*-tests revealed no significant differences in rest PrL-NAc core coherence for the prolonged versus short abstinence group at delta ($t_{10} = 0.1428$, $p = 0.8893$; Fig. 4.2C), low gamma ($t_{10} = 0.6145$, $p = 0.5526$; Fig. 4.2F), or high gamma ($t_{10} = 0.3814$, $p = 0.7109$; Fig. 4.2G) frequencies, but a trend toward a significant decrease in rest PrL-NAc core coherence

for the prolonged versus short abstinence group at theta (t_{10} = 2.065, p = 0.0658; Fig. 4.2D) and a significant decrease in rest PrL-NAc coherence for the prolonged versus short abstinence group at the beta frequency (t_{10} = 2.302, p = 0.0441; Fig. 4.2E).

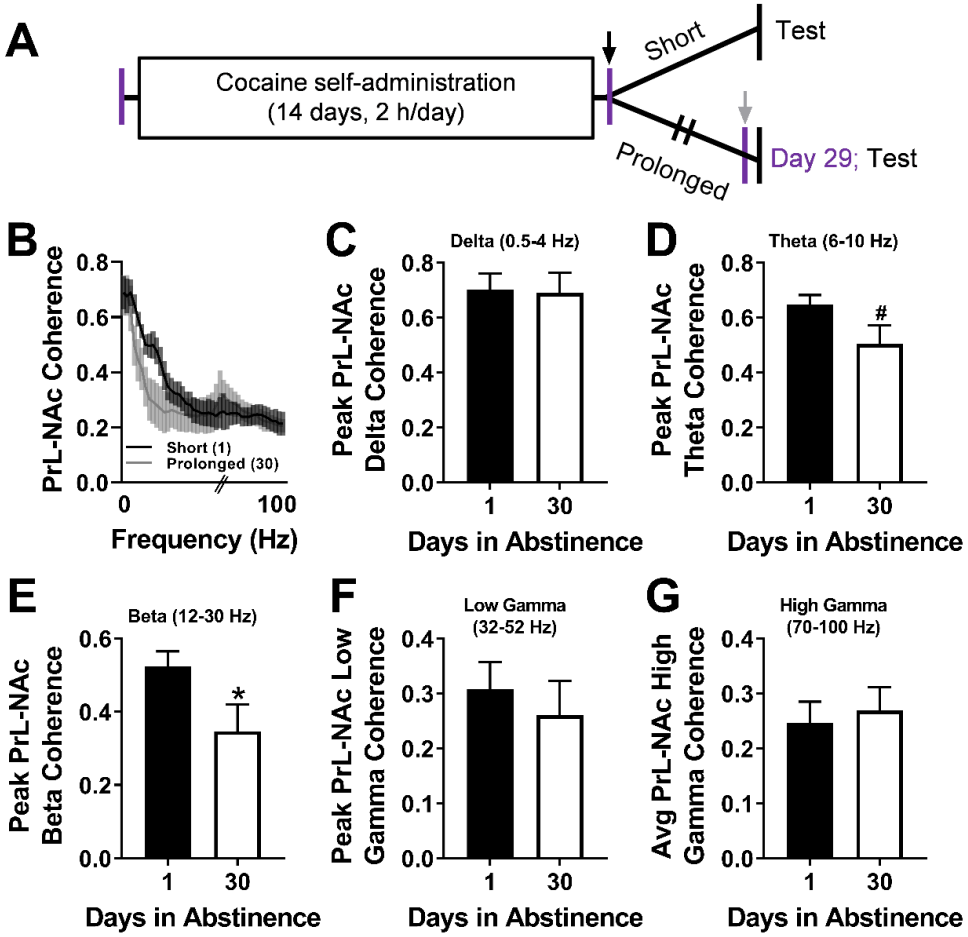

Figure 4.2 Rest PrL-NAc core coherence following short versus prolonged abstinence from cocaine. **A.** Experimental timeline with black and gray arrows indicating timepoint of rest LFP recordings for the short and prolonged abstinence groups, respectively. **B.** PrL-NAc core coherence across the frequency spectrum (0-100 Hz [54-68 Hz excluded]; 2 Hz/bin), showing reduced PrL-NAc coherence for the prolonged vs short abstinence group at lower frequencies. Error bars represent mean ± SEM. **C.** Peak PrL-NAc core coherence value in the delta range for the short vs prolonged abstinence group. **D.** Peak PrL-NAc core coherence value in the theta range. $^{\#}p<0.10$ for short vs prolonged abstinence. **E.** Peak PrL-NAc core coherence value in the beta range. $^{*}p<0.05$ for short vs prolonged abstinence. **F.** Peak PrL-NAc core coherence value in the

low gamma range. **G.** Average PrL-NAc core coherence value in the high gamma range. Error bars represent mean + SEM for **C**-**G**.

Cocaine-Seeking Behaviors are Increased Following Prolonged Cocaine Abstinence

Following cocaine self-administration, short or prolonged abstinence, and rest LFP recordings, rats underwent a three-phase test session as in Aims 1 and 2. Figure 4.3A shows a schematic of the test session consisting of CS Probes (Phase 1), Extinction (Phase 2), and Cocaine Self-Administration (Phase 3). During Phase 1 of the test, there was a significant increase in time spent ($t_{13} = 3.345$, $p = 0.0053$) and approaches toward ($t_{13} = 2.453$, $p = 0.0290$) the cocaine lever-associated quadrant of the chamber for the prolonged versus short abstinence group (Fig. 4.3B-C). Similarly, during Phase 2 of the test, there was a significant increase in number of extinction presses for the prolonged versus short abstinence group ($t_{14} = 3.455$, $p = 0.0039$; Fig. 4.3D). There was no significant difference between the short and prolonged abstinence groups in number of cocaine infusions earned during Phase 3 of the test ($t_{14} = 0.2343$, $p = 0.8181$; Fig. 4.3E). These results are consistent with prior work from our lab demonstrating that prolonged abstinence from cocaine leads to increased cue-evoked cocaine seeking (but not self-administration), i.e., incubation of cocaine craving (Hollander & Carelli, 2007; West et al., 2014).

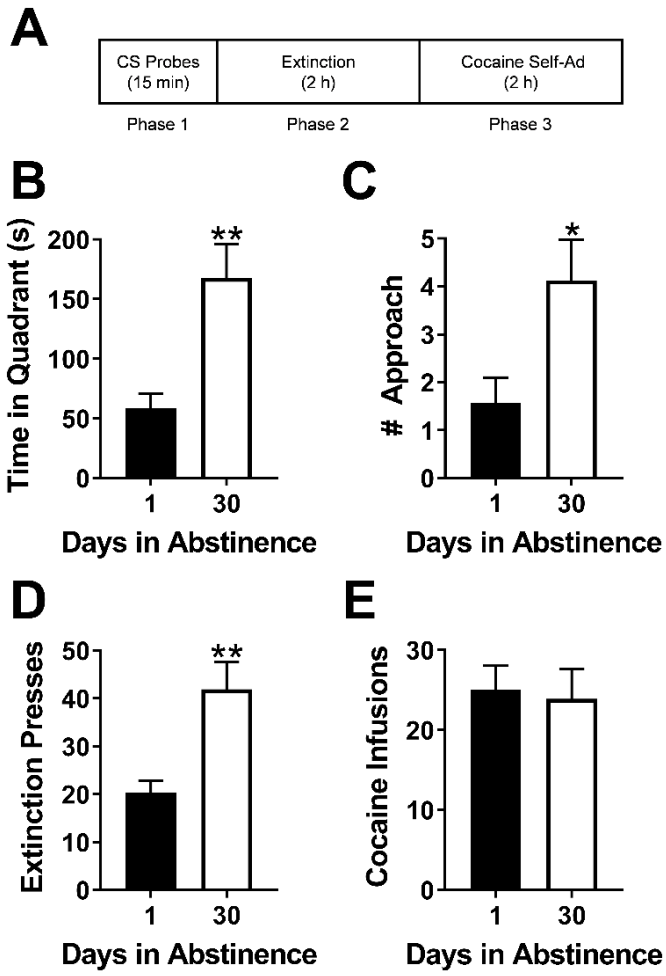

Figure 4.3 Effects of prolonged versus short abstinence on cocaine-seeking behaviors during the test. **A.** Schematic of the three-phase test session. **B.** Time spent (seconds) in the quadrant where the cocaine-associated lever was previously extended (Phase 1 of the test). **C.** Approaches toward the quadrant during Phase 1 of the test. **D.** Extinction presses during Phase 2 of the test. **E.** Cocaine infusions earned during Phase 3 of the test. *p<0.05; **p<0.01; error bars represent mean + SEM in **B-E.**

Rest PrL-NAc Core Coherence Predicts Distinct Cocaine-Seeking Behaviors During the Test

The above findings are consistent with a large body of literature in humans with SUDs and animal models showing increased cue-evoked cocaine craving and seeking, and PFC-NAc resting state hypoactivity, following extended periods (≥1 month) of abstinence from cocaine (Beveridge

et al., 2008; Hanlon et al., 2013; H. Lu et al., 2014; Wolf, 2016). Given these results, we next examined relationships between rest PrL-NAc core coherence following short or prolonged cocaine abstinence and drug-seeking behaviors during the test. Predictive relationships between rest PrL-NAc core coherence and cocaine-seeking behaviors may suggest that cocaine abstinence-induced dampening of PrL-NAc core activity at rest is related to incubated cocaine seeking. Here, Pearson's correlations were calculated between distinct cocaine-seeking behaviors during the test and rest PrL-NAc coherence values at each individual frequency (0-100 Hz; 54-68 Hz excluded; 2 Hz/bin) across one-day and one-month abstinent rats. Simple linear regression was also used to examine predictive relationships between coherence at individual frequency bands and behaviors during the test for the short and prolonged abstinence groups.

Figure 4.4 shows the relationship between rest PrL-NAc core coherence following short or prolonged abstinence and *time spent in the quadrant* during Phase 1 of the test. Fig. 4.4A shows the experimental timeline with black and gray arrows indicating timepoint of rest LFP recording for the short and prolonged abstinence groups respectively, and Figure 4.4B shows a schematic of the test session, with an arrow indicating the phase of the test in which behavior was measured (Phase 1, CS Probes). Shown in Fig. 4.4C, Pearson's correlations revealed that time spent in the quadrant was significantly ($R^2 \geq 0.4030$, $p < 0.05$) or near-significantly ($R^2 \geq 0.3210$, $p < 0.10$) *negatively* correlated with rest PrL-NAc core coherence at the following frequencies: 8-10 (theta) and 12 (beta) Hz ($p < 0.05$; light blue in Fig. 4.4C); 14-18 Hz (beta; $p < 0.10$; royal blue in Fig. 4.4C). When these correlations were calculated for the short and prolonged abstinence groups separately, negative (nonsignificant) relationships between time in the quadrant and PrL-NAc theta (8-10 Hz) coherence were present in both groups, but negative (nonsignificant) relationships between time in the quadrant and PrL-NAc beta (12-18 Hz) coherence were only present in (i.e.,

unique to) the prolonged abstinence group (not shown). Linear regression revealed that time spent in the quadrant was significantly predicted by rest PrL-NAc core coherence in the beta frequency band (R^2 = 0.4079, p = 0.0344; Fig. 4.4F). Time spent in the quadrant was not significantly predicted by rest PrL-NAc core peak delta (R^2 = 0.1347, p = 0.2668), theta (R^2 = 0.2422, p = 0.1241), low gamma (R^2 = 0.04582, p = 0.5274), or average high gamma (R^2 = 0.003457, p = 0.8637) coherence. These findings suggest that reduced rest PrL-NAc theta and beta coherence, which is observed following prolonged versus short cocaine abstinence, may predict increased behavioral responsiveness of one form (time spent in the quadrant, perhaps reflecting stimulus processing or motor behavior (Courtemanche, Fujii, & Graybiel, 2003; Jarovi, Volle, Yu, Guan, & Takehara-Nishiuchi, 2018; Paz, Bauer, & Pare, 2008)) to the cocaine-paired CS.

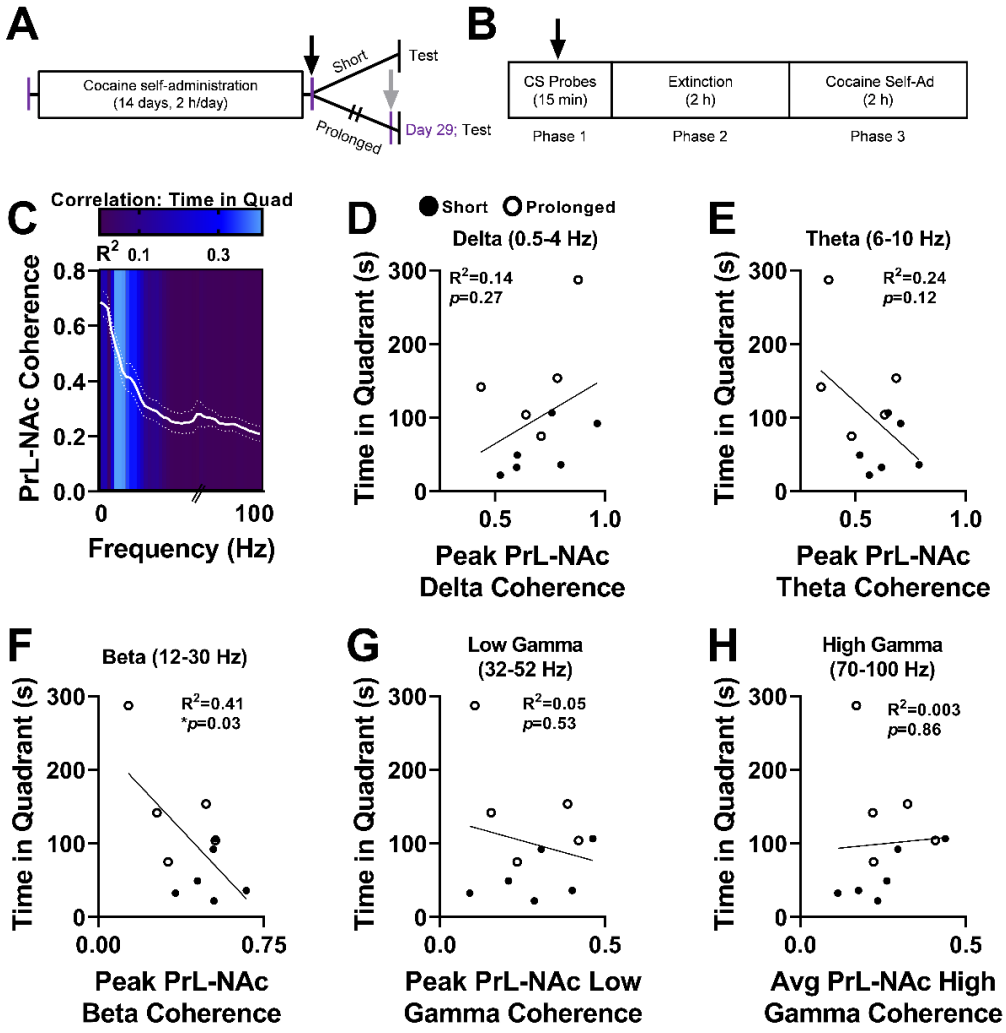

Figure 4.4 Relationship between rest PrL-NAc core coherence following short or prolonged cocaine abstinence and time spent in the quadrant during Phase 1 of the test. **A.** Experimental timeline with black and gray arrows indicating time point of rest LFP recording for the short and prolonged abstinence groups, respectively. **B.** Schematic of the three-phase test session with arrow indicating phase of test in which behavior was measured (Phase 1, CS Probes). **C.** PrL-NAc coherence across frequencies (0-100 Hz; 54-68 Hz excluded; 2Hz/bin) following short or prolonged cocaine abstinence (white line). Error bars (dotted white line) represent mean ± SEM. Coherence values at each frequency were correlated with individual rats' time spent in the quadrant (n=11) and the R^2 values for each of these correlations are represented in the color overlay (R^2 values ≥ 0.40 are significant, $p < 0.05$, light blue in the overlay). **D.** Linear regression of peak PrL-NAc core delta coherence and time in quadrant. **E.** Linear regression of peak theta coherence and time in quadrant. **F.** Linear regression of peak beta coherence and time in quadrant. *p<0.05. **G.** Linear regression of peak low gamma coherence and time in quadrant. **H.** Linear regression of average high gamma coherence and time in quadrant. Closed and open circles reflect one-day and one-month abstinent rats, respectively, in **D**-**H**.

Figure 4.5 shows the relationship between rest PrL-NAc core coherence following short or prolonged abstinence and *number of approaches toward the quadrant* during Phase 1 of the test. Fig. 4.5A shows the experimental timeline with black and gray arrows indicating timepoint of rest LFP recording for the short and prolonged abstinence groups respectively, and Figure 4.5B shows a schematic of the test session, with an arrow indicating the phase of the test in which behavior was measured (Phase 1, CS Probes). Shown in Fig. 4.5C, Pearson's correlations revealed that the number approaches toward the quadrant were significantly *positively* correlated with rest PrL-NAc core coherence at the following frequencies ($R^2 \geq 0.4370$, $p < 0.05$): 0-4 Hz (highlighted in red in Fig. 4.5C). When these correlations were calculated for the short and prolonged abstinence groups separately, significant positive correlations between PrL-NAc delta (0-4 Hz) coherence and approach behavior were present in the prolonged ($R^2 \geq 0.6590$, $p < 0.05$ for each frequency [0-4 Hz; 2 Hz/bin]), but not short abstinence group. Linear regression revealed that the number of approaches toward the quadrant were significantly predicted by rest PrL-NAc core coherence in the delta frequency range ($R^2 = 0.4315$, $p = 0.0281$; Fig. 4.5D). Shown in Figure 4.5E-H, approaches toward the quadrant were not significantly predicted by rest PrL-NAc core peak theta ($R^2 = 0.009753$, $p = 0.7727$), beta ($R^2 = 0.1240$, $p = 0.2883$), low gamma ($R^2 = 0.008163$, $p = 0.7916$), or average high gamma coherence ($R^2 = 0.08122$, $p = 0.3956$). These results suggest that higher rest PrL-NAc core coherence in the delta range is associated with increased behavioral responsiveness (approach behavior, perhaps reflecting reward anticipation (Fryer et al., 2021) or cue-induced craving (Reid et al., 2003) to the cocaine-paired CS, similar to findings from Aims 1 and 2.

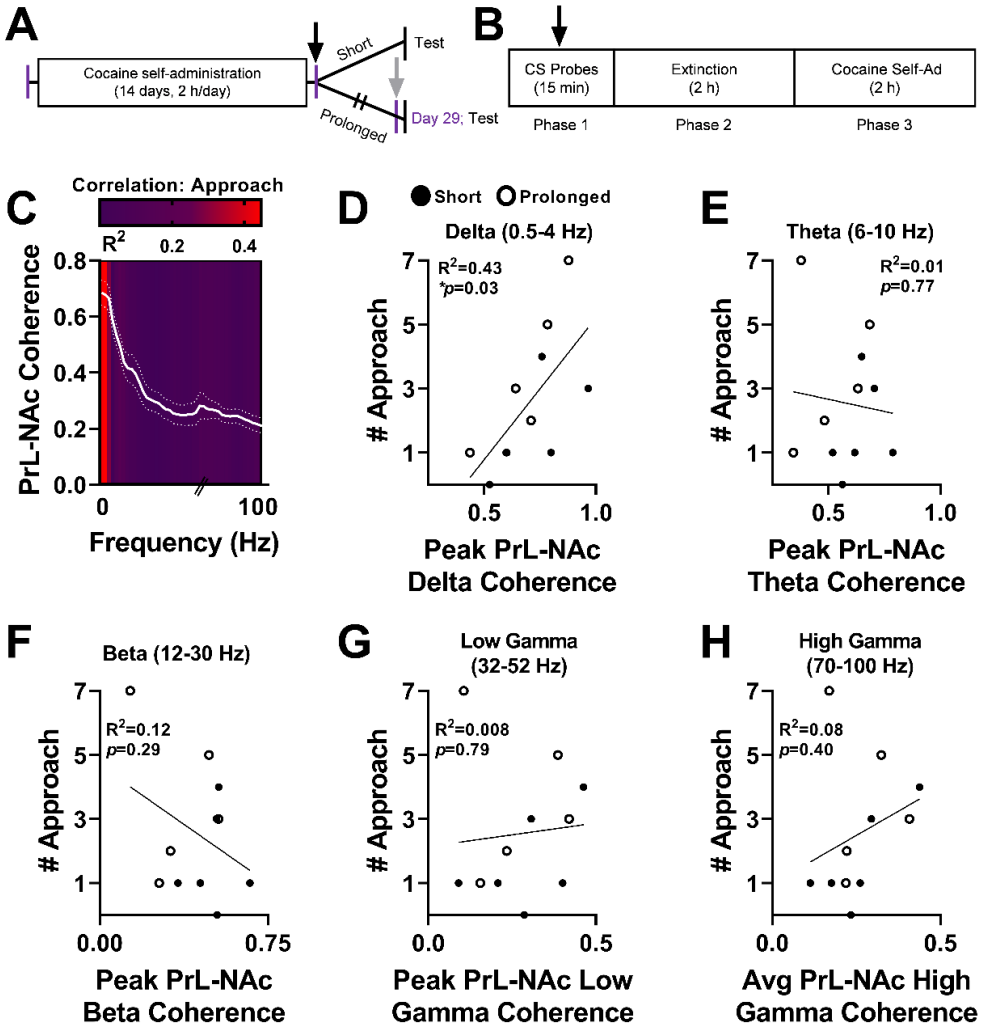

Figure 4.5 Relationship between rest PrL-NAc core coherence following short or prolonged cocaine abstinence and approaches toward the quadrant during Phase 1 of the test. **A.** Experimental timeline with black and gray arrows indicating time point of rest LFP recording for the short and prolonged abstinence groups, respectively. **B.** Schematic of the three-phase test session with arrow indicating phase of test in which behavior was measured (Phase 1, CS Probes). **C.** PrL-NAc coherence across frequencies (0-100 Hz; 54-68 Hz excluded; 2Hz/bin) following short or prolonged cocaine abstinence (white line). Error bars (dotted white lines) represent mean ± SEM. Coherence values at each frequency were correlated with individual rats' number of approaches (n=11) and the R^2 values for each of these correlations are represented in the color overlay (R^2 values ≥ 0.43 are significant, $p < 0.05$, red in the overlay). **D.** Linear regression of peak PrL-NAc core delta coherence and number of approach behaviors. *p<0.05. **E.** Linear regression of peak theta coherence and approach. **F.** Linear regression of peak beta coherence and approach. **G.** Linear regression of peak low gamma coherence and approach. **H.** Linear regression of average high gamma coherence and approach. Closed and open circles reflect one-day and one-month abstinent rats, respectively, in **D**-**H**.

Figure 4.6 shows the relationship between rest PrL-NAc core coherence following short or prolonged abstinence and extinction presses during Phase 2 of the test. Fig. 4.6A shows the experimental timeline with black and gray arrows indicating timepoint of rest LFP recording for the short and prolonged abstinence groups respectively, and Figure 4.6B shows a schematic of the test session, with an arrow indicating the phase of the test in which behavior was measured (Phase 2, Extinction). Shown in Fig. 4.6C, Pearson's correlations revealed that the number of extinction presses during Phase 2 were significantly ($R^2 \geq 0.4245$, $p < 0.05$) or near-significantly ($R^2 \geq 0.3010$, $p < 0.10$) *negatively* correlated with rest PrL-NAc core coherence at the following frequencies: 6 Hz (theta; $p < 0.05$; light blue in Fig. 4.6C); 8-10 (theta) Hz and 14-18 (beta) Hz ($p < 0.10$; royal blue in Fig. 4.6C). When these correlations were calculated for the short and prolonged abstinence groups separately, negative (nonsignificant) relationships between extinction presses during Phase 2 and rest PrL-NAc theta (6-10 Hz) coherence were present in both groups, but negative (nonsignificant) relationships between extinction presses and PrL-NAc beta (14-18 Hz) coherence were only present in (i.e., unique to) the prolonged abstinence group (not shown). Linear regression revealed that extinction presses were significantly predicted by rest PrL-NAc core coherence in the theta frequency band ($R^2 = 0.3629$, $p = 0.0498$; Fig. 4.4E). Extinction presses during Phase 2 were not significantly predicted by rest PrL-NAc core peak delta ($R^2 = 0.02922$, $p = 0.5953$), beta ($R^2 = 0.2259$, $p = 0.1396$), low gamma ($R^2 = 0.0365$, $p = 0.5736$), or average high gamma ($R^2 = 0.000601$, $p = 0.9430$) coherence. These findings suggest that reduced rest PrL-NAc theta and beta coherence, observed following prolonged cocaine abstinence (Fig. 4.2B), may be associated with incubated cue-evoked cocaine seeking (extinction presses).

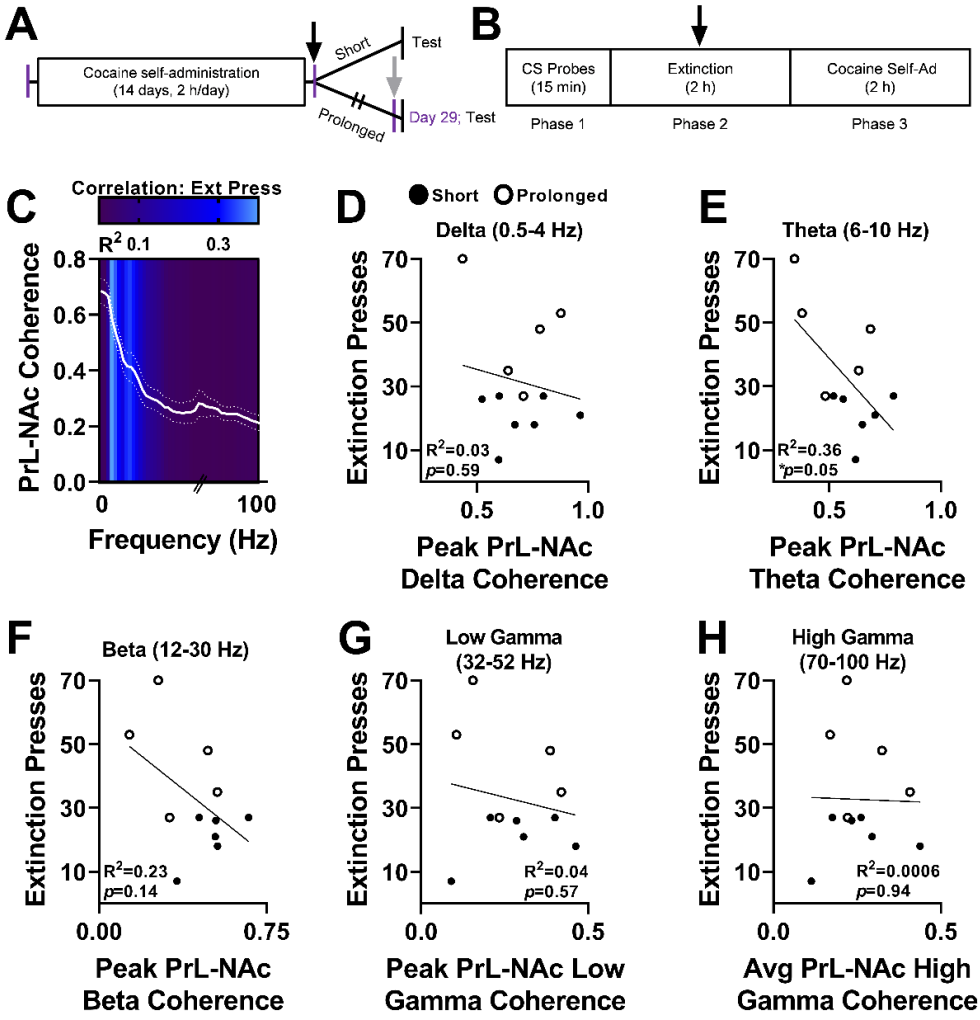

Figure 4.6 Relationship between rest PrL-NAc core coherence following short or prolonged cocaine abstinence and extinction presses during Phase 2 of the test. **A.** Experimental timeline with black and gray arrows indicating time point of rest LFP recording for the short and prolonged abstinence groups, respectively. **B.** Schematic of the three-phase test session with arrow indicating phase of test in which behavior was measured (Phase 2, Extinction). **C.** PrL-NAc coherence across frequencies (0-100 Hz; 54-68 Hz excluded; 2Hz/bin) following short or prolonged cocaine abstinence (white line). Error bars (dotted white lines) represent mean ± SEM. Coherence values at each frequency were correlated with individual rats' number of extinction presses (n=12) and the R^2 values for each of these correlations are represented in the color overlay (R^2 values ≥ 0.42 are significant, $p < 0.05$, light blue in the overlay). **D.** Linear regression of peak PrL-NAc core delta coherence and number of extinction presses. **E.** Linear regression of peak theta coherence and extinction presses. *p<0.05. **F.** Linear regression of peak beta coherence and extinction presses. **G.** Linear regression of peak low gamma coherence and extinction presses. **H.** Linear regression of high gamma coherence and extinction presses. Closed and open circles reflect one-day and one-month abstinent rats, respectively, in **D**-**H**.

There were no significant correlations between number of cocaine infusions earned during Phase 3 (Cocaine Self-Administration) of the test and rest PrL-NAc core coherence following short or prolonged abstinence at any individual frequency (not shown). Similarly, linear regression revealed that cocaine infusions earned during Phase 3 were not predicted by rest PrL-NAc core peak delta ($R^2 = 0.1430$, $p = 0.2255$), theta ($R^2 = 0.00554$, $p = 0.8182$), beta ($R^2 = 0.03601$, $p = 0.5547$), low gamma ($R^2 = 0.0264$, $p = 0.6139$), or average high gamma ($R^2 = 0.02317$, $p = 0.6367$) coherence (not shown).

Stronger CS and Extinction Press-Induced PrL-NAc Coherence Predicts Increased Cocaine-Seeking Behaviors (Phases 1, 2 of Test)

In addition to rest LFP recordings before/after cocaine self-administration and short or prolonged abstinence, *event-related LFPs* were also recorded during the test to assess activity in the PrL-NAc core circuit induced by cocaine-associated cues and drug seeking. During Phase 1 of the test, CS Probes (tone/houselight stimulus previously paired with cocaine infusion during self-administration) were randomly presented. Time interval filters were created around timestamps of the CS probes (before CS onset: -5s – 0s, 'pre-CS'; during CS presentation: 0s – 5s, 'CS-on'), and PrL-NAc core coherence values were calculated at each frequency (0-100 Hz; 2 Hz/bin) from raw LFP traces within those time intervals. Figure 4.7A shows the schematic of the test session with an arrow indicating the phase of the test in which behavior and event-related LFP were measured (Phase 1, CS Probes). Figure 4.7B-C show PrL-NAc core coherence across the spectrum (0-100 Hz; 58-64 noise removed; 2 Hz/bin) during Pre-CS (Fig. 4.7B) and CS-On (Fig. 4.7C) periods. A two-way ANOVA of PrL-NAc coherence during the Pre-CS interval revealed a main effect of group ($F_{1, 528} = 33.16$, $p < 0.0001$) but no main effect of frequency ($F_{47, 528} = 1.166$, $p = 0.2154$) nor interaction ($F_{47, 528} = 0.3285$, $p > 0.9999$). During the CS-On interval, a two-way ANOVA of PrL-

NAc core coherence revealed a main effect of group ($F_{1, 528} = 86.19$, $p < 0.0001$) and frequency ($F_{47, 528} = 2.293$; $p < 0.0001$) but no interaction ($F_{47, 528} = 0.1616$, $p > 0.9999$). However, the CS-On period appeared to increase PrL-NAc core coherence at delta for the prolonged abstinence group (gray line at 0.5-4Hz in Fig. 4.7C vs Fig. 4.7B) and reduce PrL-NAc core coherence at low gamma for the short abstinence group (black line in Fig. 4.7C vs Fig. 4.7B, and black vs gray line in Fig. 4.7C at ~40 Hz).

Given these observations, we next examined whether PrL-NAc core delta and low gamma coherence during the CS-On period predicted cocaine-seeking behaviors during Phase 1 of the test. Here, Pearson's correlations were calculated between cocaine-seeking behaviors during Phase 1 (time spent and approaches toward the quadrant) and PrL-NAc coherence during CS-On at each individual frequency (0-100 Hz; 58-64 Hz excluded; 2 Hz/bin) across one-day and one-month abstinent rats (Fig. 4.7D-E). *Time spent in the quadrant* was significantly positively correlated with PrL-NAc core coherence during CS-On at the following frequencies ($R^2 \geq 0.3834$, $p < 0.05$, highlighted in red in Fig. 4.7D): 0-2 Hz (delta), 40 Hz (low gamma). *Approaches* toward the quadrant were significantly positively correlated with PrL-NAc core coherence during CS-On at delta frequencies (0-2 Hz; $R^2 \geq 0.4710$, $p < 0.05$, highlighted in red in Fig. 4.7E).

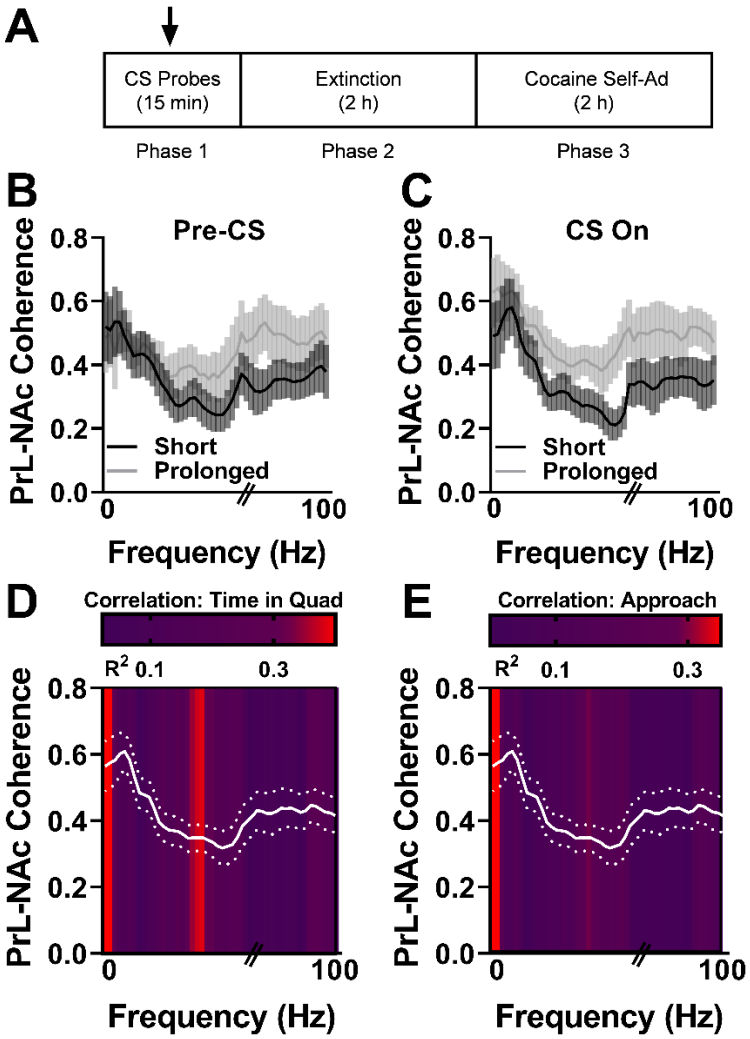

Figure 4.7 Relationships between *event-related* PrL-NAc coherence following short or prolonged cocaine abstinence and cocaine-seeking behaviors during Phase 1 of the test. **A.** Schematic of the test session with arrow indicating test phase in which behavior and event-related LFP were measured. **B.** PrL-NAc core coherence during the 'Pre-CS' time interval (-5 – 0s relative to CS onset) across the spectrum (0-100 Hz; 58-64 Hz excluded; 2 Hz/bin) for the short and prolonged abstinence groups. **C.** PrL-NAc core coherence during the 'CS-On' time interval (0 – 5s relative to CS onset) across the spectrum (0-100 Hz; 58-64 Hz excluded; 2 Hz/bin) for the short and prolonged abstinence groups. **D-E.** PrL-NAc coherence *during CS-On* across frequencies (0-100 Hz; 58-64 Hz excluded; 2Hz/bin) following short or prolonged cocaine abstinence (white line). Coherence values at each frequency were correlated with individual rats' time spent (n=12, **D**) and approaches toward (n=12, **E**) the quadrant and the R^2 values for each of these correlations are represented in the color overlays (R^2 values ≥ 0.38 are significant, $p < 0.05$, red in the overlays). Error bars represent mean ± SEM in **B-E**.

During Phase 2 of the test (Extinction), time interval filters were created around timestamps of extinction presses (-2.5 – 2.5 s relative to lever press), and PrL-NAc core coherence values were calculated at each frequency (0-100 Hz; 2 Hz/bin) from raw LFP traces within those time intervals. Figure 4.8A shows the schematic of the test session with an arrow indicating the phase of the test in which behavior and event-related LFP were measured (Phase 2, Extinction). Figure 4.8B shows extinction press-induced PrL-NAc core coherence across the spectrum (0-100 Hz; 58-64 noise removed; 2 Hz/bin). A two-way ANOVA of PrL-NAc coherence during extinction press revealed a main effect of group ($F_{1, 528} = 105.2$, $p < 0.0001$) and frequency ($F_{47, 528} = 2.660$, $p < 0.0001$), but no group x frequency interaction ($F_{47, 528} = 0.8538$, $p = 0.7444$). Shown in Fig. 4.8C, Pearson's correlations revealed that the number of extinction presses during Phase 2 of the test were significantly positively correlated with extinction press-induced PrL-NAc core coherence at low gamma frequencies ($R^2 \geq 0.3574$, $p < 0.05$): 32-48 Hz (highlighted in red in Fig. 4.8C).

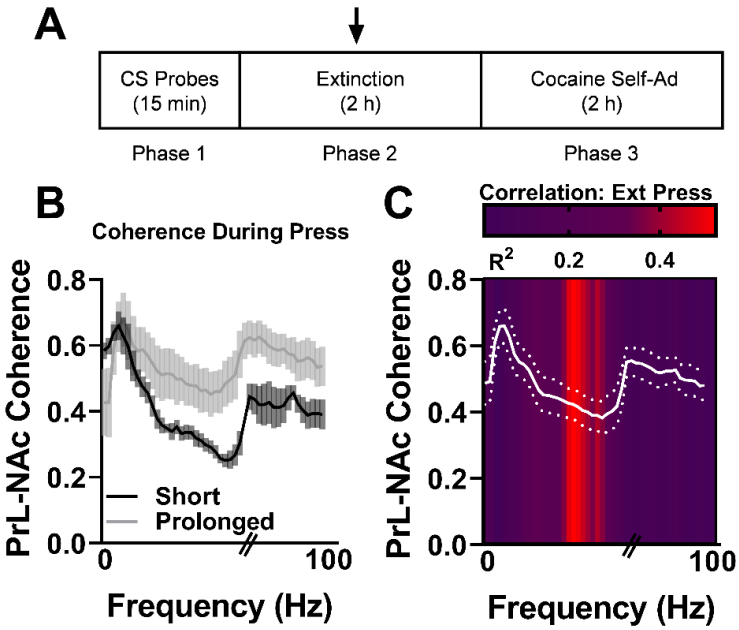

Figure 4.8 Relationships between *event-related* PrL-NAc coherence following short or prolonged cocaine abstinence and extinction presses during Phase 2 of the test. **A.** Schematic of the test

session with arrow indicating test phase in which behavior and event-related LFP were measured. **B.** PrL-NAc core coherence during the extinction press time interval (-2.5 – 2.5s relative to lever press) across the spectrum (0-100 Hz; 58-64 Hz excluded; 2 Hz/bin) for the short and prolonged abstinence groups. **C.** PrL-NAc coherence during extinction press across frequencies (0-100 Hz; 58-64 Hz excluded; 2Hz/bin) following short or prolonged cocaine abstinence (white line). Coherence values at each frequency were correlated with individual rats' extinction presses (n=13) and the R^2 values for each of these correlations are represented in the color overlay (R^2 values $\geq$ 0.36 are significant, $p < 0.05$, red in the overlay). Error bars represent mean ± SEM in **B-C**.

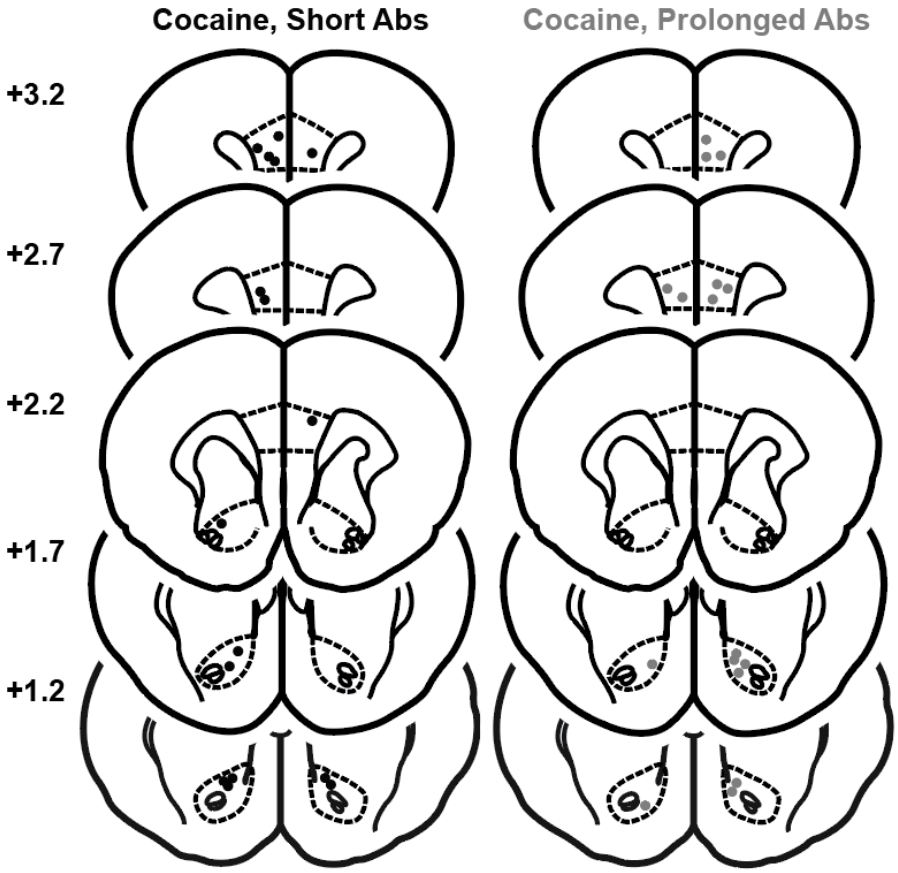

Figure 4.9 Histology. Electrode tip placements in the PrL and NAc core for rats in the short (gray) and prolonged (black) abstinence groups.

Discussion

The current study examined the effects of short (one-day) versus prolonged (one-month) experimenter-imposed cocaine abstinence on PrL-NAc coherence at rest and during discrete elements of our cocaine-seeking/taking test, as well as relationships between PrL-NAc coherence and cocaine seeking. We used *in vivo* electrophysiology to record resting state LFPs in the PrL and NAc core before and immediately following cocaine self-administration and short or prolonged abstinence. We then recorded event-related LFPs in this circuit during a test session consisting of presentations of the cocaine-paired CS (Phase 1), extinction (Phase 2), and resumption of cocaine self-administration (Phase 3). Rest PrL-NAc coherence was reduced in the beta frequency band following prolonged (versus short) cocaine abstinence, accompanied by increased cocaine-seeking behaviors. Additionally, reduced PrL-NAc theta and beta coherence predicted increased time spent in the cocaine-associated quadrant and extinction presses during the test, while delta was positively correlated with approach behavior. Finally, while rest PrL-NAc coherence was reduced following prolonged cocaine abstinence, event-related coherence in this circuit during CS probes and extinction pressing was increased and positively correlated with cocaine seeking at delta and low gamma frequencies. These data reveal bidirectional relationships between PrL-NAc oscillatory dynamics and cocaine seeking mediated by whether LFPs are measured at rest or induced by cocaine-associated cues or drug seeking. The implications of these findings are discussed below.

Consistent with our prior work (Hollander & Carelli, 2005, 2007; West et al., 2014), we found significantly increased time spent and approaches toward the cocaine-associated quadrant during Phase 1 of the test and extinction presses during Phase 2 of the test for the prolonged versus short abstinence group, but no group difference in cocaine self-administration behavior during Phase 3. These results are also consistent with numerous studies showing incubation of cue-elicited

cocaine craving and seeking (typically under extinction conditions) following protracted cocaine abstinence (Conrad et al., 2008; Dong et al., 2017; Lee et al., 2013; Loweth et al., 2014; Ma et al., 2014; Parvaz et al., 2016; Purgianto et al., 2013; Wolf, 2016). While our previous work revealed an increase in PrL and NAc core activation by cocaine-associated cues and cocaine-seeking behaviors (Cameron & Carelli, 2012; Hollander & Carelli, 2005, 2007; West et al., 2014), here, we observed a *reduction* in PrL-NAc LFP coherence *at rest* following prolonged versus short cocaine abstinence, particularly in the beta frequency band with a trend toward reduced theta. Reduced rest PrL-NAc core coherence following prolonged cocaine abstinence is in line with neuroimaging studies showing reduced rsFC between the PFC and striatum (Gu et al., 2010; Hu et al., 2015) in individuals with CUD compared to matched non-substance using controls, and reduced rsFC between the NAc and mPFC in rats following one-month abstinence from cocaine versus sucrose self-administration (H. Lu et al., 2014). Interestingly, while in Aims 1 and 2 we observed reduced rest PrL-NAc coherence across delta, beta, low and high gamma frequencies following *cocaine versus saline* self-administration, when we differentiated between *short versus prolonged cocaine abstinence* here, reduced PrL-NAc core coherence was significant only at the beta frequency. These findings suggest reduced rest PrL-NAc beta coherence may be a unique neural underpinning of incubation of cocaine craving.

We also examined relationships between rest PrL-NAc core coherence following short or prolonged abstinence and cocaine-seeking behaviors during the test. We found significant *negative* correlations between rest PrL-NAc theta and beta coherence with time spent in the cocaine-associated quadrant during Phase 1 of the test and extinction presses during Phase 2 of the test. Interestingly, negative relationships between cocaine seeking and PrL-NAc theta coherence were observed in both short and prolonged groups while the *beta* relationship was unique to the

prolonged abstinence group, although correlations were not significant when analyzed for the short and prolonged abstinence groups separately and therefore should be interpreted with caution. Nevertheless, considerations such as these (with larger sample size) may be important for future studies examining the effects of tACS on cocaine seeking following variable periods of abstinence, which represents a crucial goal for maintaining the clinical relevance of this work. We postulate that the significant negative correlations between rest PrL-NAc coherence at theta and beta frequencies and cocaine-seeking behaviors may be consistent with prominent theories suggesting a role of prefrontal theta and beta oscillations in top-down cognitive control (Cavanagh & Frank, 2014; Cooper et al., 2019; Engel & Fries, 2010; Riddle et al., 2021; Zavala et al., 2018). However, although prior work indicates rats that have undergone incubation of cocaine craving are less sensitive to punishment-induced suppression of cocaine seeking (Gancarz-Kausch et al., 2014), the test procedure used in the current study did not specifically recruit top-down executive control processes. As such, the relationships between diminished rest PrL-NAc theta and beta coherence and augmented cue-evoked cocaine seeking may reflect other oscillatory underpinnings of cocaine seeking which can be further examined in future studies. Interestingly, we also observed a *positive* relationship between rest PrL-NAc delta coherence and approach behavior which was driven by the prolonged abstinent group. These findings are consistent with data from Aims 1 and 2 and previous work implicating prefrontal delta oscillations in cue-induced cocaine craving in humans with CUD (Reid et al., 2003). Critically, although prior work has shown optogenetic stimulation in the delta frequency range (1 Hz) of the PrL-NAc core projection induced LTD and inhibited incubation of cocaine craving (Ma et al., 2014), we hypothesize that delta frequency tACS may augment cue-induced craving following prolonged abstinence from short access cocaine self-administration. This hypothesis can be tested in future studies.

While rest PrL-NAc coherence was reduced following prolonged versus short cocaine abstinence, we observed apparent increases in PrL-NAc coherence during cocaine cue presentation periods (Phase 1 of the test) and cocaine-seeking behaviors (extinction presses during Phase 2 of the test) for the prolonged abstinence group. These results are consistent with previous work from our lab showing increased phasic activity in the PrL and NAc core during presentation of cocaine-associated cues and cocaine seeking (Cameron & Carelli, 2012; Hollander & Carelli, 2005, 2007; West et al., 2014). Likewise these findings are consistent with work from Wolf and colleagues showing synaptic changes in the NAc core and PrL-NAc core projection following prolonged cocaine abstinence (Conrad et al., 2008; Loweth et al., 2014; Ma et al., 2014; Purgianto et al., 2013; Scheyer et al., 2014; Wolf, 2010, 2016), which are required for incubated cocaine-seeking behavior (Conrad et al., 2008; Loweth et al., 2014; Ma et al., 2014), and increase NAc responsiveness to incoming glutamate (Purgianto et al., 2013) such as that released from PFC regions in response to cocaine-associated stimuli (Wolf, 2016). When we examined relationships between event-related PrL-NAc coherence and cocaine-seeking behaviors, we found significant *positive* correlations between event-related PrL-NAc core coherence at delta and low gamma frequencies and cocaine-seeking behaviors during Phases 1 and 2 of the test. These findings seem to be consistent with previous work linking delta oscillations with cue-induced cocaine craving in humans with CUD (Reid et al., 2003), and other work indicating a role of NAc gamma oscillations in reward anticipation in rats (Donnelly et al., 2014; van der Meer & Redish, 2009). Interestingly, in Aim 2 we found *negative* correlations between PrL-NAc low gamma coherence *at rest* and extinction presses, but in this Aim we show *positive* correlations between *event-related* PrL-NAc coherence at this frequency and cocaine-seeking behaviors. These data support the need to

examine shifts in *rest and event-related* oscillatory dynamics during prolonged cocaine abstinence, how these may be modulated by tACS, and the resulting effect on cocaine seeking in future studies.

Critically, although reduced rsFC in cortico-striatal networks is reported in individuals with CUD (Gu et al., 2010; Hu et al., 2015), when neuroimaging was completed immediately after a cue-induced craving test (wherein cocaine-associated stimuli were shown), rsFC between the ventral striatum (which includes the NAc) and PFC was *increased* in currently abstaining individuals with CUD compared with matched controls (Wilcox et al., 2011). We believe our data showing reduced PrL-NAc coherence at rest but increased circuit coherence during presentation of cocaine-associated stimuli and cocaine seeking following prolonged versus short cocaine abstinence are in line with these findings. Furthermore, to our knowledge the current study is the first to demonstrate these context-dependent bidirectional shifts in PrL-NAc LFP signaling following prolonged cocaine abstinence and their relationship to cue-evoked cocaine seeking in rats. A remaining question is whether a shared oscillatory mechanism underlies these abstinence-related opposing shifts in PrL-NAc signaling. One possibility is cross-frequency interactions between lower frequency (e.g., beta) top-down oscillatory signals and higher frequency (e.g., gamma) bottom-up activity which can be influenced by experimental task demands (Buschman & Miller, 2007; Richter et al., 2017; von Stein & Sarnthein, 2000). Future work in collaboration with the Fröhlich lab will therefore examine cross-frequency interactions (e.g., phase-amplitude coupling) at rest and during cocaine seeking and their modulation by prolonged cocaine abstinence. These analyses are of particular interest as we move forward with tACS studies given recent work from the Fröhlich lab showing cross-frequency tACS targeting specific functionally relevant phase-amplitude coupling modulated performance in a cognitive control task (Riddle et al., 2021).

Finally, notable experimental differences between the current study and Aims 1 and 2, as well as limitations related to control groups and sample size preclude holistic interpretations about the PrL-NAc oscillatory dynamics underlying cue-elicited cocaine seeking following short or prolonged abstinence. Future studies should therefore employ a within-subject design to examine rest and cocaine cue-induced LFP signaling and its relationship to cocaine seeking before and following prolonged cocaine abstinence. This will allow us to better assess the role of distinct LFP frequency bands in incubation of cocaine craving and how tACS affects the neurocircuitry underlying this behavior.

CHAPTER 5

GENERAL DISCUSSION

SUD is a chronically relapsing disease characterized by compulsion to seek and take drug, reduced control over intake, and emergence of negative emotional states during periods of abstinence (Koob & Le Moal, 1997; Koob & Volkow, 2010). In individuals with CUD and animal models, prolonged abstinence from cocaine also leads to profound changes in PFC and NAc activity linked with cognitive impairments, cue-induced drug craving, and persistent vulnerability to relapse (Beveridge et al., 2008; Goldstein & Volkow, 2002, 2011; Hanlon et al., 2013; Wolf, 2016). Given these findings, an emerging strategy for treating SUD involves using noninvasive brain stimulation to normalize drug-induced aberrations in brain activity. While TMS and tDCS delivered to the PFC may reduce drug craving and promote sustained abstinence in individuals with SUD (Hanlon et al., 2018; Hone-Blanchet et al., 2015; Mahoney et al., 2020; Terraneo et al., 2016), we believe a stronger, mechanism-driven approach involves combining *in vivo* electrophysiology with tACS to target specific neural activity patterns that are disrupted following prolonged abstinence from cocaine. Remarkably, Daughters and colleagues (2020) recently demonstrated recruitment, retention, and administration feasibility of tACS in individuals with SUDs undergoing intensive outpatient treatment (87% retention rate), and beneficial effects of tACS on inhibitory control in these patients (Daughters et al., 2020). With the goal of performing more detailed parametric analyses of tACS effects on drug seeking and other behaviors associated

with SUD, our lab collaborated with Dr. Flavio Fröhlich to develop a rat model of tACS. We recently published data showing the efficacy of 80 Hz tACS administered over the PrL to reverse cocaine-induced deficits in PrL-NAc signaling and restore behavioral flexibility (West et al., 2021). In this dissertation, three specific aims were completed to investigate the role of the PrL-NAc core circuit in cocaine-seeking behaviors following short or prolonged abstinence and the feasibility of tACS to modulate PrL-NAc oscillatory dynamics and cocaine seeking in rats.

Summary of Experimental Findings

Aim 1 examined the effects of 80 Hz tACS administered over the PrL on resting state PrL-NAc coherence and cocaine-seeking behaviors. Briefly, we used *in vivo* electrophysiology to record LFPs in the PrL and ipsilateral NAc core at rest following one-month abstinence from cocaine or saline self-administration. We then applied 80 Hz tACS (or sham) to target specific neuronal activity patterns that were disrupted following one-month abstinence from cocaine and examined the effects of this treatment on PrL-NAc core oscillatory dynamics and cocaine seeking. PrL-NAc coherence was reduced at multiple frequency bands (including high gamma) following one-month abstinence from cocaine compared with saline controls, consistent with recent work from our lab (West et al., 2021). Given these findings, and our recent data showing 80 Hz tACS restores cocaine-induced deficits in PrL-NAc coherence and behavioral flexibility (West et al., 2021), we hypothesized that 80 Hz tACS would reduce cocaine seeking in this task. While 80 Hz tACS (versus sham) increased PrL-NAc coherence across frequencies, it actually *increased* and was positively correlated with cocaine seeking during the test. These results indicate that tACS targeting high gamma frequencies may actually augment cue-evoked cocaine craving.

Aim 2 build upon this work and examined the effects of 16 Hz tACS administered over the PrL on rest PrL-NAc core coherence and cocaine seeking. As in Aim 1, we recorded LFPs in the PrL and NAc core at rest following one-month abstinence from cocaine or saline self-administration. In Aim 2 however, we applied 16 Hz tACS or sham to target a lower frequency oscillation that was dampened following one-month abstinence from cocaine and examined the effects of this treatment on PrL-NAc coherence and cocaine seeking. Rest PrL-NAc coherence was reduced at multiple frequency bands (including beta) following one-month abstinence from cocaine versus saline, consistent with Aim 1. Given these findings and the proposed role of beta oscillations in top-down cognitive control (Engel & Fries, 2010; Richter et al., 2017; Riddle et al., 2021; Zavala et al., 2018), we hypothesized that 16 Hz tACS would reduce cocaine seeking in our task. While 16 Hz tACS reversed cocaine-induced deficits in PrL-NAc beta coherence, it also appeared to increase delta and theta frequencies. We found positive correlations between rest PrL-NAc coherence following treatment and behavioral responses to the cocaine-paired CS (Phase 1 of test), similar to Aim 1. Interestingly, however, in Aim 2 higher PrL-NAc beta coherence and greater 16 Hz tACS-induced increases in this frequency predicted significantly *less* cocaine seeking under extinction (Phase 2 of test). Collectively, these results suggest that tACS protocols which selectively and maximally entrain beta oscillations may reduce cocaine seeking following prolonged abstinence.

While results from Aim 2 were exciting, we realized that we needed to better characterize relationships between PrL-NAc oscillatory dynamics and cocaine seeking to set the foundation for future studies that can more effectively use tACS to modulate behaviors linked with SUD. Therefore, Aim 3 examined the effects of one-day versus one-month experimenter-imposed cocaine abstinence on PrL-NAc coherence at rest and during discrete elements of our test, as well

as relationships between PrL-NAc activity and cocaine-seeking behaviors. We recorded resting state LFPs in the PrL and NAc core before and immediately following cocaine self-administration and short or prolonged abstinence. We then recorded event-related LFPs during a test session consisting of presentations of the cocaine-paired CS (Phase 1), extinction (Phase 2), and resumption of cocaine self-administration (Phase 3). Rest PrL-NAc coherence was reduced in the beta band following prolonged (versus short) cocaine abstinence, accompanied by increased cocaine-seeking behaviors. Additionally, reduced PrL-NAc theta and beta coherence predicted increased time spent in the cocaine-associated quadrant and extinction presses during the test (negative correlation), while delta was positively correlated with approaches toward the quadrant. Notably, while rest PrL-NAc coherence was reduced following prolonged cocaine abstinence, event-related coherence in this circuit during CS probes and extinction presses was increased and positively correlated with cocaine seeking at delta and low gamma frequencies. These data reveal bidirectional relationships between PrL-NAc oscillatory dynamics and cocaine seeking mediated by whether LFPs are measured at rest or induced by cocaine-associated cues or drug seeking.

General Implications, Limitations, and Future Directions

In Aims 1 and 2, we found tACS increased rest PrL-NAc coherence non-specifically. As described above, we believe the broad-band increase in rest PrL-NAc core coherence following 80 Hz tACS may reflect a general effect of this high frequency stimulation on cells in the PrL-NAc projection rather than a frequency-specific entrainment of oscillatory activity. Indeed, we recently showed that 83 Hz optogenetic stimulation of cell bodies in the PrL that project to the NAc core induced neuronal activity in the NAc core time-locked to photostimulation (West et al., 2021), perhaps indicating this high frequency is sufficient to induce a general increase in PrL-NAc circuit

activity. Additionally, while 16 Hz tACS reversed cocaine-induced deficits in PrL-NAc coherence at the beta frequency specifically, it appeared to increase PrL-NAc coherence at delta and theta frequencies as well. This improvement in frequency-specificity for 16 Hz tACS compared to 80 Hz tACS is promising and demonstrates target engagement in our rodent tACS model. However, future experiments should determine whether specificity of tACS effects on PrL-NAc oscillatory dynamics is improved by rational design (Frohlich, 2014) of experimental parameters such as individualized stimulation frequency, dose (i.e., number of stimulations per session), and duration (i.e., number of sessions) which aim to achieve durable modulation of PrL-NAc circuit dynamics (Ekhtiari et al., 2019; Herrmann et al., 2013).

In Aim 1, although 80 Hz tACS increased PrL-NAc coherence relative to sham-treated rats, it actually increased cocaine seeking during the test. As described above, these results are consistent with the notion that activation of PFC-NAc circuitry differentially modulates behavior depending on the experimental conditions recruiting PFC and NAc regions (Jasinska et al., 2015). That is, in the presence of cocaine-paired cues in Aim 1, strengthening PrL-NAc signaling with 80 Hz tACS augmented cocaine seeking, whereas when this neural circuit was recruited by executive control processes, strengthening PrL-NAc signaling with 80 Hz tACS reversed cocaine-induced deficits in behavioral flexibility (West et al., 2021). In support, when operant behavior previously reinforced by cocaine was paired with delivery of shock, stimulation of the PFC reduced cocaine seeking (Chen et al., 2013), but in the presence of cocaine cues activation of the PFC (Kalivas, 2008) and NAc (Cornish & Kalivas, 2000; McFarland et al., 2003) promotes cocaine seeking. Together these findings highlight the importance of context when designing tACS methods for modulating behaviors associated with CUD.

In Aim 2, although no significant group differences in cocaine-seeking behaviors were observed for the tACS versus sham group, the level of entrainment of PrL-NAc beta coherence by 16 Hz tACS significantly predicted reduced cocaine seeking. That is, reduction of putatively incubated cocaine craving was determined by the degree to which we effectively enhanced coherent beta oscillatory activity in the PrL-NAc circuit with 16 Hz tACS. As such, we propose that tACS protocols which selectively entrain beta oscillations in this circuit may reduce cocaine seeking following prolonged abstinence. In addition to using individualized tACS parameters to improve frequency-specificity of tACS effects on neural signaling in future studies, it will also be important to examine the effects of tACS on behavioral flexibility, decision making, motivated behavior for non-drug reinforcers, and other behaviors that are impaired in SUDs. Future studies should also use behavioral models that can directly examine the interplay between cue-induced drug craving and diminished executive function during cocaine abstinence, and effects of tACS on these symptoms, to develop multifaceted SUD treatment strategies.

Related to these proposed future directions, Aim 3 revealed bidirectional relationships between PrL-NAc oscillatory dynamics and cocaine seeking influenced by whether LFPs were measured at rest or induced by cocaine-associated cues or drug seeking. We believe these data are in line with neuroimaging studies in humans with CUD showing reduced rsFC in cortico-striatal networks at baseline (Gu et al., 2010; Hu et al., 2015), but increased rsFC between the ventral striatum and PFC when neuroimaging was completed immediately after a cue-induced craving task (Wilcox et al., 2011). To our knowledge, Aim 3 is the first study to demonstrate these context-dependent opposing shifts in PrL-NAc LFP activity following prolonged cocaine abstinence and their relationship to cue-evoked cocaine seeking in rats. As described in Chapter 4, it remains to be determined if a shared oscillatory mechanism underlies these abstinence-related bidirectional

shifts in PrL-NAc signaling. One potential mechanism is cross-frequency interactions between lower frequency top-down oscillatory signals and higher frequency bottom-up activity which can be modulated by experimental task demands (Buschman & Miller, 2007; Richter et al., 2017; von Stein & Sarnthein, 2000). Ongoing work in collaboration with the Fröhlich lab is examining cross-frequency phase-amplitude coupling at rest and during cocaine seeking and its modulation by prolonged cocaine abstinence. Critically, recent work from the Fröhlich lab demonstrated that cross-frequency tACS targeting specific phase-amplitude coupling modulated performance in a cognitive control task (Riddle et al., 2021). As such, analysis of phase-amplitude coupling in our task may inform future development of cross-frequency tACS for reducing cocaine seeking in rats.

While administration of tACS over the PrL increased coherent activity in the PrL-NAc circuit in Aims 1 and 2, this stimulation method is less specific than other techniques and may have downstream effects on other regions via alternative PrL projections. Given the vast neural circuitry underlying cocaine seeking (Dong et al., 2017; Kalivas, 2008; Koob & Volkow, 2010; McFarland & Kalivas, 2001; Wolf, 2016), future studies will also need to determine how oscillatory dynamics in these additional circuits are linked to drug seeking following prolonged abstinence, and how tACS affects this behavior and associated neurocircuitry. Additionally, our tACS protocols in Aims 1 and 2 were administered following a uniform abstinence duration (one month) in rats, while in clinical populations undergoing substance use treatment, abstinence durations likely vary. As such, it will also be important to examine relationships between oscillatory activity and cocaine seeking following varying durations of abstinence and how these may be differentially targeted by tACS. Finally, it is difficult to draw definitive conclusions about the PrL-NAc oscillatory dynamics underlying cue-evoked cocaine seeking following short or prolonged abstinence due to differences in experimental design between the three Aims of this

dissertation. Future studies should therefore employ a within-subject design to assess resting state and cocaine cue-induced LFP activity and its relationship to cocaine seeking before and following protracted cocaine abstinence. This will enable us to determine the role of distinct oscillatory frequency bands in incubation of cocaine craving and how tACS affects the circuit dynamics underlying this behavior.

Concluding Remarks

In individuals with CUD and animal models, prolonged cocaine abstinence is associated with profound changes in PFC and NAc activity that contribute to cognitive impairments and persistent vulnerability to relapse (Beveridge et al., 2008; Goldstein & Volkow, 2002, 2011; Hanlon et al., 2013; Wolf, 2016). An emerging strategy for treating SUD therefore involves using noninvasive brain stimulation to normalize drug-induced disruptions in brain activity. While TMS and tDCS targeting PFC regions may dampen drug craving in individuals with SUDs (Hanlon et al., 2018; Hone-Blanchet et al., 2015; Mahoney et al., 2020; Terraneo et al., 2016), we believe a stronger approach involves combining *in vivo* electrophysiology with tACS to target specific circuit-level neuronal activity patterns that are disrupted following prolonged cocaine abstinence. The studies described herein indicate that reduced *rest* PrL-NAc beta coherence may be a unique oscillatory underpinning of incubation of cocaine craving, and tACS protocols which selectively and maximally entrain beta oscillations may reduce cocaine seeking following prolonged abstinence. Future work should determine whether target engagement of oscillatory activity by tACS is improved by rational design of experimental parameters such as individualized stimulation frequency, dose, and duration to achieve lasting modulation of PrL-NAc circuit dynamics (Ekhtiari et al., 2019; Frohlich, 2014; Herrmann et al., 2013).

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