Preclinical Evidence for Intersectional Impacts of HIV and Cocaine Use Disorders on Behavior and Neuroimmune Function

by

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ABSTRACT

Cocaine use disorders (CUDs) and human immunodeficiency virus (HIV) are a common comorbidity, although it is largely unknown whether HIV interacts with cocaine abstinence to uniquely alter neuroimmune function and whether HIV may modulate the efficacy of medications intended to treat CUDs. My dissertation research demonstrates using preclinical rodent models of drug self-administration and craving that systemic exposure to the HIV protein gp120 produces a unique profile of neuroimmune changes within the nucleus accumbens core (NAc core) that is distinct from early cocaine abstinence alone. After a protracted period of abstinence, gp120 exposure abolished the effect of the dopamine D3 receptor (D3R) partial agonist MC-25-41, which successfully attenuated cue-induced cocaine seeking in non-exposed rats. Further probing the role of downstream, intracellular neuroimmune function on cue-induced cocaine seeking, I examined the role of the nuclear factor kappa B (NF-κB) signaling pathway within the NAc core on cue-induced cocaine seeking after a period of protracted abstinence across sex and reinforcer type. I demonstrated that knockdown of the p65 subunit of NF-κB results in a decrease in cue-induced cocaine seeking in males, but not in females. This effect was specific to cocaine, as p65 knockdown did not affect cue-induced sucrose seeking in either males or females. Moreover, I examined expression levels of the extracellular matrix enzyme MMP-9 within the NAc core, as it is regulated by NF-κB and is an important mediator of cue-induced cocaine seeking and associated synaptic plasticity. I demonstrated that males express higher levels of MMP-9 within the NAc

compared to females, and that p65 knockdown decreases NAc core MMP-9 in males but not females among cocaine cue-exposed animals. Altogether, these results suggest that immunotherapeutic medications may be useful tools in the treatment of CUDs, particularly among males that are disproportionately impacted by HIV.

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CHAPTER 1

INTRODUCTION

Over the last decade, cocaine overdose rates have increased dramatically within the United States and worldwide, particularly in combination with synthetic opioids (National Institute on Drug Abuse, 2021; Ritchie & Roser, 2018). Specifically, cocaine overdose deaths involving synthetic opioids have risen to nearly 16,000 per year within the United States. In the wake of the opioid epidemic, psychostimulant overdose deaths are continuing to rise partly due to the lack of evidence-based, FDA-approved therapeutics that treat psychostimulant use disorders (Ronsley et al., 2020). Substance Use Disorders (SUDs) are broadly characterized by symptoms of drug misuse and dependence, such as the development of withdrawal and tolerance after prolonged drug use, repeated failed attempts to quit or control one's drug use, social and interpersonal problems related to one's drug use, among many others. Importantly, one criterion that was recently added to the Diagnostic and Statistical Manual (DSM)-V for SUDs is drug "craving" (Ronsley et al., 2020). Drug craving is generally defined as the subjective desire to use drug (Tiffany & Wray, 2012), and drug craving is thought to, in part, underly drug motivation and relapse vulnerability (Baker, Morse, & Sherman, 1986; Robinson & Berridge, 2003).

Much preclinical research has been devoted to understanding the neurobiological underpinnings of drug craving for the purposes of identifying potentially novel therapeutic targets for medications development (Koob & Volkow, 2016; Pickens et al.,

2011). In humans with cocaine use disorders (CUDs), binge cocaine use results in a "crash" of increased sleeping and depression, followed within days by an emerging increase in cocaine craving which can lead to relapse (Gawin & Kleber, 1986). Similarly, during withdrawal from a daily cocaine self-administration regimen in rats, cocaine-seeking behavior becomes progressively stronger during the course of prolonged abstinence (Childress et al., 1999; Gawin & Kleber, 1986). This phenomenon, first modeled by our lab (Tran-Nguyen et al., 1998), is known as the "incubation of craving" effect (Grimm et al., 2001) and is thought to contribute to relapse. Many studies have identified neurobiological adaptations that accompany this incubation of drug craving (X. Li et al., 2015; Pickens et al., 2011), yet medications that successfully treat drug craving and relapse in humans are still lacking. Another important, common, and often understudied variable that can significantly impact treatment outcomes is the presence of comorbidities. One such comorbidity is human immunodeficiency virus (HIV), which remains a pervasive public health concern in the United States and worldwide, particularly among lower socioeconomic minority populations (Durvasula & Miller, 2014; National Institute on Drug Abuse, 2012). Cocaine abuse, which is associated with risky behaviors such as unsafe sexual practices, can increase HIV risk (Centers for Disease Control and Prevention, 2018). Notably, over 1 million people in the U.S. are currently living with HIV and 1 in 7 are unaware of their HIV status (Linley et al., 2010). While modern antiretroviral therapy is a major advancement in the treatment of HIV, these drugs do not readily cross the blood brain barrier, rendering the brain particularly

vulnerable to chronic HIV infection (Atluri et al., 2015). Importantly, cocaine abuse can increase blood brain barrier permeability (Zhang et al., 1998) and facilitate invasion (An & Scaravilli, 1997) and viral replication (Sahu et al., 2015) of HIV in the brain even during the early asymptomatic stages of HIV infection. Thus, with no FDA-approved medications for treating CUDs, individuals who have CUDs and become infected with HIV are particularly vulnerable to the chronic neurological consequences of combined cocaine and HIV. For instance, neuroinflammation occurs with exposure to either cocaine (Buch et al., 2011; Crews, Zou, & Qin, 2011; Cui, Shurtleff, & Harris, 2014) or HIV and its protein products (Bruce-Keller et al., 2008; Kesby et al., 2014, 2016, 2017; Melendez et al., 2016; Purohit et al., 2011; Y. Yang et al., 2010). Nevertheless, little is known about how cocaine and HIV interact to dysregulate neuroimmune function nor whether interaction effects impact vulnerability to relapse. The studies described herein attempt to clarify the behavioral and neurobiological interactions between HIV and cocaine with the overall goal of understanding how neuroimmune function within the reward system contributes to drug-seeking behavior and how comorbidities such as HIV, which significantly perturb neuroimmune function, modulate the efficacy of medications intended to treat CUDs.

CHAPTER 2

REVIEW OF THE LITERATURE

Cocaine is a highly addictive psychostimulant drug that is derived from the leaves of the coca plant (*Erythroxylum coca*), with the most common routes of self-administration

including intranasal, smoking, and intravenous injection (Gossop et al., 1994). Cocaine interacts with the mesocorticolimbic reward system by blocking the cellular reuptake of monoamine neurotransmitters such as dopamine, serotonin, and norepinephrine, which are released by distinct subpopulations of neurons within the midbrain that project to corticolimbic structures such as the nucleus accumbens (NAc), prefrontal cortex (PFC), amygdala (AMY), hippocampus (HPC), among others (Brodie & Dunwiddie, 1990; Koob & Volkow, 2010; Scheel-Krüger et al., 1977). Studies in rodents have demonstrated that the rewarding and reinforcing properties of cocaine are likely due to pharmacological blockade of monoamine transporters such as the dopamine transporter (DAT), the serotonin transporter (SERT), and the norepinephrine transporter (NET) (Hall et al., 2002; Kuhar et al., 1991; Rocha et al., 1998; Sora et al., 2001; Volkow et al., 1997; Xu et al., 2000). Indeed, such findings have led to the proposed use of psychostimulant drugs with similar mechanisms of action to treat cocaine dependence (Rush & Stoops, 2012), although such treatments may not be efficacious in promoting sustained cocaine abstinence (Castells et al., 2016). While the acute pharmacokinetic and pharmacodynamic effects of cocaine are well studied (Cone, 1995; Hedaya & Wei-Jian, 1997; Ma et al., 1999; Mulé, 1984), the cellular adaptations and molecular mechanisms underlying vulnerability to cocaine relapse, particularly the aforementioned “incubation of craving” effect, are not as thoroughly understood. Improving our understanding of the unique neurobiological milieu of cocaine abstinence and craving may reveal novel and effective therapeutic targets for medications development.

The Neurobiology of Cue-Induced Cocaine Seeking: A Focus on Glutamate and Dopamine Interactions within the Reward System

Drug relapse in humans can occur following a protracted period of abstinence (Hunt et al., 1971), and this is often triggered by exposure to drug-associated cues that can stimulate drug craving (O'Brien et al., 1992). Similar to humans, protracted periods of drug withdrawal in rodents are associated with time-dependent increases in drug-seeking behavior, a phenomenon known as the "incubation of drug craving" (Abdolahi et al., 2010; Bienkowski et al., 2004; Grimm et al., 2001, 2002; Neisewander et al., 2000; Shalev et al., 2001; Shepard et al., 2004; Tran-Nguyen et al., 1998). Glutamatergic plasticity that occurs over the course of drug withdrawal and abstinence has been implicated across several major drugs of abuse (Kalivas et al., 2009; Scofield et al., 2016; van Huijstee & Mansvelder, 2014), and these synaptic adaptations, particularly within the NAc, are thought to drive cue-motivated drug seeking. Several studies have shown that after chronic drug self-administration and withdrawal, NAc synapses accumulate GluA2-lacking calcium-permeable AMPA receptors (CP-AMPARs), which enhances post-synaptic excitability and facilitates the incubation of drug seeking (Conrad et al., 2008; Wolf & Tseng, 2012). In line with these observations, cue-induced cocaine seeking is associated with time-dependent increases in neuronal activation within the NAc (Pickens et al., 2011). Moreover, drug withdrawal downregulates the function and expression of glial glutamate transporters within the NAc, which normally regulate extracellular glutamate levels and subsequent post-synaptic glutamate signaling (Roberts-Wolfe &

Kalivas, 2015). Drug cue-elicited glutamatergic stimulation of medium spiny neurons (MSNs) within the NAc, particularly via prelimbic cortex (PrL) afferents, critically mediates reward seeking in response to contingent cues (Di Ciano et al., 2001). Indeed, several studies have shown that within the core subregion of the NAc (NAc core), this PrL glutamate input mediates cue- and drug-induced reinstatement of cocaine-, nicotine-, and heroin-seeking as well as associated changes in dendritic spine morphology and physiology (Gipson, Kupchik, et al., 2013; Gipson, Reissner, et al., 2013; LaLumiere & Kalivas, 2008; McFarland et al., 2003; Shen et al., 2011; Smith et al., 2017). Restoration of the glial glutamate transporter GLT-1 [i.e., excitatory amino acid transporter-2 (EAAT-2)], which is responsible for about 90% of glutamate uptake within the brain (Haugeto et al., 1996), can attenuate cue-induced drug seeking (Knackstedt et al., 2010; Namba et al., 2020; Reissner et al., 2015). As well, chemogenetic activation of astrocytes, which are the primary source of GLT-1-mediated glutamate uptake, inhibits cue-induced reinstatement of cocaine seeking (Scofield et al., 2015). Altogether, these studies indicate that drug abstinence and withdrawal are associated with persistent dysregulations in glutamatergic homeostasis and that restoring normal glutamatergic signaling can inhibit cue-motivated drug seeking.

In addition to glutamate, persistent alterations to dopamine signaling within the mesocorticolimbic reward system may underlie the incubation of drug craving and contribute to relapse. Specifically, the dopamine D3 receptor (D3R) has been implicated in both human and animal studies of cocaine-induced changes in dopamine

neurotransmission (Neisewander et al., 2014). D3Rs, which are G_i-coupled receptors that inhibit downstream adenylyl cyclase activity, are uniquely localized within the mesocorticolimbic reward system, where high levels of expression are found within the striatum, VTA, and other limbic structures (Sokoloff et al., 2006). Following cocaine self-administration, rats show time-dependent increases in D3R binding within the NAc core (Neisewander et al., 2004), and many preclinical studies have demonstrated that pharmacological inhibition of D3Rs reduces drug-seeking behavior (Cheung et al., 2013; Di Ciano et al., 2003; Higley et al., 2011; Jordan et al., 2019; Powell et al., 2018, 2020; Vorel et al., 2002; Xi et al., 2006; You et al., 2019). Interestingly, acute D3R agonist treatment decreases VTA dopamine neuron firing and extracellular dopamine levels within the frontal cortex of rats (Gobert et al., 1996), while systemic D3R antagonist treatment enhances cocaine-induced increases in NAc dopamine while also reducing cue-induced cocaine seeking in rats (Xi & Gardner, 2007). Moreover, D3R-deficient mutant mice exhibit higher levels of basal dopamine within the ventral striatum compared to controls (Koeltzow et al., 1998). Human imaging studies corroborate these preclinical findings, demonstrating that cocaine-dependent individuals exhibit higher levels of D3R binding and expression within the striatum (Boileau et al., 2015; Payer et al., 2014; Staley & Mash, 1996). Inhibition of D3Rs may also improve cognitive function, which is also impaired among those with CUDs (Millan et al., 2010; Nakajima et al., 2013). Altogether, such findings suggest that inhibition of D3Rs may normalize striatal dopamine transmission and reduce cocaine motivation.

Many converging lines of evidence suggest that inhibition of D3R-mediated signaling could interact with glutamatergic plasticity to reduce cocaine seeking. Within the NAc, dendritic spines of GABAergic MSNs form a "synaptic triad" with dopaminergic and glutamatergic afferents. Specifically, cortical glutamatergic afferents tend to make asymmetric contact with dendritic spine heads, while dopaminergic terminals tend to form symmetric synapses with dendritic spine necks of MSNs within the NAc (Freund et al., 1984; Spiga et al., 2014; Zahm, 1992). VTA train stimulation, which mimics dopamine cell firing, inhibits NAc excitatory postsynaptic potentials (EPSPs) elicited by PFC stimulation, which is likely mediated by dopamine D2 receptors (D2Rs) on glutamatergic terminals (Bamford et al., 2004; Brady & O'Donnell, 2004). Protracted withdrawal from cocaine self-administration in rats is associated with decreased D2R and increased D3R cell surface expression within the NAc core (Conrad et al., 2010), which may contribute to dysregulated glutamate transmission during cue-induced cocaine seeking. D3Rs are localized near the post-synaptic density of glutamatergic synapses within NAc and can also function as autoreceptors within the VTA (Sokoloff et al., 2013). Importantly, D3R inhibition normalizes activity of hyper-responsive glutamatergic neurons within the PrL (Sokoloff et al., 2013) and increases dopamine within the NAc (Huang et al., 2019). Cocaine consumption during cue-induced cocaine seeking suppresses cue-evoked glutamatergic plasticity of dendritic spines within the NAc, which is likely dopamine-mediated (Spencer et al., 2017; Suto & Wise, 2011). Interestingly, rats with a history of cocaine self-administration tested under extinction

conditions exhibit elevated extracellular dopamine levels within the NAc core during the first 60 mins of testing and a parallel, within-session decline in active lever pressing despite persistently elevated glutamate levels (Suto et al., 2010). Moreover, the inhibition of L-type calcium channels within the VTA, possibly through disinhibition of VTA-NAc dopamine projections, inhibits cue-induced cocaine seeking through increasing NAc core dopamine levels (Addy et al., 2018). Other studies have also shown that systemic inhibition of calcium channels reduces drug-seeking behavior (Biala & Budzynska, 2006; Uhrig et al., 2017). Taken altogether, it's possible that stimulation of NAc dopamine release via D3R inhibition may suppress cue-evoked glutamatergic plasticity and cocaine seeking behavior. As described below, glial immunomodulation may be important in the global regulation of these dopamine-glutamate interactions and may represent a novel treatment strategy to treat CUDs.

Immunomodulation of Learning, Memory, and Synaptic Plasticity: Cytokines as a Case Example

Glia play diverse roles in dynamically modulating synaptic plasticity, learning, and memory beyond their "traditional" roles in supporting tissue homeostasis (Achour & Pascual, 2010; Perea et al., 2014; Temburni & Jacob, 2001). For example, neurochemical signaling molecules such as glutamate and dopamine mediate neuron-glia crosstalk that can alter downstream immunomodulatory signaling. As illustrated in Figure 1, microglia, which continuously survey their environment with ramified processes, express both glutamate and dopamine receptors and can release proinflammatory factors in response to

rapid changes in extracellular neurotransmitter levels (Hagino et al., 2004; H. Liu et al., 2016; Murugan et al., 2011). Similarly, astrocytes express such receptors and dynamically respond to rapid changes in synaptic neurotransmitter levels through modulation of neurotransmitter uptake (Duan et al., 1999) and through gliotransmission via adenosine triphosphate (ATP) and other transmitters (Harada et al., 2016). As well, astrocytes are highly sensitive to immunomodulatory signals and this has indirect consequences on astrocytic regulation of synaptic transmission (Cekanaviciute & Buckwalter, 2016). Together, microglia and astrocytes can orchestrate potent modulatory control over synaptic plasticity through glial cell-derived immunomodulatory factors such as cytokines.

Cytokines have been extensively studied for their role in learning, memory, and synaptic plasticity (Levin & Godukhin, 2017), and the outcome of these processes depend on the specific cytokine, its concentration within the brain, receptors available for cytokine binding and activation of signal transduction pathways, and the conditions underlying cytokine release (Goshen & Yirmiya, 2007). For example, Beattie et al., (2002) and Stellwagen et al., (2005) have demonstrated that glial tumor necrosis factor alpha (TNFα) facilitates membrane insertion of calcium-permeable α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) glutamate receptors (CP-AMPARs) and internalization of γ-aminobutyric acid type A ($GABA_A$) receptors within the hippocampus, leading to enhanced excitatory synaptic transmission. In contrast, Lewitus et al., (2014) found that TNFα exerts an opposite effect at striatal synapses, where TNFα

internalizes CP-AMPARs and reduces corticostriatal synaptic strength. These investigators demonstrated that microglia-induced TNFα release depresses excitatory synaptic activity within the ventral striatum through internalization of AMPARs and that this process is associated with cocaine-induced locomotor sensitization (Lewitus et al., 2016). Another example of region-specific regulation of synaptic plasticity showed that TNFα secretion in response to peripheral nerve injury enhances excitatory synaptic connectivity within the spinal cord but impairs this connectivity within the hippocampus (Y. Liu et al., 2017). These studies provide clear evidence that immunomodulatory signals such as TNFα crucially regulate synaptic plasticity in a brain region-specific manner, which may have important implications for understanding the pathophysiology of SUDs.

Like TNFα, microglia and astrocytes also release interleukin-6 (IL-6) within the central nervous system (CNS), a cytokine involved in modulating learning and memory (Choi et al., 2014; Y. Dong & Benveniste, 2001; Ye & Johnson, 1999). Early studies demonstrated that acute IL-6 exposure inhibits long-term potentiation (LTP) within the hippocampus likely through inhibition of mitogen-activated protein kinase/extracellular signal-regulated kinase (MAPK/ERK) signaling (A. J. Li et al., 1997; Tancredi et al., 2002). In addition, overexpression of IL-6 in astrocytes of mice results in reduced LTP in the dentate gyrus (Bellinger et al., 1995). However, *in vitro* and *in vivo* studies examining IL-6 expression during the induction of hippocampal LTP show that LTP induces an upregulation of IL-6 mRNA that is localized to non-neuronal cells such as astrocytes

(Balschun et al., 2004; Jankowsky et al., 2000) and inhibition of IL-6 signaling results in improved performance in hippocampus-dependent memory tasks (del Rey et al., 2013). Consistent with this finding, IL-6 knockout mice exhibit enhanced performance in a radial arm maze task compared to wild type mice, which is correlated with hippocampal choline acetyltransferase activity (Braida et al., 2004).

Interleukin-1β (IL-1β) is also critically involved in hippocampal-dependent learning and synaptic plasticity. Within the hippocampus, fear conditioning and LTP upregulate IL-1β and systemic administration of small concentrations of IL-1β can enhance learning and memory (Balschun et al., 2003; del Rey et al., 2013; Goshen et al., 2007; Schneider et al., 1998). Additionally, acute intra-hippocampal administration and chronic overexpression of IL-1β can impair fear conditioning and spatial learning as well as hippocampal LTP similar to IL-6 (Gonzalez et al., 2009; Hein et al., 2010; Loscher et al., 2003; MacHado et al., 2010; Moore et al., 2009; F. M. Ross et al., 2003; Vereker et al., 2000). Despite these seemingly convergent and consistent findings, studies examining learning (e.g., fear conditioning) and hippocampal plasticity using IL-1 receptor (IL-1R) knockout mice have demonstrated mixed effects (Avital et al., 2003; Koo & Duman, 2009; Murray et al., 2013). This is likely the result of complex interactions with other cytokines within the IL-1 family, which is a very broad family of closely related cytokines (Dinarello, 2018).

These studies provide clear evidence that cytokines can exert a dynamic modulatory role within the nervous system over synaptic plasticity, learning, and

memory. They also highlight that the specific cytokine and conditions in which neural circuits are exposed to them are paramount when interpreting these findings. This complexity is a primary reason why elucidating the role of immunomodulation and neuron-glia crosstalk in facilitating the formation and persistence of addiction-related behaviors remains challenging.

Immunomodulation of Cocaine-Induced Synaptic Plasticity and Behavior: A Focus on the NF-κB Pathway

Drugs of abuse dysregulate immune system function both within the brain and throughout the periphery, and such aberrant changes in immune function may underly the pathophysiology of substance use disorders (SUDs) and associated comorbidities (Namba et al., 2021). For example, individuals with CUDs have altered serum levels of pro- and anti-inflammatory cytokines (Araos et al., 2015; Moreira et al., 2016; Zaparte et al., 2019), and cocaine users exhibit elevated expression of microglial activation markers such as cluster of differentiation 68 (CD68) within the striatum and hippocampus when examined post-mortem (Little et al., 2009). Similarly, non-human primates that self-administer cocaine intravenously display elevated expression of the microglial activation marker translocator protein 18 kDa (TSPO) within the striatum (H. R. Smith et al., 2019). In rodents, both experimenter-delivered and self-administered cocaine produce significant changes in the expression of various neuroimmune markers across the mesocorticolimbic system. For example, both intravenous cocaine self-administration and acute, experimenter-delivered cocaine upregulate interleukin (IL)-1β mRNA expression

within the ventral tegmental area (VTA) of rodents (Brown et al., 2018; Northcutt et al., 2015). Additionally, mice receiving sub-chronic administration of cocaine exhibit increases in IL-6, tumor necrosis factor alpha (TNFα), and nuclear factor kappa B (NF-κB) protein expression within the striatum (Ang et al., 2008; Lin et al., 2011; Russo et al., 2009; R. Zhu et al., 2018).

NF-κB is a DNA-binding protein complex that broadly regulates the transcription of a wide variety of genes, including cytokines and key addiction-related genes (Crews et al., 2017; Namba et al., 2021). In short, NF-κB exists in an inactivated state within the cytoplasm when bound to the inhibitory protein IκBα. Various upstream signals (e.g., pro-inflammatory cytokines, glutamate, etc.) can promote phosphorylation and degradation of IκBα by the enzyme complex IκB kinase (IKK), liberating NF-κB and permitting its translocation into the nucleus (Gilmore, 2006). Here, NF-kB can rapidly promote or repress the transcription of a wide array of genes, including immune mediators such as cytokines and genes that are known regulators of cue-motivated drug-seeking behavior (Bond et al., 1998; Chiechio et al., 2006; Kalivas, 2009; Richter et al., 2002; Sitcheran et al., 2005; A. C. W. Smith et al., 2014). Among these genes is the extracellular matrix enzyme matrix metalloproteinase-9 (MMP-9), which modulates learning, memory, and synaptic plasticity (Bozdagi et al., 2007; Knapska & Kaczmarek, 2015; Szklarczyk et al., 2002). Importantly, MMP-9 enzymatic activity is upregulated within the NAc core of rats following extinction from cocaine self-administration and is further increased in response to cocaine cue exposure (A. C. W. Smith et al., 2014).

Moreover, MMP-9 inhibition is associated with reductions in cue-induced cocaine seeking and concomitant increases in dendritic spine head diameter within the NAc core (A. C. W. Smith et al., 2014). We and others have previously demonstrated that NF-κB pathway signaling within the nucleus accumbens (NAc), which can promote MMP-9 gene expression (Bond et al., 1998; Hozumi et al., 2001; T. Wang et al., 2018), also regulates conditioned drug-seeking behavior. Specifically, experimenter-delivered cocaine upregulates the p65 subunit of NF-κB within the NAc, and inhibition of upstream IKK signaling attenuates cocaine conditioned place preference (CPP) and associated dendritic spine plasticity (Russo et al., 2009). Similarly, inhibition of IKK within the NAc core suppresses cue-induced reinstatement of extinguished nicotine seeking and activation of IKK prevents the antioxidant *N*-acetylcysteine from inhibiting this behavior (Namba et al., 2020).

Preclinical studies have also implicated neuroinflammation and NF-κB signaling in the pathophysiology of other disorders and diseases that are commonly comorbid with CUDs. For example, chronic social defeat stress, which induces a depression-like behavioral phenotype in mice, increases IKK activity within the NAc, and this is correlated with depression-like behaviors (Christoffel et al., 2011). Moreover, this study showed that inhibition of NAc IKK signaling reverses these depression-like behaviors, while increasing IKK activity promotes social avoidance behavior. Another study demonstrated that pharmacological inhibition of NF-κB prior to acute and chronic stress in rats prevents stress-induced decreases in measures of neurogenesis within the HPC as

well as IL-1β-induced decreases in adult hippocampal progenitor proliferation *in vitro* (Koo et al., 2010). While mesocorticolimbic neuroimmune function has been extensively studied across neuropsychiatric disorders that are commonly comorbid with CUDs (Hodes et al., 2015; Hori & Kim, 2019), such processes remain poorly understood in cases of comorbid HIV and CUDs.

The Neurobiological and Behavioral Intersections of HIV and CUDs

According to the United States Centers for Disease Control and Prevention, over 1 million people in the United States are currently living with HIV and 1 in 7 of those affected are unaware of their HIV status (Centers for Disease Control and Prevention, 2019). Globally, nearly 40 million people are currently living with HIV, and individuals who inject drugs are at a 22 times greater risk for acquiring HIV (UNAIDS, 2019). Importantly, HIV still remains a significant public health concern among minority and socioeconomically disadvantaged populations (Durvasula & Miller, 2014; National Institute on Drug Abuse, 2020). As well, drug misuse (particularly psychostimulant misuse) is associated with higher rates of risky behaviors such as unsafe sexual practices that can increase HIV risk (Centers for Disease Control and Prevention, 2018).

Modern antiretroviral therapy (ART) has proven successful at suppressing viral load and mitigating the transition to acquired immunodeficiency syndrome (AIDS), allowing many individuals living with HIV to live relatively normal lives. However, ART does not readily cross the blood-brain barrier (BBB), rendering the CNS vulnerable to chronic HIV infection (Atluri et al., 2015). Specifically, drugs of abuse such as cocaine

can increase BBB permeability (Zhang et al., 1998) and facilitate invasion (An & Scaravilli, 1997) and viral replication (Sahu et al., 2015) of HIV in the brain even during the early asymptomatic stages of HIV infection. This process can happen rapidly and lead to chronic CNS infection that results in neuroimmune activation and, in many cases, HIV-associated neurocognitive disorders (HAND) (Hong & Banks, 2015; Zayyad & Spudich, 2015). Cocaine facilitates HIV replication through a NF-κB-dependent mechanism (Fiume et al., 2012; Sahu et al., 2015) and impairs the innate immune response of astrocytes to infiltrating viruses such as HIV through increased oxidative stress when tested *in vitro* (Cisneros et al., 2018). Drugs of abuse also exacerbate the effects of the HIV proteins *trans*-activator of transcription (Tat) and gp120 in inducing oxidative stress, microgliosis, and astrogliosis (Aksenov et al., 2001, 2003; Samikkannu et al., 2015; Shah et al., 2013; Zeng et al., 2018). Moreover, HIV Tat primes and activates proinflammatory responses in microglia via the NOD-like receptor family pyrin domain containing 3 (NLRP3) inflammasome (Chivero et al., 2017), and dopamine can activate NF-κB and prime the NLRP3 inflammasome in macrophages (Nolan et al., 2020) (however, see Zhu et al., 2018, where astrocytic D2Rs were shown to restrict neuroinflammation and NLRP3 inflammasome activation). This could be exacerbated by drugs of abuse that promote dopamine transmission within areas of the brain that are particularly vulnerable to HIV, such as the striatum (Nolan & Gaskill, 2019). Clinically, the mitochondrial protein translocator protein (TSPO), which has garnered interest as a biomarker of neuroinflammation in human imaging studies (Werry et al., 2019), is

upregulated in people living with HIV (PLWH) and increased TSPO within the hippocampus, amygdala, and thalamus is associated with impaired cognition (Vera et al., 2016). Other studies suggest that impaired cognitive function is associated with enhanced TSPO binding in frontal and temporal regions of the brain in PLWH (Garveya et al., 2014; Rubin et al., 2018). It remains unclear whether combined HIV and active drug use, compared to drug abstinence, produces differential profiles of neuroinflammation and immune activation to confer increased vulnerability to HAND and drug relapse. Nevertheless, many preclinical studies suggest that HIV and its protein products may exacerbate drug reward and reinforcement as well as drug-induced neuroadaptations that may impede successful SUD treatment outcomes.

Growing evidence suggests that HIV and its protein products interact with brain reward circuitry to contribute to dysregulated reward-seeking behavior and cognition. For example, HIV-1 transgenic rats, which constitutively express HIV-1 viral proteins and model many HIV-associated neurological abnormalities observed in humans (McLaurin et al., 2018; Moran et al., 2014; Vigorito et al., 2015), exhibit increased dopamine affinity at striatal dopamine transporters and a leftward shift in the ascending limb of the cocaine dose response function, suggesting enhanced sensitivity to the reinforcing properties of cocaine (McIntosh et al., 2015). Another study using this transgenic rat model showed that these animals exhibit enhanced hyperexcitability of pyramidal neurons within the PFC following abstinence from cocaine (Wayman et al., 2016). As well, inducible Tat expression within the brain of transgenic mice is associated with enhanced METH-

induced microglia activation as well as METH-induced locomotor sensitization (Kesby et al., 2017). Other studies using the inducible Tat mouse model have shown that induced Tat expression within the brain increases the magnitude of cocaine CPP and is sufficient to reinstate extinguished cocaine CPP (Paris, Carey, et al., 2014). Similar effects have been observed in females, albeit in an estrous cycle phase-dependent manner (Paris, Fenwick, et al., 2014). Similarly, inducible gp120 expression within the mouse brain is associated with the formation of METH CPP at lower METH doses compared to non-transgenic controls (Kesby et al., 2014). Many other preclinical studies have also demonstrated impaired cognitive function in animals exposed to HIV proteins and drugs of abuse, mimicking the cognitive dysfunction observed in humans with HAND (Kesby et al., 2016; Kesby, Heaton, et al., 2015; Kesby, Markou, et al., 2015; Moran et al., 2014; Paydary et al., 2016). Taken together, such studies suggest that HIV and its protein products may increase one's sensitivity to the rewarding and reinforcing properties of psychostimulants and impair cognitive function.

In contrast to the studies above, a recent study using the HIV transgenic rat model suggests that HIV promotes a motivational state of apathy (Bertrand et al., 2018). However, one significant limitation to this study and studies like it is that the HIV transgenic rat model constitutively expresses HIV proteins throughout the CNS *prior* to the establishment of stable drug self-administration, which may produce profoundly different effects on mesocorticolimbic circuit function and neuroimmune signaling compared to the induction of viral protein expression within the CNS *after* drug self-

administration is established or during a period of abstinence. Indeed, some individuals with preexisting SUDs contract HIV collaterally through drug use and risky behaviors associated with drug use (Centers for Disease Control and Prevention, 2018). Regardless, it is possible that HIV-induced perturbations to neuroimmune signaling described above may underly these neurobehavioral intersections between HIV and SUDs. Thus, immunopharmacology may be a promising avenue of research into medications that inhibit the long-term neurocognitive deficits observed with chronic HIV infection as well as drug craving and relapse in cases of comorbid SUDs (Ambrosius et al., 2019; Roederer et al., 1992; Tripathi et al., 2020). Interestingly, ART itself possess anti-inflammatory properties (Hileman & Funderburg, 2017), but drugs of abuse may interact pharmacologically with ART to reduce its effectiveness in both suppressing viral load and minimizing cognitive deficits associated with HAND (Kumar et al., 2015). Altogether, these studies suggest that HIV and substance abuse may interact synergistically to impair the efficacy of ART and to promote addiction-related behaviors. However, it remains unclear how HIV may alter the neurobiological and behavioral sequelae of drug abstinence and the incubation of drug craving. Thus, the present studies sought to address these knowledge gaps.

Research Aims and Hypotheses

The present studies aimed to examine 1) whether the novel dopamine D3R partial agonist MC-25-41 decreases cue-motivated cocaine seeking in rats with a history of protracted cocaine abstinence and HIV gp120 exposure and 2) whether MC-25-41

modulates NAc core neuroimmune function. Due to the known effects MC-25-41 in suppressing high-effort cocaine, but not sucrose, self-administration (Powell, Namba, et al., 2020), we hypothesized that D3Rs facilitate motivation for cocaine, in part due to dysregulation of neuroimmune function. Therefore, we predicted that the dopamine D3R antagonist MC-25-41 would suppress cue-induced cocaine seeking and that combined cocaine abstinence and gp120 exposure would uniquely alter NAc core neuroimmune function (Brailoiu et al., 2017; Dahal et al., 2015; McLaurin, Cook, et al., 2018; Melendez et al., 2016). These experiments inform our overarching question by revealing whether a prior history of HIV gp120 produces long-lasting deficits in neuroimmune function and whether treatment with a D3R partial agonist can restore these deficits and also inhibit cocaine motivation similar to unexposed animals.

In addition to these experiments, we further investigated whether viral-mediated inhibition of NF-κB pathway signaling within the NAc core decreases cue-induced cocaine seeking and associated changes in MMP-9 expression in a 1) sex-specific and 2) reinforcer-specific manner. Given previous work demonstrating a role for NF-κB and MMP-9 signaling in drug-conditioned behavior (Namba et al., 2020; Russo et al., 2009; Smith et al., 2014), we hypothesized that NAc core NF-κB signaling would mediate cue-induced cocaine seeking and MMP-9 expression. Thus, we predicted that inhibition of NF-κB signaling within the NAc core would suppress cue-motivated cocaine seeking and MMP-9 expression. These experiments further inform our overarching question and the results from the previous experiments by elucidating whether striatal NF-κB pathway

signaling, which mediates cocaine-induced potentiation of HIV replication (Sahu et al., 2015), exerts modulatory control over reward seeking behavior and downstream neuroimmune function in a sex- and reinforcer-specific capacity, thus indicating a potential cellular and molecular mechanism through which HIV dysregulates drug-motivated behavior.

CHAPTER 3

EXPERIMENT 1 METHODS & MATERIALS

Subjects

Adult male Sprague-Dawley rats (N = 55; 201-225 g upon arrival, Charles River Laboratories, Hollister, CA, USA) were housed individually in a temperature- and humidity-controlled vivarium on a 14:10 hour reverse light:dark cycle. Animals had *ad libitum* access to water for the duration of the study but had restricted access to food, maintained at 85% of their free-feeding weight, during self-administration to facilitate cocaine acquisition. All experiments were conducted during the dark phase. All procedures were approved by and performed in accordance with the Institutional Animal Care and Use Committee of Arizona State University and the National Institutes of Health's *Guide for the Care and Use of Laboratory Animals*.

Drugs

Cocaine hydrochloride (NIDA Drug Supply Program, RTI International, Research Triangle Park, NC, USA) was dissolved in sterile saline to a stock concentration of 10 mg/kg, which was further diluted with saline to 0.75 mg/kg and filtered through 0.2 μm

syringe filters. HIV-1 IIIB gp120 was obtained from the NIH AIDS Reagent Program and was diluted in 1X phosphate buffered saline (PBS) to a final concentration of 45 ng/μL. MC-25-41 was dissolved in 20% cyclodextrin + 3% 1M HCl to a final concentration of 10 mg/kg as previously described (Powell, Namba, et al., 2020).

Surgical Procedures

All animals were implanted with intracranial guide cannulae and cocaine animals also received jugular vein catheters as previously described (Namba et al., 2020). Briefly, animals were anesthetized using vaporized isoflurane (2-3%), and cocaine animals received a sterile polyurethane catheter that was inserted 2.5 mm into the right jugular vein. The opposite end of the catheter was burrowed subcutaneously between the shoulder blades and attached to a cannula that was secured within a harness. Intracranial guide cannulae were stereotaxically implanted 1 mm dorsal to the right lateral ventricle (A/P: -0.8 mm, M/L: -1.6 mm, D/V: -2.6 mm) and secured to the skull using anchor screws and dental acrylic. All animals received buprenorphine (0.05 mg/kg/mL, s.c.) and meloxicam (1 mg/kg/mL, s.c.) at the end of surgery. Meloxicam was administered for 1 day post-operatively. Catheterized animals also received cefazolin (100 mg/mL, 0.1 mL, i.v.) and heparin (70 U/mL, 0.1 mL, i.v.), dissolved in saline, for 5 days post-operatively. Heparin alone was administered daily before and after each self-administration session to maintain catheter patency. Animals were given a maximum of 5 days of recovery prior to beginning self-administration.

Cocaine and Sucrose Self-Administration, Forced Abstinence, and Cue Reactivity

Animals were placed in individual operant conditioning chambers equipped with one active and one inactive lever, a cue light above each lever, a house light, a tone generator, and a food receptacle (30 × 24 × 21 cm; Med Associates Inc., St. Albans, VT, USA). Prior to self-administration, animals were habituated to their respective chambers for 1 hour with both levers retracted. Animals underwent 2-hour training sessions 6 days/week and were food-restricted as described above. Self-administration initially began on a fixed-ratio (FR) 1 schedule of reinforcement where one active lever response delivered a single infusion of cocaine (0.75 mg/kg/infusion, i.v., over 6 sec) or a sucrose pellet (45 mg/pellet, Bio-Serv, Flemington, NJ, USA) paired with a compound stimulus light and auditory tone (500 Hz). Each reinforcer was followed by inactivation of the light and tone cues and illumination of the house light to signal a 20-sec time-out period during which lever responses yielded no consequences. Inactive lever responses resulted in no reinforcer or associated cues, but responses were still recorded. Within a session, the schedule of reinforcement advanced to a variable ratio (VR) 2, 3, and 5, sequentially, where a reinforcer was delivered after an average number of active lever responses, according to the corresponding ratio, was achieved. Here, a minimum of 5 reinforcers earned was required within a reinforcement schedule within a given hour to advance to the next reinforcement schedule. Advancement to the next starting schedule between sessions required animals to end on a higher schedule than the starting one for 3 consecutive sessions. Stability criteria at the end of self-administration were defined as achieving at minimum 10 reinforcers on a VR5 starting schedule and ≤15% variability in

reinforcers earned across 3 consecutive sessions with no upward or downward trends. Some animals underwent 5 days of forced abstinence and were sacrificed on day 6 for brain tissue collection and protein quantification. Other animals underwent 21 days of forced abstinence, followed by a 1-hour cue reactivity test where active lever responses resulted in presentation of the light/tone cues but no cocaine or sucrose reinforcers. These animals were then sacrificed immediately following cue reactivity testing for brain tissue collection for protein quantification.

Microinjection Procedures

Beginning on day 1 of abstinence, all animals received a daily microinjection of gp120 (45 ng/μL, 1.0 μL, 0.5 μL/min, i.c.v.) or vehicle (1X PBS, 1.0 μL, 0.5 μL/min, i.c.v.) into the right lateral ventricle for 5 consecutive days. Some animals were then sacrificed the day after the fifth infusion (i.e., on day 6 of abstinence) to examine NAc cytokine, chemokine, and growth factor expression proximal to sub-chronic gp120 exposure. After 21 days of forced abstinence, remaining animals received a systemic injection of MC-25-41 (10 mg/kg/mL, i.p.) or vehicle (1 mL/kg of 20% cyclodextrin + 3% HCl in saline, i.p.) 10 minutes prior to undergoing the 1-hour cue reactivity test. This dose of MC-25-41 is based on results of our previous study showing a significant MC-25-41-induced reduction in cocaine self-administration at this dose (Powell, Namba, et al., 2020).

NAc core Tissue Processing and Measurement of Cytokine, Chemokine, and Growth Factor Expression

Animals were deeply anesthetized with isoflurane until respiration ceased prior to rapid decapitation on day 6 of abstinence or immediately after cue reactivity testing. A 2 mm-thick dissection of the NAc core was collected over ice and homogenized in an ice-cold RIPA lysis buffer solution containing protease and phosphatase inhibitors (Sana Cruz Biotechnology, Dallas, TX, USA). Tissue homogenates were centrifuged at 10,000 x g for 5 minutes and the supernatants were collected and stored at -80°C. Samples were diluted 1:1 in 1X PBS and cytokine, chemokine, and growth factor expression levels were determined using the Rat Cytokine/Chemokine 27-Plex Discovery Assay® Array (Eve Technologies, Calgary, AB Canada). Targets for this assay included eotaxin, epidermal growth factor (EGF), fractalkine (CX3CL1), interferon gamma (IFNγ), interleukin (IL)-1α, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-10, IL-12p70, IL-13, IL-17A, IL-18, IP-10, GRO/KC (CXCL1), tumor necrosis factor alpha (TNFα), granulocyte colony-stimulating factor (G-CSF), granulocyte-macrophage colony-stimulating factor (GM-CSF), monocyte chemoattractant protein-1 (MCP-1), Leptin, LIX (CXCL10), macrophage inflammatory protein (MIP)-1α, MIP-2, RANTES (CCL5), and vascular endothelial growth factor A (VEGF-A). IL-12p70 and MCP-1 levels were undetectable in every sample tested and were thus excluded from analyses. Each sample was run in duplicates and the average of each pair of readings was used as the final measure for each sample across all targets.

Data Analysis

Cocaine infusions and sucrose pellets earned as well as active and inactive lever presses across each of the qualifying sessions of self-administration were analyzed by repeated measures two-way ANOVAs, with “session” (self-administration session), “group”, and “lever” (active vs. inactive) as factors. Active and inactive lever presses during cue reactivity were analyzed with a three-way ANOVA, with “treatment” (vehicle vs. MC-25-41), “microinjection” (control vs. gp120), and “lever” (active vs. inactive) as factors. Tukey’s or Bonferroni’s multiple comparisons tests were conducted to examine specific group differences when appropriate. Cytokine, chemokine, and growth factor expression was examined using principal component analysis (PCA). A two-component PCA model was used here to represent the relationships between neuroimmune factors within a two-dimensional plane, such that the variables that explain the largest degree of variance within the data are grouped within principal component 1 (PC1) and the variables that explain the next largest degree of variance are grouped within PC2 along an orthogonal axis. Positively correlated variables (expressed as vectors within the PC1-PC2 plane) cluster within the same quadrant or, at the very least, on the same side of one of the PCs. In other words, positively correlated variables form small angles between their vectors (e.g., highly correlated vectors would appear overlapping). Negatively correlated variables are positioned opposite one another, and their vectors form large angles (i.e., close to 180°). Variables whose vectors meet near 90° are not likely to be correlated. The axes on the loading plots represent the degree each vector (i.e., variable) is correlated positively or negatively with a particular PC. Data were centered and scaled for this

analysis, and we retained the first two PCs which had eigenvalues greater than 1.0. The original data for each animal was projected onto the newly constructed, two-dimensional space in the PC scores plots. The PC scores are the result of a mathematical computation that represents each observation as a value weighted by the relative contribution of each loading factor. These PC scores were calculated using the linear combinations that defined each PC, allowing for the examination of clusters, trends, and outliers within the data based on treatment conditions. All analyses were conducted at the $\alpha = 0.05$ significance level where appropriate using GraphPad Prism 9.0 software.

CHAPTER 4

EXPERIMENT 1 RESULTS

Cocaine and Sucrose Self-Administration

Rats were trained to self-administer cocaine or sucrose for 2 hours/day for ≥12 days prior to intracerebroventricular gp120 administration and forced abstinence (Figures 2A, 4A for timelines of experimental procedures). For cocaine animals that underwent 5 days of abstinence, a repeated-measures two-way ANOVA for cocaine infusions earned revealed a significant main effect of session ($F_{(11,88)} = 6.161$, $p < 0.0001$) but no significant main effect of group or group-by-session interaction. *Post hoc* analysis of the session main effect revealed a significant increase in cocaine infusions earned on sessions 6-12 relative to session 1 (Dunnett's test, $^*p < 0.05$, Figure 2B). A repeated measures two-way ANOVA for active lever presses revealed a significant main effect of session ($F_{(11,88)} = 2.619$, $p = 0.0062$) but no main effect of group or group-by-session interaction.

Post hoc analysis of the session main effect revealed no significant differences in active lever presses from sessions 2-12 compared to session 1 (Figure 2C). For sucrose animals that underwent 5 days of abstinence, a repeated-measures two-way ANOVA for sucrose pellets earned revealed a significant main effect of session ($F_{(11,110)} = 23.35$, $p < 0.0001$) but no significant main effect of group or group-by-session interaction. *Post hoc* analysis of the session main effect revealed a significant increase in sucrose pellets earned on sessions 2-12 relative to session 1 (Dunnett's test, $^*p < 0.05$, Figure 2D). A repeated measures two-way ANOVA for active lever presses revealed a significant main effect of session ($F_{(11,110)} = 13.15$, $p < 0.0001$) but no significant main effect of group or group-by-session interaction. *Post hoc* analysis of the session main effect revealed a significant increase in active lever presses from sessions 2-9 compared to session 1 (Dunnett's test, $^*p < 0.05$, Figure 2E). No differences in inactive lever presses were observed across any of the groups. For cocaine animals that underwent 21 days of forced abstinence and cue reactivity testing, repeated-measures two-way ANOVAs for cocaine infusions earned (Figure 4B) and active lever presses (Figure 4C) revealed no significant main effects of session and group, and no significant group-by-session interactions. Similarly, no differences in inactive lever presses were detected. These results verify that the random assignment of animal subjects to each treatment condition produced similar profiles of self-administration prior to experimental manipulations.

Cocaine Cue Reactivity

We sought to determine whether exposure to gp120 during cocaine abstinence would alter the therapeutic efficacy of MC-25-41 in attenuating cue-induced cocaine seeking (Figure 4D). A three-way ANOVA revealed significant main effects of microinjection type ($F_{(1,23)} = 5.153$, $p = 0.0329$), drug treatment ($F_{(1,23)} = 14.77$, $p = 0.0008$), and lever ($F_{(1,23)} = 97.76$, $p < 0.0001$), as well as significant microinjection-by-treatment ($F_{(1,23)} = 5.195$, $p = 0.0322$) and lever-by-treatment ($F_{(1,23)} = 10.03$, $p = 0.0043$) interaction effects. *Post hoc* analysis revealed that MC-25-41 attenuated cue-induced cocaine seeking in unexposed rats but failed to do so in gp120-exposed rats (Tukey's test, $^{*}p < 0.05$ relative to inactive lever presses, $^{\#}p < 0.05$ relative to active lever presses of every other group, Figure 4D). Taken together, these results suggest that MC-25-41 significantly decreases cue-induced cocaine seeking and that gp120 exposure prevents this effect.

Principal Component Analysis of NAc core Cytokine, Chemokine, and Growth Factor Expression

Principal component analysis (PCA) was used to perform an exploratory examination of the impacts of cocaine abstinence and HIV gp120 exposure history on NAc core neuroimmune function. Many of these neuroimmune substrates highly correlate with one another, indicating a high degree of multicollinearity within the data. PCA, which compresses large data sets of many interrelated or interdependent variables into a smaller number of composite dimensions (Sainani, 2014), is useful for this type of exploratory analysis. Figure 3 depicts the loading and principal component (PC) score

plots of NAc core neuroimmune expression for the 5-day abstinence animals. Table 1 provides the percent contribution of each neuroimmune factor to the variability explained for each PC. The first two PCs explain 51.94% of the variation in the dataset. Clear and distinct clustering between cocaine and sucrose rats is observed in the PC score plot along PC1. In contrast, PC2 does not parse the data in a clearly distinguishable manner. Figure 5 depicts the loading and PC score plots of NAc core neuroimmune expression for the 21-day abstinence and cue reactivity animals. Table 2 provides the percent contribution of each neuroimmune factor to the variability explained for each PC. The first two PCs explain 60.25% of the variation in the dataset. While less clear than in the 5-day animals, the data appears to be clustered primarily along PC1 by microinjection type (i.e., control vs. gp120).

CHAPTER 5

EXPERIMENT 1 DISCUSSION

The present study is the first to demonstrate that a history of CNS exposure to the HIV protein gp120 during early abstinence impairs the efficacy of a therapeutic compound that is otherwise successful at suppressing cocaine-seeking behavior in rodents (Powell, Namba, et al., 2020). Specifically, rats that received sub-chronic i.c.v. microinfusions of gp120 during the first 5 days of cocaine abstinence showed altered NAc core neuroimmune function and resistance to treatment with the novel dopamine D3 receptor (D3R) partial agonist MC-25-41. While a history of gp120 exposure alone did not potentiate cue-induced cocaine seeking, the observed effects on MC-25-41 efficacy

raises important questions regarding medications development efforts for the treatment of psychostimulant use disorders among populations of individuals suffering from comorbidities such as HIV.

MC-25-41 Reduces Cue-Motivated Cocaine Seeking

The dopamine D3 receptor (D3R) has long been implicated in cue-motivated drug seeking (Neisewander et al., 2014; Pilla et al., 1999). These receptors are particularly enriched along mesocorticolimbic reward circuits (Heidbreder et al., 2005; Sokoloff et al., 2006) and appear to underlie drug-seeking behavior in several preclinical rodent models of drug abuse (Cervo et al., 2003; Galaj et al., 2015; Jordan et al., 2019; Vorel et al., 2002; Xi et al., 2006; You et al., 2019). Notably, human brain imaging studies have shown enhanced binding of D3Rs in cocaine-dependent individuals (Boileau et al., 2015; Matuskey et al., 2014; Payer et al., 2014), and rats exhibit increased D3R expression and binding within the striatum following protracted abstinence from cocaine that is accompanied by progressive enhancement of cocaine-seeking behavior (Conrad et al., 2010; Neisewander et al., 2004; Tran-Nguyen et al., 1998). Interestingly, this increase in D3R expression and binding may depend on associative learning processes, as rats that form novel, conditioned associations between cocaine and environmental stimuli exhibit increased D3R expression within striatum (Le Foll et al., 2002). Considering the high affinity of D3Rs for dopamine compared to other dopamine receptors (e.g., >20-fold higher compared to D2Rs), it is likely that even small changes in receptor expression can have significant effects on synaptic activity and, subsequently, motivated behavior

(Nakajima et al., 2013; Sokoloff et al., 1990). Taken together, D3Rs are a promising target for drug development to help treat psychostimulant misuse, and various preclinical assessments have provided significant evidence in support of this approach.

Several studies examining the role of D3Rs in facilitating cocaine-seeking behavior have demonstrated that attenuating D3R signaling may be an effective treatment strategy to reduce drug craving and relapse in humans (Galaj et al., 2018; Neisewander et al., 2014). Specifically, preclinical rodent studies have demonstrated that inhibition of D3R signaling with antagonists or partial agonists attenuates cocaine self-administration under high- but not low-effort schedules of reinforcement and that these effects are not necessarily due to direct pharmacological attenuation of cocaine's acute reinforcing properties (Caine et al., 2012; Di Ciano et al., 2003; Gál & Gyertyán, 2003; Galaj et al., 2014; Powell et al., 2018; Xi et al., 2006). Moreover, we and others have previously shown that inhibition of D3Rs decreases conditioned responding for cocaine-paired cues (Gilbert et al., 2005; Gyertyán et al., 2007; Powell et al., 2018; Powell, Namba, et al., 2020) as well as cocaine-induced reinstatement of cocaine seeking (Achat-Mendes et al., 2010; Vorel et al., 2002; Xi et al., 2006). Interestingly, several studies have also demonstrated that D3R antagonists may effectively attenuate drug seeking behaviors across other drugs of abuse [e.g., opioids (Jordan et al., 2019; You et al., 2019), methamphetamine (Higley et al., 2011), nicotine (Andreoli et al., 2003), and alcohol (Vengeliene et al., 2006)]. Taken together, these studies demonstrate considerable

translational potential for the development of novel D3R pharmacotherapeutics that attenuate drug craving and subsequent relapse in humans.

We and others have sought to develop novel D3R compounds that have an extended half-life and favorable pharmacokinetic and pharmacodynamic properties that demonstrate preclinical efficacy at reducing drug craving and drug-seeking behavior. For example, we have recently reported that the novel D3R partial agonist LS-3-134, which is an arylamide phenylpiperazine that exhibits over 160-fold selectivity for D3Rs over D2Rs (Rangel-Barajas et al., 2014), inhibits cocaine self-administration on a high-effort schedule of reinforcement and cue-induced reinstatement of cocaine seeking (Powell et al., 2018). Similarly, we have previously demonstrated that the arylamide phenylpiperazines OS3-106 and WW-III-55, which both act as D3R partial agonists with high selectivity for D3Rs, inhibit cocaine self-administration (Cheung et al., 2013). Specifically, WW-III-55 selectively inhibited cocaine self-administration only under a high-effort schedule, which is consistent with other reports mentioned previously. The present study examined the therapeutic efficacy of a novel D3R partial agonist, MC-25-41, that exhibits a high affinity for D3Rs (D3R K_i = 0.50 nM), 1486-fold increased selectivity for D3Rs over D2Rs, an extended half-life of >60 m in human and rat liver microsome assays, and only 19.4% maximum activity in the forskolin-dependent adenylyl cyclase activity inhibition assay (Chen et al., 2019; Powell, Namba, et al., 2020). In line with our previous findings showing reductions in cocaine motivation with MC-25-41 treatment (Powell, Namba, et al., 2020), we show that MC-25-41 significantly

attenuates cue-induced cocaine seeking after a period of protracted abstinence. Taken together, our findings contribute to a wide breadth of literature implicating D3R inhibitors as a potentially effective pharmacotherapeutic to treat CUDs. However, MC-25-41 failed to attenuate cocaine seeking in rats with a history of CNS gp120 exposure, which may be due in part to gp120-induced impairments in neuroimmune function described above. Regardless, the results from our behavioral experiments suggest that comorbidities such as HIV need to be built into future preclinical assessments of novel medications to better understand the scope of their efficacy within clinical populations.

A History of gp120 Exposure Impairs the Efficacy of MC-25-41

The present findings are the first to show that a history of HIV gp120 exposure within the CNS during early cocaine abstinence can impair the efficacy of a novel therapeutic that is otherwise successful at suppressing cocaine motivation. Most studies examining the preclinical efficacy of novel medications to treat CUDs do not account for comorbidities that often occur within clinical populations. For example, nearly half of young adults who have a mental illness also meet the diagnostic criteria for a SUD, and over 60% of adolescents enrolled in community-based SUD treatment programs also meet the DSM criteria for other mental disorders (Hser et al., 2001; Kelly & Daley, 2013; Ross & Peselow, 2012). A large, multi-site study within the U.S. of over 10,000 HIV-positive adults found a SUD prevalence rate of 48%, with a 20% rate of polysubstance use and an 11% prevalence rate of CUDs, which is substantially higher than the national average (Hartzler et al., 2017). Many studies have characterized the deleterious effect of

substance misuse on HIV treatment outcomes, which includes reduced antiretroviral treatment (ART) adherence and healthcare utilization as well as increased difficulty in managing viral load (for review, see Durvasula & Miller, 2014). Regardless of treatment adherence (Rasbach et al., 2013), studies have shown that cocaine can impair the efficacy of ART, possibly through direct drug-drug interactions (Kumar et al., 2015). Thus, with no FDA-approved medications that adequately treat CUDs, individuals with comorbid HIV and CUDs are particularly vulnerable to poorer health outcomes.

Enhanced neuroinflammation causes aberrant changes to dopaminergic and glutamatergic synapses, which could promote cue-induced cocaine seeking. For example, ceftriaxone, which inhibits cue-induced reinstatement of cocaine seeking and restores the expression of GLT-1 (Knackstedt et al., 2010; Trantham-Davidson et al., 2012), attenuates TNFα expression in microglia within a model of traumatic brain injury (Lim et al., 2021). Ceftriaxone also attenuates tobacco smoke withdrawal-induced increases in anxiety-like behavior as well as NF-κB and TNFα mRNA expression within the PFC, VTA, and NAc (Hammad et al., 2021). Considering proinflammatory cytokines such as TNFα, IL-1β, and IFNγ as well as oxidative and nitrosative stress can decrease GLT-1 expression (Tilleux & Hermans, 2007), it is possible ceftriaxone's anti-inflammatory properties at least partially contribute to its restorative effects on glutamate homeostasis. Interestingly, gp120 Tg mice (Melendez et al., 2016) and fetal human astrocytes exposed to gp120 (Z. Wang et al., 2003) exhibit downregulation of striatal GLT-1 expression and

glutamate uptake, which raises the question of whether a history of gp120 exposure can impair the restoration of GLT-1 by drugs like ceftriaxone.

Alongside dysregulation of glutamate homeostasis within the striatum, neuroinflammation may also contribute to impaired dopamine transmission, which could further disrupt the balance of dopamine-glutamate interactions that regulate motivated behavior. Indeed, a vast array of studies converge onto the conclusion that proinflammatory cytokines can impair dopamine neurotransmission (for review, see Felger & Treadway, 2017). For example, chronic interferon alpha (IFNα) administration in rhesus monkeys is associated with anhedonia-like behavior as well as reduced dopamine release and D2R binding within the striatum (Felger et al., 2013). As well, low-dose IL-1β treatment in rats decreased motivation to consume palatable food on a FR-5 schedule of reinforcement without altering overall appetite (Nunes et al., 2014). LPS treatment in mice can also increase the metabolic degradation of monoamines such as dopamine and serotonin within the NAc (Van Heesch et al., 2014). One recent study found that HIV Tg rats, which experience chronic neuroinflammation prior to cocaine exposure, fail to choose cocaine over sucrose and show diminished responding for sucrose, suggesting a state of apathy (Bertrand et al., 2018). However, these rats did not have a history of cocaine self-administration and abstinence prior to HIV protein exposure. Thus, it remains unclear whether drug-induced disruption of glutamate homeostasis and neuroimmune function *prior* to HIV exposure renders NAc synapses vulnerable to glutamate-driven hyperexcitability, diminished dopamine signaling and

inhibitory tone over glutamatergic afferents, and subsequent hyperexcitability of postsynaptic medium spiny neurons that drive cue-induced cocaine seeking. While we did not observe a significant increase or decrease in cue-motivated cocaine seeking among gp120-exposed rats, gp120-induced impairments in NAc dopamine release could have accounted for the failed efficacy of MC-25-41 observed in gp120-exposed rats. Future studies examining how HIV protein exposure *after* a period of drug use and abstinence impacts dopamine-glutamate interactions are warranted and would reveal important insights into novel mechanisms that can be leveraged for the development of medications intended to treat comorbid HIV and CUDs.

HIV gp120-Induced Neuroimmune Dysregulation in the NAc Core

Through principal component analysis (PCA) of cytokine, chemokine, and growth factor expression within the NAc core of cocaine-withdrawn rats exposed to gp120, we discovered that gp120 alone produces a distinct profile of neuroimmune changes compared to unexposed sucrose rats. Moreover, we revealed that early cocaine abstinence alone also impairs neuroimmune function regardless of gp120 exposure. Many studies have demonstrated that HIV proteins such as gp120 and Tat can induce neurotoxicity and neuroinflammation within the striatum and other mesocorticolimbic structures. For example, exposure of rat hippocampal cell cultures to Tat protein increases oxidative stress and neuronal degeneration, which is potentiated by cocaine (Aksenov et al., 2006). Tat also induces oxidative stress as well as microgliosis and astrogliosis within the rat striatum (Aksenov et al., 2001, 2003). Within human mesencephalon/glia cell culture

preparations that are rich in dopamine neurons, gp120 also induces neurodegeneration and oxidative stress (Hu et al., 2009), implicating dopamine neurons as a susceptible cell population that is impacted by gp120 exposure. Similar to Tat, cocaine potentiates cellular toxicity, oxidative stress, and NF-κB pathway activation induced by gp120 exposure within rat primary astrocyte cell cultures (Y. Yang et al., 2010). In contrast to these studies, one study investigating i.c.v. administration of gp120, using a dose two-times greater than the dose used in the present study found no evidence of neurodegeneration after either 1, 7, or 14 days of administration (Bagetta et al., 1994). Nevertheless, a gp120 dosing regimen similar to that used in the present study found spatial learning impairments (J. Dong et al., 2008). Thus, it is unlikely that the observed effects of gp120 on NAc core neuroimmune function and subsequent cocaine-seeking behavior are due to accompanying neurodegeneration and neurotoxicity.

Recent studies using transgenic animal models that constitutively express HIV proteins such as gp120 and Tat provide further insights into how these viral proteins impact mesocorticolimbic neuroimmune function. One recent study utilizing MicroPET imaging in gp120 transgenic (Tg) mice found that, similar to PET studies among people living with HIV (Hammoud et al., 2005), gp120 Tg mice exhibit increased radioligand binding to translocator protein (TSPO; a microglial activation marker) within the striatum, hypothalamus, and hippocampus in response to a LPS challenge compared to wild type controls (Young et al., 2022). Another recent study found that morphine treatment in gp120 Tg mice increases RNA expression of CCL2, CCL5, CXCL10, TNFα,

and IFNγ within the hippocampus (Canonico et al., 2022), suggesting that drugs of abuse may enhance neuroinflammation within key limbic structures that regulate motivated drug-seeking behavior. Nevertheless, a key question that remains is whether abstinence is a key phase in the addiction cycle that significantly modulates the CNS neuroimmune response to HIV. Recently, de Guglielmo et al., 2020 showed that HIV transgenic rats, which constitutively express 7 of the 9 HIV proteins including gp120 and Tat, exhibit enhanced escalation of methamphetamine (METH) self-administration after a 1-month period of abstinence as well as increased progressive ratio responding for METH. These rats also showed enhanced neuroinflammation within the prefrontal cortex, including differential expression of TNFα, inflammasome pathways, and type I and II interferon pathways. In line with these findings, we observed differential expression of IFNγ, eotaxin, IL-1α, CX3CL1, IL-18, among other factors, in gp120-exposed rats during early abstinence. We did not find gp120-induced potentiation of cocaine seeking behavior following a period of protracted abstinence, but we did observe a distinct profile of NAc core neuroimmune changes among gp120-exposed rats that was primarily driven by differential expression of CXCL5, CXCL2, IL-2, CCL3, IL-1α, IFNγ, among many others. Importantly, MC-25-41 did not appear to restore the profile of NAc neuroimmune function within gp120-exposed rats, which could be why MC-25-41 failed to attenuate cocaine seeking in gp120-exposed rats. Nevertheless, our findings corroborate previous work that demonstrates neuroinflammation induced by HIV protein exposure within the

CNS and highlight drug abstinence as a critical period in the addiction cycle that can modulate HIV-induced neuroimmune and behavioral dysfunction.

CHAPTER 6

EXPERIMENT 2 METHODS & MATERIALS

Subjects

Male and female Sprague-Dawley rats (N = 58, 201–225 g) were obtained from Charles River Laboratories and were housed on a 14:10 reverse light:dark cycle in a temperature- and humidity-controlled vivarium. All rats had ad libitum access to water throughout the study and began food restriction (maintained at 85% of their free-feeding weight) prior to self-administration training. All experiments were conducted during the dark phase. Seven rats were excluded from the study due to misplaced viral infusions within the surrounding nucleus accumbens shell (NAcSh) or dorsal striatum (dSTR) or due to clogged guide cannulas. Figure 6D depicts viral microinfusion placement for rats included in this study. Six cocaine rats were excluded from the study due to intravenous catheter failure or failure to acquire cocaine self-administration. A power analysis based on prior work examining the effects of viral vector-mediated inhibition NAc NF-κB pathway signaling on cocaine CPP (Cohen, 1973; Lakens, 2013; Russo et al., 2009) revealed an effect size of $\eta^2_p = 0.304$ (i.e., Cohen's $f = 0.5913$). A total sample size N = 25 was determined based on an a priori power of $1- \beta = 0.80$ for a 2x2 factorial analysis. All procedures were approved by the Arizona State University Institutional Animal Care and Use Committee and were conducted in accordance with the National Institutes of

Health's Guide for the Care and Use of Laboratory Animals. A timeline of experimental procedures described herein is provided in Figure 6A.

Drugs

Cocaine hydrochloride (RTI International, Research Triangle Park, NC, USA) was dissolved in sterile saline to a stock solution of 10 mg/kg, which was further diluted with sterile saline to 0.75 mg/kg. Post-operative medications were all diluted with sterile saline to their appropriate doses (see below).

Surgical Procedures

All animals underwent implantation of intracranial guide cannulae aimed at the NAc core, and cocaine self-administering animals also received jugular vein catheters as previously described (Namba et al., 2020; Powell, Namba, et al., 2020). Briefly, animals were anesthetized using 2-3% isoflurane and a sterile polyurethane catheter that was inserted 2.5 mm into the right jugular vein of cocaine animals. The other end of the catheter was tunneled subcutaneously through the skin dorsal to the shoulder blades, where it was attached to a cannula that was secured within a harness (Instech Laboratories, Plymouth Meeting, PA, USA). Intracranial guide cannulae (P1 Technologies, Roanoke, VA, USA) were bilaterally inserted to terminate 2 mm dorsal to the dorsomedial NAc core (+1.5 mm A/P, +/- 1.5 mm M/L, and -5.0 mm D/V) and were secured to the skull with dental acrylic. Sham injectors were inserted into the cannulae and protected with dust caps. Immediately after surgery, all animals received buprenorphine (0.05 mg/kg/mL, s.c.) and meloxicam (1 mg/kg/mL, s.c.) post-operatively,

followed by an additional day of meloxicam treatment. Catheterized animals also received cefazolin (10 mg/0.10 mL, i.v.) and heparin (7 units/0.10 mL, i.v.) for 5 days post-operatively, followed by daily heparin twice per day throughout cocaine self-administration to maintain catheter patency. Catheter patency was assessed as needed with methohexital sodium (0.835 mg/0.5 mL, i.v.).

Cocaine and Sucrose Self-Administration, Forced Abstinence, and Cue-Induced Reward Seeking

Animals were trained to self-administer either cocaine or sucrose within operant conditioning chambers containing two levers, a cue light above each lever, a house light, a tone generator, and a food receptacle (Med Associates, Inc, Fairfax, Vermont, USA). One day prior to self-administration, rats were placed inside their respective chambers for 1 hr to allow them to habituate to the environment. Rats began training (2-hrs/day, 6 days/week) on a fixed-ratio (FR)-1 schedule of reinforcement, where one active lever press resulted in a single infusion of cocaine (0.75 mg/kg, i.v.) over 6 sec or the delivery of a sucrose pellet (45 mg/pellet), either of which was paired with the illumination of a cue light and an auditory tone. Six seconds after reinforcer delivery, the light and tone cues were inactivated and a house light was illuminated that signaled a 20-sec timeout period, where active lever presses resulted in no programmed consequences. Within each session, the schedule of reinforcement advanced sequentially from a FR-1 to a variable-ratio (VR)-2, VR-3, and VR-5 as the animals achieved 5 reinforcers within a given hour on a given schedule. The starting schedule between sessions advanced from a FR-1 to a

VR-2, VR-3, and VR-5 if the animals ended the previous 3 sessions on a higher schedule than the starting schedule. These 3 sessions were considered qualifying sessions to advance to the next starting schedule if animals self-administered at least 10 reinforcers/session. Daily sucrose consumption was not verified; however, rats did not leave behind sucrose pellets in the food receptacles beyond the initial FR-1 training phase. Animals were moved into forced abstinence for 21 days once they were stable on a VR-5 starting schedule, where the variability in cocaine infusions or sucrose pellets earned across three consecutive sessions was ≤15% with no upward or downward trends. After 21 days of forced abstinence, rats were tested for cue-induced cocaine or sucrose seeking. Here, rats were placed into their operant chambers for 1 hr, where active lever presses resulted in cocaine- or sucrose-associated cues only. Immediately after cue testing, rats were euthanized for brain tissue collection. We chose this self-administration paradigm because we have demonstrated previously that it produces robust and stable self-administration (Powell, Namba, et al., 2020). As well, we have shown that VR schedules can produce significantly greater response rates during a cue-induced reinstatement test employing an FR-1 cue reinforcement schedule (Acosta et al., 2008). We chose our abstinence timepoint specifically because we and others have established that 21 days of cocaine abstinence is associated with the incubation of cocaine craving effect (Powell, Vannan, et al., 2020; Reichel & Bevins, 2009; Thiel et al., 2012; Tran-Nguyen et al., 1998; Venniro et al., 2016). Thus, we wanted to examine the effect of

manipulating NAc core NF-κB pathway function on cue-motivated cocaine seeking at this critical timepoint of potentiated drug motivation.

Lentiviral Administration and Validation

On day 1 of abstinence, rats received intra-NAc core microinfusions of either a control lentivirus (pLKO.1-puro-CMV-TurboGFP™ positive control lentiviral particles; 10^9 VP/mL, SHC003H, Sigma-Aldrich) or a RELA shRNA lentivirus (pLKO.1-puro-CMV-TurboGFP™-RELA shRNA lentiviral particles; 10^9 VP/mL, TRCN0000235832, Sigma-Aldrich) designed to inhibit expression of the p65 subunit of NF-κB (Garancher et al., 2020; Stanisavljevic et al., 2011). Microinjection needles were inserted 2 mm below the bottom of the cannulae into the dorsomedial NAc core (Figure 6B). Lentivirus was infused at a rate 0.20 µL/min for 10 min and the needle remained in the tissue for another 5 min to allow for complete diffusion of the virus. Validation of p65 knockdown within the NAc core from cocaine- and sucrose-naïve rats was assessed via quantitative immunohistochemistry (Figure 6C).

Tissue Collection, Immunohistochemistry, and Confocal Imaging

Immediately after cue testing, rats were euthanized with pentobarbital (390 mg/kg, 1 mL/kg) and transcardially perfused with 1X phosphate-buffered saline (PBS) and 4% paraformaldehyde (PFA). Brains were stored in 4% PFA overnight and transferred to a 30% sucrose solution for at least 2 days. 40 µm coronal sections of the NAc with visible cannula tracts were collected and stored in 1X PBS + 0.01% sodium azide. For immunostaining of p65 and MMP-9, NAc sections were washed in 1X PBS

and blocked in 1X PBS + 0.1% Triton-X 100 (PBST) + 5% normal donkey serum (NDS) for 2 hrs. Tissue sections were then incubated in 1X PBST + 5% NDS along with anti-p65 (Abclonal, NO:A19653, 1:500) or anti-MMP-9 primary antibody (Abcam, ab228402, 1:500) overnight and then washed 3 x 10 mins with 1X PBST. Next, tissue sections were incubated in 1X PBST + 5% NDS along with secondary antibody (goat anti-rabbit Alexa Fluor® 568, preabsorbed, ab175696, 1:1,000) for 1 hr, washed 3 x 10 mins in 1X PBST, and then mounted and cover-slipped using Vectashield® antifade mounting medium containing DAPI. A Zeiss LSM800 confocal microscope was used to collect z-stack images of p65 or MMP-9 (568 nm), TurboGFP™ (tGFP, 488 nm), and DAPI (405 nm) within the dorsomedial NAc core and NAcSH at 20x magnification. Cannula placement and anatomical precision of viral infusions were visually inspected prior to imaging (using the anterior commissure as an anatomical landmark), and any animals with GFP expression outside of the NAc core were excluded from the study (n = 7). Colocalization of p65 and tGFP and total immunoreactivity (IR) of MMP-9 were quantified using ImageJ.

Data Analysis

Validation of lentiviral knockdown of p65 within the NAc core was analyzed via Student's unpaired t-test. Cocaine infusions and sucrose pellets earned as well as lever presses for each criterion-qualifying session of self-administration were analyzed via repeated measures two-way ANOVAs. Cue-induced cocaine and sucrose seeking were analyzed via three-way ANOVAs, with virus (control vs. shRNA), sex (male vs. female),

and lever (active vs. inactive) as fixed factors. MMP-9 expression was analyzed with two-way ANOVAs, with sex and virus or virus and brain region as fixed factors. Simple linear regression was used to analyze the correlation of NAc core and NAcSh MMP-9 expression with cue-induced cocaine and sucrose seeking. Post-hoc Tukey's, Bonferroni's, or Dunnett's tests were conducted where appropriate to examine significant group differences. The ROUT method for examining potential outliers was utilized where deemed necessary. All tests were run at an $\alpha = 0.05$ significance level using GraphPad Prism version 9.0, and all data are represented as the mean ± SEM. G*Power version 3.1.9.7 was used to conduct the power analysis.

CHAPTER 7

EXPERIMENT 2 RESULTS

Lentiviral Knockdown of NF-κB p65 Expression within the NAc Core

To verify lentiviral knockdown of p65, we examined the degree of colocalization between p65 and tGFP within the NAc core. Lentiviral infusions were administered bilaterally into the dorsomedial NAc core (Figure 6B). An unpaired t-test revealed a significant reduction in colocalization between p65 and tGFP in shRNA-treated rats relative to control rats ($t_{(9)} = 3.352$, *p = 0.0085; Figure 6C). Specifically, the *RELA* shRNA resulted in over 80% reduction in p65 expression within tGFP+ cells, which corroborates previous observations in other studies utilizing this specific vector (Garancher et al., 2020; Stanisavljevic et al., 2011).

Cocaine and Sucrose Self-Administration and Cue-Induced Reward Seeking

A repeated measures two-way ANOVA of cocaine infusions earned during self-administration prior to virus treatment revealed significant main effects of treatment group ($F_{(3,21)}$ = 3.428, p = 0.0328) and session ($F_{(11,228)}$ = 2.805, p = 0.0019), but no session-by-group interaction (Figure 7A). The main effect of session was likely due to an increase in infusions earned during sessions 7-12 compared to session 1 regardless of group (Dunnett's test, *p<0.05), whereas a *post hoc* Bonferroni's test of the group main effect failed to reveal any group differences. A repeated-measures two-way ANOVA of active lever presses during self-administration for cocaine animals revealed a significant main effect of session ($F_{(11,231)}$ = 5.127, p < 0.0001) and a significant session-by-group interaction $F_{(33,231)}$ = 2.503, p < 0.0001), but no main effect of group. The interaction may have been spurious, as there did not appear to be any time-dependent differences in response rate patterns across groups and *post hoc* analysis showed only one difference between CTRL-males and shRNA-females on session 11 (Tukey's test, *p<0.05; Figure 7B). No differences were observed in inactive lever presses among the groups. A repeated-measures two-way ANOVA of sucrose reinforcers earned during self-administration at each qualifying session revealed a significant main effect of session ($F_{(11,198)}$ = 12.50, p < 0.0001) and a significant session-by-group interaction ($F_{(33,198)}$ = 2.962, p < 0.0001), but no significant main effect of group. A *post hoc* Tukey's test showed that sucrose pellets earned did not differ significantly between groups except at sessions 10 and 12, where shRNA-males consumed more sucrose than shRNA-females prior to receiving their respective viral infusions (*p < 0.05, Figure 7C). A repeated

measures two-way ANOVA of active lever presses for sucrose animals revealed significant main effects of session ($F_{(11,198)} = 9.731$, $p < 0.0001$) and a significant session-by-group interaction ($F_{(33,198)} = 8.14$, $p < 0.0001$), but no significant main effect of group. *Post hoc* analysis of the interaction revealed differences among the groups during early (2-6) and late sessions (10-12; Tukey's test, $p<0.05$; Figure 7D) where in general males showed a typical acquisition curve of gradual increase in response rates across sessions, whereas females showed the highest active lever responses rates early during training and later stabilized at a relatively lower rate of responding. No differences in inactive lever presses were observed.

Following self-administration, viral infusion, and forced abstinence, rats underwent a 1-hr cue-induced cocaine or sucrose seeking test as measured by active lever presses. A three-way ANOVA of active and inactive lever presses during the cocaine cue test revealed significant main effects of virus ($F_{(1,21)} = 8.762$, $p = 0.0075$) and lever ($F_{(1,21)} = 134.3$, $p < 0.0001$) as well as significant virus-by-sex ($F_{(1,21)} = 6.30$, $p = 0.0203$) and sex-by-lever interactions ($F_{(1,21)} = 4.579$, $p = 0.0443$) (Figure 8A). Both interactions were due to the low active lever response rate of the shRNA-male group compared to all other conditions. Indeed, this was the only group that failed to show a difference in active versus inactive lever responses, whereas all other groups showed higher active lever responses than inactive lever responses (Tukey's test, $*p<0.01$). Furthermore, the active lever response rate of shRNA-male group was lower than that of all other groups, (Tukey's test, $*p<0.01$). A three-way ANOVA of active lever presses during the sucrose

cue test revealed a significant main effect of lever ($F_{(1,18)}$ = 163.5, p < 0.0001) and significant virus-by-sex ($F_{(1, 18)}$ = 9.468, p = 0.0065) and sex-by-virus-by-lever interactions ($F_{(1, 18)}$ = 4.687, p = 0.0441). A Tukey's multiple comparisons test revealed no significant differences among the groups in active lever presses and significantly greater active lever pressing relative to inactive lever pressing for each group (Figure 8B). The significant interactions observed here were likely due to nonsignificant trends in active lever presses toward a decrease in males and an increase in females due to shRNA treatment. Taken together, these results suggest that knockdown of NAc core p65 attenuates cue-induced cocaine seeking in male, but not female, rats and that this effect is likely reinforcer-specific.

NAc Core Matrix Metalloproteinse-9 (MMP-9) Expression

A two-way ANOVA of total intensity of MMP-9 immunoreactivity (IR) from the NAc core of cocaine animals revealed significant main effects of virus ($F_{(1, 21)}$ = 13.22, p = 0.0015) and sex ($F_{(1, 21)}$ = 5.422, p = 0.03), as well as a significant virus-by-sex interaction ($F_{(1, 21)}$ = 4.394, p = 0.0484) (Figure 9B). Here, CTRL-males showed significantly greater NAc core MMP-9 IR compared to all other groups (Tukey's test, **p < 0.01). For sucrose animals, a two-way ANOVA of MMP-9 IR revealed a significant main effect of sex ($F_{(1, 17)}$ = 20.82, *p = 0.0003) but no significant main effect of virus or significant virus-by-sex interaction (Figure 10B). Taken together, these results demonstrate that male rats exhibit significantly greater levels of MMP-9 within the NAc

core than females and that p65 knockdown significantly reduces MMP-9 levels within the NAc core in males following cocaine abstinence and cue-induced cocaine seeking.

NAcSh Matrix Metalloproteinse-9 (MMP-9) Expression

To determine whether our observed virus-induced changes and sex differences in NAc core MMP-9 expression were brain region-specific, we examined MMP-9 expression within the NAcSh. A two-way ANOVA of NAcSh MMP-9 IR between cocaine males and females revealed a significant main effect of sex ($F_{(1, 21)} = 39.85$, *p < 0.0001) but no significant main effect virus or significant virus-by-sex interaction, indicating that males exhibit higher MMP-9 IR within the NAcSh (Figure 11B). Among cocaine males, a two-way ANOVA revealed significant main effects of brain region ($F_{(1, 20)} = 18.42$, p = 0.0004) and virus ($F_{(1, 20)} = 7.91$, p = 0.0108) and a significant brain region-by-virus interaction ($F_{(1, 20)} = 10.93$, p = 0.0035). Here, NAc core MMP-9 IR in CTRL-males was significantly greater than all other groups (Tukey's test, **p < 0.01; Figure 11C). Among cocaine females, a two-way ANOVA revealed a significant main effect of brain region ($F_{(1, 22)} = 34.49$, *p < 0.0001) but no significant main effect of virus or significant brain region-by-virus interaction, indicating that females exhibited higher MMP-9 IR within the NAc core compared to the NAcSh. No outliers were detected here using the ROUT method at Q = 1% (Figure 11D). NAcSh MMP-9 expression could not be examined among sucrose rats because MMP-9 IR was completely undetectable in all but 2 rats across both males and females. Altogether, these results indicate that intra-NAc core shRNA treatment in males did not significantly reduce MMP-9 expression within

the neighboring NAcSh, and that males exhibit significantly greater MMP-9 expression in the NAcSh compared to females akin to the NAc core.

Correlations Between NAc Core and NAcSh MMP-9 Expression and Cue-Induced Cocaine Seeking

Given the significant sex- and reinforcer-specific effect of intra-NAc shRNA treatment on both MMP-9 expression and cue-induced cocaine seeking, we examined whether NAc MMP-9 IR correlated linearly with cue-induced cocaine and sucrose seeking. Simple linear regression analyses of cue-induced cocaine seeking and NAc core MMP-9 IR among males (Figure 12A) and females (Figure 12B) revealed no significant linear relationships. Similarly, no significant linear relationships were detected between cue-induced sucrose seeking and NAc core MMP-9 IR among males (Figure 12C) and females (Figure 12D). No significant linear relationships between cue-induced cocaine seeking and NAcSh MMP-9 IR were detected among males (Figure 13A) and females (Figure 13B). Taken together, these results suggest that total expression levels of NAc MMP-9 do not significantly correlate with cue-induced cocaine or sucrose seeking for both males and females.

CHAPTER 8

EXPERIMENT 2 DISCUSSION

The present study is the first to describe sex differences and reinforcer specificity regarding the regulatory role of ventral striatal NF-κB signaling in cue-induced reward seeking and associated MMP-9 expression. Specifically, we demonstrate that viral

knockdown of the p65 subunit of NF-κB within the rat NAc core decreases cue-induced cocaine seeking in males but not females. These findings extend previous studies demonstrating a role for NF-κB in cocaine conditioned place preference (Russo et al., 2009) and cue-induced reinstatement of nicotine seeking (Namba et al., 2020). Concomitantly, we demonstrate that males exhibit significantly greater levels of MMP-9 expression within the NAc core than females, that p65 knockdown significantly reduces MMP-9 expression in males but not females, and that this effect is specific to cocaine.

Inhibition of NAc NF-κB Suppresses Cue-Induced Drug Seeking

Drug-induced upregulation of various constituents of the NF-κB pathway within the brain and the peripheral immune system is a consistent finding across numerous *in vivo* and *in vitro* studies (Ang et al., 2008; Crews et al., 2017; Lepsch et al., 2009; López-Pedrajas et al., 2015; Russo et al., 2009). As well, drug-induced dysregulation of the function and expression of various cytokines that activate NF-κB are well documented (Crews et al., 2017; Cui, Shurtleff, & Harris, 2014; Hofford, Russo, & Kiraly, 2019; Jacobsen, Hutchinson, & Mustafa, 2016; Namba et al., 2021). We have previously demonstrated in rats that inhibition of IκB kinase (IKK) within the NAc core during extinction following nicotine self-administration attenuates cue-induced reinstatement of nicotine seeking, and that constitutive activation of IKK prevents the antioxidant *N*-acetylcysteine from attenuating this behavior (Namba et al., 2020). In addition, we showed a concomitant upregulation of tumor necrosis factor alpha (TNFα) protein expression within the NAc core following extinction training, which can stimulate

canonical NF-κB signaling (Namba et al., 2020; Shih et al., 2015). In corroboration, Adeluyi et al., 2019 demonstrated that nicotine withdrawal induces increases in TNFα mRNA expression within the NAc of mice. Other studies implicate enhanced TNFα gene expression within the central amygdala of rats in opioid or alcohol withdrawal (Freeman et al., 2012; O'Sullivan et al., 2019). While such studies suggest that drug withdrawal may promote neuroinflammation, it is largely unknown how neuroimmune function changes across cocaine abstinence. We demonstrate here that inhibiting NAc core NF-κB signaling during cocaine abstinence reduces cue-motivated cocaine-seeking behavior. Taken together, it is possible that immunomodulatory interventions during cocaine abstinence may be effective in reducing cocaine relapse vulnerability, and future studies that further probe this phenomenon across drugs of abuse are warranted.

Sex-Dependent Role of NAc NF-κB Signaling in Cue-Motivated Drug Seeking

Ovarian hormones likely regulate cue-motivated drug seeking as well as neuroimmune function. However, similar to our findings in control rats (Figure 8), others have found no sex differences in cue-induced cocaine seeking (Bechard et al., 2018; Fuchs et al., 2005; Weber et al., 2018) (although see Zhou et al., 2014). Nevertheless, female behavior varies with estrous cycle phase, as females tested during estrus, when estradiol levels are rising (Emanuele, Wezeman, & Emanuele, 2003), show enhanced extinction responding relative to males and non-estrus females (Kerstetter et al., 2008). Moreover, females exhibit enhanced incubation of cue-induced cocaine seeking when cocaine is paired with cues during the estrus phase (Johnson et al., 2019). Furthermore,

females given extended access to cocaine demonstrate enhanced incubation of cocaine seeking during the estrus phase (Corbett et al., 2021; Nicolas et al., 2019). Altogether, these findings suggest that estrous cycle phases significantly modulate the behavioral expression of cue-motivated cocaine seeking, but that this process is likely dependent on behavioral parameters such as drug access conditions, drug training dose, and length of withdrawal.

While the present study did not reveal sex differences in cue-induced cocaine seeking among control rats, sex-differences were observed as a result of NAc core p65 knockdown. This interesting finding is not surprising given the role of ovarian hormones in regulating neuroimmune function and NF-κB signaling (Kalaitzidis & Gilmore, 2005; Yilmaz et al., 2019). For example, 17β-estradiol (E2) treatment in BV2 microglia cells inhibits oxidative stress, reduces nuclear translocation of NF-κB, and attenuates ethanol-induced neuroinflammation and neurotoxicity in the postnatal rat brain (Khan et al., 2017). Similarly, E2 reduces neuroinflammation and NF-κB activation in a rat model of cerebral ischemia (Wen et al., 2004). Progesterone also exhibits similar anti-inflammatory properties. For instance, progesterone protects rats against effects of repeated mild traumatic brain injury and associated neuroinflammation, oxidative stress, and impairments in cognition (Webster et al., 2015). Like E2, progesterone also suppresses lipopolysaccharide (LPS)-induced neuroinflammation and NF-κB activation in BV2 microglia (Lei et al., 2014). Unlike E2, several studies suggest that elevated progesterone levels can suppress cocaine-seeking behavior in females (Feltenstein et al.,

2009; Feltenstein & See, 2007; Mello et al., 1997), and peak progesterone levels tend to coincide with the proestrus phase of the estrous cycle (Emanuele, Wezeman, & Emanuele, 2003). Moreover, while many studies indicate progesterone and E2 can suppress NF-κB, other studies have shown that these hormones activate NF-κB under certain conditions (Mo et al., 2013; Stice et al., 2012). Indeed, NF-κB bidirectionally regulates genes that are critically involved in cue-motivated drug seeking, such as the astrocytic glutamate transporter GLT-1 (Karki et al., 2013; Lee et al., 2008; Scofield et al., 2016; Sitcheran et al., 2005). While some studies show that progesterone and E2 are positive regulators of GLT-1 (Pawlak, Brito, Küppers, & Beyer, 2005; Nematipour et al., 2020), chronic drug use also produces significant disruptions in the menstrual/estrous cycle (King et al., 1990; Mello et al., 1997). Thus, how ovarian hormones interact with NF-κB to promote or repress the expression of addiction-related genes is largely unknown.

While this study did not examine the role of the estrous cycle in modulating cue-induced cocaine seeking, it is possible that heterogeneity in circulating ovarian hormones across the female rats at the time of cue reactivity testing could have introduced variability into the cocaine-seeking behavior of the females, thus diminishing our ability to detect an effect of shRNA treatment. However, cycling ovarian hormones are likely not the only potential source of sex differences observed in this study. Other factors, such as microglial activity, may be involved in the sex difference observed in this study that may or may not be linked to circulating hormone levels. A recent study examining sex

differences in the analgesic effects of morphine found that morphine reduces pain sensitivity more effectively in males than in females, which is associated with higher basal microglia activation within the periaqueductal gray of females compared to males (Doyle et al., 2017). Importantly, antagonism of toll-like receptor 4 (TLR4), which can activate the NF-κB pathway (Kawai & Akira, 2007), attenuated the development of morphine tolerance in males but not in females. Interestingly, several studies indicate that males may be uniquely susceptible to immune insults during early development (Osborne et al., 2018). For example, one study found that chronic maternal stress increases placental inflammation in males but not in females and that maternal anti-inflammatory treatment attenuates this effect. As well, this study found that early prenatal stress significantly reduces dopamine D2 receptor gene expression within the NAc of males, which is partially rescued by maternal anti-inflammatory treatment (Bronson & Bale, 2014). Collectively, these studies point towards a hypothesis that males, under certain conditions, may be uniquely sensitive to immunomodulation and immune insults compared to females. Indeed, future studies that systematically address this possible sex-specific mechanism are warranted.

Sex-Dependent Regulation of NAc MMP-9 Expression and Associated Synaptic Plasticity

In addition to sex-dependent effects on cue-induced cocaine seeking, we show that inhibition of NAc core p65 expression also produced sex-specific effects on MMP-9 expression. Very few studies have examined sex differences in the expression of MMPs

within the mesocorticolimbic reward system and their subsequent role in addiction-related behaviors. However, one study demonstrated that the acquisition of nicotine CPP was associated with significantly elevated levels of MMP-9 within the hippocampus of female rats, but not after 5 days of withdrawal and nicotine context re-exposure (Natarajan et al., 2013). In contrast, cue-induced reinstatement of extinguished cocaine seeking in male rats is associated with increased MMP-9 expression within the NAc (Smith et al., 2014). Nevertheless, another study has shown that female MMP-9 knockout mice exhibit less motivation to seek alcohol during withdrawal and when access to alcohol is limited (Stefaniuk et al., 2017). Taken together, these studies suggest MMP-9 may be involved in the acquisition, maintenance, and/or persistence of addiction-related behaviors in females. Indeed, studies have shown that ovarian hormones can modulate the expression of MMP-9 (J. R. Li & Shen, 2015; Santos et al., 2011), and it is possible that complex hormone-neuroimmune interactions may underly the mechanistic underpinnings of MMP-9 regulation of cocaine-induced synaptic plasticity and behavior in females. It is largely unknown whether there are significant baseline sex differences in MMP expression across the brain. However, we report here that females, regardless of drug history or viral manipulation, express significantly lower levels of MMP-9 within the NAc core compared to males. It is possible that we could not detect shRNA-induced reductions in MMP-9 among the females due to low basal levels of expression, where sensitivity to detect such changes would be reduced. Thus, we cannot definitively rule out

the possibility that MMP-9 regulates drug-induced plasticity and behavior among females as it does in males.

Many studies in male rodents have demonstrated a clear role for MMP-9 in the regulation of addiction-related behaviors and associated synaptic plasticity processes. MMP-9 mRNA can be transported to, and locally translated and released from, dendrites in an activity-dependent manner (Dziembowska et al., 2012) and MMP-9 modulates cell-surface expression of glutamate receptors to control dendritic spine plasticity (Gawlak et al., 2009; Szepesi et al., 2014). As well, MMP-9 activity facilitates the formation of silent synapses within the hippocampus, and silent synapse formation within the NAc is thought to underly the retrieval and reconsolidation of cocaine memories (Magnowska et al., 2016; Wright et al., 2019). Cocaine-primed reinstatement of cocaine CPP in rats is associated with increased expression of MMP-9 within the medial prefrontal cortex (Brown et al., 2008) and broad-spectrum, systemic inhibition of MMPs disrupts the acquisition and cocaine-primed reinstatement of cocaine CPP (Brown et al., 2007). Inhibition of NAc core MMP-9 is also associated with attenuated cue-induced reinstatement of cocaine seeking (Smith et al., 2014). Parallel to these studies conducted in male rodents, we demonstrated here that a decrease in cue-induced cocaine seeking was associated with a concomitant decrease in NAc core MMP-9 expression in male rats (Figure 9). Altogether, these convergent findings, coupled with the distinct lack of female data in the literature, highlight the need for future investigations into sex differences in

MMP expression across the brain and the physiological role such differences play in the regulation of drug-induced synaptic plasticity and behavior.

Despite observing sex-, reinforcer-, and brain region-specific effects on MMP-9 expression, we did not observe significant correlations of MMP-9 expression with cue-induced cocaine seeking behavior. It is important to note that the antibody used for our MMP-9 IHC experiments stains for total levels of MMP-9 protein, as opposed to only activated MMP-9. Furthermore, behavior varies during the session, but MMP-9 assessment is limited to a single, post-session time point. A previous study by other investigators examining the role of NAc MMP activity in cue-induced reinstatement of drug seeking using in vivo zymography found that NAc core MMP activity is rapidly increased during the first 15 min of cue-induced cocaine, nicotine, and heroin seeking (Smith et al., 2014). Moreover, this study showed that NAc core MMP-9 protein expression is significantly elevated after 45, but not 15, minutes of cue-induced reinstatement of cocaine seeking. Pharmacological inhibition of NAc core MMP-9 also reduced cue-induced cocaine, but not sucrose, seeking as well as dendritic spine head diameter and postsynaptic excitability. Altogether, such findings suggest that MMP-9 activity and expression not only mediate neuronal plasticity within the NAc core, but also changes dynamically within a cue-induced cocaine seeking session. Thus, while we did not observe significant correlations between total NAc core MMP-9 expression and cue-induced cocaine seeking, it is possible that shRNA treatment could have altered MMP-9 activation dynamics during cue-induced cocaine seeking test. Such changes in MMP-9

activation may indeed correlate better with cue-induced cocaine seeking rather than changes in total protein expression.

CHAPTER 9

GENERAL DISCUSSION

The overarching aims of this dissertation were to examine the neuroimmune sequelae within the NAc core of combined cocaine abstinence and systemic HIV gp120 exposure and to probe whether a history of HIV gp120 exposure alters the therapeutic efficacy of the D3R partial agonist MC-25-41 within a rodent model of cue-elicited cocaine seeking behavior. Moreover, we probed the role of downstream, intracellular neuroimmune function in cue-motivated cocaine seeking across sex and reinforcer type by using a viral vector-mediated approach to perturb NF-κB pathway signaling within the NAc core. In Experiment 1, we discovered that sub-chronic, systemic exposure to the HIV protein gp120 produced neuroinflammation within the rat NAc core, which contrasts with a profile of immunosuppression observed in the NAc core of rats in early abstinence from cocaine self-administration. Interestingly, we did not observe a unique synergistic interaction between gp120 and early cocaine abstinence on neuroimmune function within the NAc core. Among cocaine self-administering rats that underwent 3-weeks of abstinence, treatment with the D3R partial agonist MC-25-41 prior to cue reactivity testing successfully attenuated cocaine-seeking behavior akin to our previous findings with this compound (Powell, Namba, et al., 2020). However, a history of gp120 exposure blocked the attenuating effect of MC-25-41 treatment on cue-induced cocaine seeking.

Exploratory examination of the neuroimmune signaling within the NAc core using a broad-spectrum cytokine array approach revealed cocaine abstinence-induced changes in neuroimmune signaling after 5 days of abstinence as well as gp120-induced changes in neuroimmune signaling after 21 days of cocaine abstinence and cue reactivity testing. In Experiment 2, we discovered that intra-NAc core knockdown of the NF-κB protein subunit p65 attenuated cue-induced cocaine, but not sucrose, seeking in male rats. Moreover, we observed a significant reduction in the expression of the extracellular matrix enzyme MMP-9 in the NAc core of male, but not female, rats following viral knockdown of p65. Importantly, male rats exhibited significantly higher levels of NAc core and NAcSh MMP-9 expression among both cocaine and sucrose rats compared to female rats. Taken altogether, these results suggest that cocaine abstinence may produce significant impairments in neuroimmune function within the brain's reward circuitry and that HIV may impair the therapeutic efficacy of otherwise-successful medications intended to treat CUDs. Importantly, mechanistic insights gained from these studies indicate that males, who are already at a much greater risk for HIV (Centers for Disease Control and Prevention, 2019), may be more responsive to immunopharmacological therapeutics that target cocaine- and HIV-induced immune dysregulation.

HIV-Induced Dysregulation of Learning, Memory, and Synaptic Plasticity Across the Mesocorticolimbic System

Psychostimulant misuse is a significant risk factor for HIV, which can complicate psychostimulant use disorder treatment efforts. The direct effects of cocaine on the

pathophysiology and neuroinflammatory effects of HIV have been well documented (Aksenov et al., 2006; Bagetta et al., 2004; Yang et al., 2010; Yao et al., 2009). Nevertheless, the impact of combined HIV and cocaine abstinence on neuroimmune function and subsequent relapse remain poorly understood. Many studies have utilized transgenic rodent models that constitutively express HIV proteins throughout the CNS to study the effects of HIV on addiction-like behaviors (Bertrand et al., 2018; de Guglielmo et al., 2020; Hoefer et al., 2015; Kesby et al., 2014; Wayman et al., 2016). However, one limitation of such approaches is that animals are chronically exposed to these HIV proteins *prior* to the establishment of drug self-administration and withdrawal, which may produce a different profile of neuroadaptations within mesocorticolimbic circuitry compared to exposure to HIV proteins *after* chronic drug exposure and abstinence. Indeed, many individuals that misuse psychostimulants contract HIV through engaging in risky behaviors associated with drug misuse (e.g., needle sharing, risky sexual practices; Centers for Disease Control and Prevention, 2021). Despite the limitation of microinfusions lacking other important HIV proteins (e.g., Tat), one significant advantage of the gp120 protein exposure approach used in the present experiments is the ability to maintain temporal control over HIV protein exposure within the CNS. This HIV protein was selected for these experiments because of its significant role in facilitating viral entry into the CNS and for its well-known effects on neuroinflammation (Atluri et al., 2015; Bagetta et al., 1994; Bansal et al., 2000; Shah et al., 2013; Strazza et al., 2011). Nevertheless, many studies using the aforementioned transgenic rodent models have

revealed important insights into how HIV and its protein products may dysregulate synaptic plasticity within the reward system.

Among virally-suppressed individuals living with HIV, upwards of 40% eventually develop cognitive dysfunction in the form of HIV-associated neurocognitive disorder (HAND) (Cysique & Brew, 2011; Heaton et al., 2010), which can range from asymptomatic neurocognitive impairment to HIV-associated dementia. Rodent models, such as the HIV transgenic (Tg) rat, recapitulate many neurocognitive features of HAND (Vigorito et al., 2015). For example, HIV Tg rats, which constitutively express 7 of the 9 HIV proteins, display spatial learning and memory impairments when tested in the hippocampus-dependent Morris water maze (Vigorito et al., 2007, 2013). Similarly, the HIV Tat Tg mouse, where Tat expression is induced within astrocytes upon doxycycline administration, show impaired dendritic spine density and excitatory postsynaptic field potentials within the hippocampus as well as performance in the Morris water maze (Fitting et al., 2013). Interestingly, Tg mice that constitutively express gp120 in astrocytes show learning deficits in an attentional set-shifting task that parallel those observed in humans, where both mice and humans that used methamphetamine showed the greatest deficits (Kesby et al., 2015). Within the striatum, HIV gp120 Tg mice exhibit impaired glutamate uptake and expression of GLT-1 (Melendez et al., 2016) as well as a decrease in predominantly mushroom spines on medium spiny neurons (MSNs) (Speidell et al., 2020). HIV Tg rats also exhibit reductions in mushroom spines as well as increases in stubby spines at the distal dendritic branches of NAc MSNs (McLaurin, Cook, et al.,

2018), where the majority of PFC-derived glutamatergic and VTA-derived dopaminergic inputs are found (Spiga et al., 2014). Such distal spines can form asymmetric synapses with glutamatergic inputs at the spine head and symmetric synapses with dopaminergic inputs at the spine neck (Spiga et al., 2014; Freund et al., 1984; Zahm, 1992). Thus, a gp120-induced reduction in distal mushroom spines could result in an increase in the ratio of glutamate inputs to dopamine inputs and reduce basal dopamine levels at these synapses.

While many studies have implicated phasic release of dopamine into the NAc core as necessary and sufficient to induce cue-induced cocaine seeking (Phillips et al., 2003; Solecki et al., 2013; Stuber et al., 2005), other studies alternatively suggest that NAc core dopamine may produce a suppressive (or "satiating") effect over cocaine seeking (Spencer et al., 2017; Suto et al., 2010; Suto & Wise, 2011). Studies examining extracellular dopamine and glutamate levels within the NAc core during cue-induced cocaine seeking consistently show that dopamine levels remain persistently elevated for upwards of an hour or longer across a test session (Neisewander et al., 1996; Suto et al., 2010), while glutamate levels rise within the first 15 minutes and decline over time (returning to near-baseline levels after 1-2 hrs of testing) parallel to the within-session decline in active lever presses (Gipson, Reissner, et al., 2013; LaLumiere & Kalivas, 2008; A. C. W. Smith et al., 2017). These temporal patterns of NAc core dopamine and glutamate levels are consistent with the hypothesis that maintenance of increased dopamine overflow within the NAc core across a cue-induced cocaine seeking test could

provide negative feedback to tone down glutamate release onto postsynaptic MSNs. Indeed, several converging lines of evidence support this hypothesis. For example, a recent study demonstrated that the L-type calcium channel inhibitor isradipine inhibits cue-induced cocaine seeking through an increase in NAc core dopamine overflow (Addy et al., 2018). Interestingly, the effects of isradipine were abolished by intra-NAc core microinfusion of flupenthixol, which is a non-selective dopamine receptor antagonist. Cocaine abstinence is associated with decreased basal dopamine levels within the NAc (Parsons et al., 1991). As well, protracted withdrawal from cocaine decreases D2R and increases D3R cell surface expression within the NAc core (Conrad et al., 2010), and dopamine will preferentially bind to high-affinity dopamine receptors such D3Rs at low concentrations (Maramai et al., 2016). Altogether, it is possible that impaired NAc core dopamine transmission during abstinence and a shift towards elevated D3R binding (Neisewander et al., 2004) may contribute to glutamate-driven hyperexcitability of MSNs during cue-induced cocaine seeking. Indeed, D3R inhibition normalizes activity of hyper-responsive glutamatergic neurons within the PrL (Sokoloff et al., 2013) and increases dopamine within the NAc (Huang et al., 2019). D3R knockout mice also exhibit increased NAc dopamine levels (Koeltzow et al., 1998), and stimulation of the VTA can inhibit NAc EPSPs induced by PFC stimulation likely through a D2R autoreceptor-dependent mechanism on presynaptic glutamatergic terminals (Bamford et al., 2004; Brady & O'Donnell, 2004). Taken together, if gp120 impairs the synaptic triad architecture within the NAc core and reduces dopaminergic stimulation of PrL glutamate

inputs, this may prevent MC-25-41 from reducing cue-induced cocaine seeking. This disruption of homeostatic dopamine-glutamate interactions may be caused in part by dysregulations in neuroimmune function within the striatum, as described in more detail below.

Immunomodulation of Striatal Synaptic Plasticity

Immune regulation of neurophysiology, behavior, and cognition has been a topic of intensive study for the last four decades. In 1991, Ronald S. Smith published an essential paper titled "The Immune System is a Key Factor in the Etiology of Psychosocial Disease," where he highlights early evidence for the involvement of immunomodulation of CNS function and references the emergence of AIDS-related dementia as one of many lines of evidence in support of this hypothesis (R. S. Smith, 1991). Early studies have demonstrated that cytokines such as TNFα, IL-1, IL-2, and IFNγ can exert actions directly within the brain to modulate basic physiological processes such as temperature regulation, sleep, pain perception, feeding behavior, among others (Balkwill & Burke, 1989; Plata-Salaman, 1989; R.S. Smith, 1991). More recently, many studies have elucidated the cellular and molecular mechanisms through which immune signaling modulates synaptic physiology and subsequent behavioral processes. For example, IL-1β, a potent pro-inflammatory cytokine, modulates excitatory transmission within the hippocampus through numerous mechanisms, such as regulating AMPA receptor surface expression, NMDA receptor-mediated currents, and calcium conductance (Lai et al., 2006; Viviani et al., 2006; Yang et al., 2005). Other pro-

inflammatory cytokines such as IL-6, IFNγ, and IL-18, inhibit excitatory synaptic transmission or promote inhibitory postsynaptic potentials within the hippocampus (Curran & O'Connor, 2001; Flood et al., 2019; Tancredi et al., 2002). While such studies suggest that these proinflammatory cytokines can impair excitatory synaptic transmission and associated learning and memory processes, other studies demonstrate that the modulatory effects of such immune signaling are brain-region specific.

In contrast to the hippocampus, pro-inflammatory cytokines such as IL-1β, TNFα, and interferons exert differential modulatory effects over synaptic plasticity within the striatum. For example, IL-1β increases spontaneous EPSPs and inhibits GABA transmission in rat corticostriatal brain slices, suggesting that IL-1β promotes hyperexcitability of striatal MSNs within the NAc (Rossi, Furlan, et al., 2012; Rossi, Studer, et al., 2012). In a murine model of multiple sclerosis, known as experimental autoimmune encephalomyelitis (EAE) and characterized by striatal neuroinflammation, mice exhibit upregulation of IL-1β and impaired dopamine transmission within the striatum that is recovered by IL-1 receptor antagonism (Gentile et al., 2015). Within this EAE model, TNFα also promotes postsynaptic excitability in MSNs and enhances AMPAR phosphorylation (Centonze et al., 2009) and TNFα i.c.v injections also mimic these synaptic alternations (Haji et al., 2012). Such immunomodulatory factors, which are released by astrocytes and microglia, also regulate dopaminergic transmission within the striatum. For example, synaptically-released dopamine within the NAc of mice activates calcium signaling in astrocytes, leading to the release of adenosine that binds to

presynaptic A1 receptors to inhibit excitatory synaptic transmission (Corkrum et al., 2020). While such evidence suggests that TNFα promotes excitatory postsynaptic excitability, other studies suggest that TNFα suppresses excitatory signaling within the striatum and that NAc TNFα release following cocaine administration in mice serves an adaptive compensatory mechanism to reduce cocaine-induced sensitization and associated plasticity (Lewitus et al., 2014, 2016). Such discrepancies suggest that the role of individual cytokines on neuronal plasticity and behavior is likely brain region-specific and dependent on the disease model.

In addition to glutamate receptors, glial cells also express both D1- and D2-like receptors and dopaminergic stimulation of immune cells can modulate their neuroimmune output (Kawano et al., 2018; Xia et al., 2019). For example, dopamine receptor stimulation on microglia can attenuate nitric oxide release in response to lipopolysaccharide (LPS) exposure, which is a potent proinflammatory stimulus (B. Wang et al., 2019). This anti-inflammatory potential for dopamine may be due to stimulation of low-affinity dopamine receptors (e.g., D1Rs and D2Rs) under high dopamine conditions (Pacheco, 2017). Indeed, stimulation of both microglial and astrocytic D2Rs has been shown to reduce neuroinflammation through modulation of angiotensin signaling and through an αB-crystallin-dependent mechanism, respectively (Dominguez-Meijide et al., 2017; Shao et al., 2013). Similarly, D1R signaling suppresses NLRP3 inflammasome activation and associated increases in IL-1β in striatum-derived mouse microglia and astrocytes (Yan et al., 2015). In contrast to lower-affinity dopamine

receptors, the high-affinity D3R may exacerbate neuroinflammation, although the literature here is less clear. For example, D3R knockout mice exhibit reduced microglial activation within the striatum in response to LPS treatment and reduced inducible nitric oxide synthase expression compared to wild types, suggesting a proinflammatory role of glial D3Rs (Montoya et al., 2019). However, D3R knockout mice exhibit elevated NAc TNFα and IL-6 mRNA expression and depressive behaviors (J. Wang et al., 2020), and intra-NAc knockdown of D3Rs promotes depressive-like behaviors and proinflammatory microglial activation in mice (J. Wang et al., 2022). Taken together, it is possible that low levels of extracellular dopamine within the striatum, observed during periods of withdrawal (Kuhar & Pilotte, 1996), preferentially stimulate high-affinity D3Rs to alter neuroimmune function and drug seeking. Indeed, D3R binding is progressively increased over a period of protracted abstinence (Neisewander et al., 2004). Furthermore, inhibition of striatal D3Rs may ameliorate this effect and contribute to the decrease in cocaine seeking we observed in the present study, and neuroinflammation induced by gp120 exposure may impair this attenuating effect.

Immunopharmacology to Treat SUDs and Associated Comorbidities

Immune system dysfunction among cocaine-dependent individuals is a consistent finding across numerous studies (Araos et al., 2015; Maza-Quiroga et al., 2017; Moreira et al., 2016; Zaparte et al., 2019), and clinical investigations into immunomodulatory therapeutics to treat CUDs have shown some promise. For example, the antioxidant *N*-acetylcysteine (NAC), which inhibits cue-induced cocaine and nicotine seeking (Namba

et al., 2020; Reissner et al., 2015), has shown some efficacy in clinical studies at reducing cocaine use and cravings among cocaine-dependent individuals (LaRowe et al., 2013; Mardikian et al., 2007). NAC inhibits TNFα-induced NF-κB activation (Oka et al., 2000) and we have shown that NAC also attenuates TNFα within the NAc core of rats in withdrawal from nicotine (Namba et al., 2020). Taken together, it is possible that NAC's anti-inflammatory activity may contribute to its therapeutic efficacy. While the poor bioavailability of NAC is a therapeutic hurdle, alternative delivery vehicles such as nanoparticles can improve NAC's anti-inflammatory activity and thus may improve its therapeutic potential for CUDs (Markoutsa & Xu, 2017). In contrast to NAC, other immunomodulatory therapeutics have shown checkered success between preclinical and clinical studies across drugs of abuse. For example, while several preclinical studies show that the phosphodiesterase inhibitor ibudilast inhibits drug-seeking behavior (Beardsley et al., 2010; Charntikov et al., 2015; Poland et al., 2016; Snider et al., 2013), a recent randomized, placebo-controlled study of methamphetamine-dependent individuals showed no efficacy of ibudilast in facilitating abstinence (Heinzerling et al., 2019). Similarly, cannabidiol, which has anti-inflammatory properties (Morissette et al., 2021) and reduces cocaine self-administration across numerous preclinical studies (Rodrigues et al., 2020), failed to reduce cocaine craving and relapse among individuals with CUDs in a randomized, placebo-controlled study (Mongeau-Pérusse et al., 2021). Despite these disparate findings, several studies have demonstrated that the use of anti-inflammatories as adjunctive therapeutics may improve neuropsychiatric treatment outcomes

(Akhondzadeh et al., 2007; Arabzadeh et al., 2015; Bauer et al., 2018; Berk et al., 2014). Indeed, future studies that examine the effiacy of anti-inflammatories as adjunctive therapeutics for SUDs are warranted. Importantly, the present findings highlight the need for such studies to consider sex as an important biological variable that may modulate the therapeutic efficacy of immunomodulatory medications.

Concluding Remarks

We demonstrate with the present collection of studies that a subchronic history of HIV protein exposure within the CNS can produce enduring neurobiological and behavioral adaptations that can diminish the therapeutic efficacy of a dopamine D3R partial agonist treatment that is otherwise successful at suppressing cocaine motivation. As well, we provide evidence that this treatment-resistance may be due in part to neuroimmune dysfunction within the mesolimbic reward system produced by HIV protein exposure. Further probing the role of mesolimbic neuroimmune function in regulating drug-seeking behavior, we show that suppression of NAc NF-κB signaling inhibits drug-seeking behavior only in males. As well, we show that inhibition of this critical neuroimmune signaling pathway sex-specifically inhibits downstream expression of the extracellular matrix enzyme MMP-9, which critically modulates drug-seeking behavior and associated synaptic plasticity. These effects were specific cocaine seeking, as viral suppression of NAc NF-κB signaling had no effect on sucrose seeking. This body of work highlights the need for future studies to consider not only sex as an important biological variable within preclinical SUD research, but also comorbidity status. As

highlighted numerous times throughout this dissertation, comorbidities such as depression, anxiety, chronic pain, HIV, etc. interact uniquely with chronic drug use to produce distinct neurobiological adaptations that may impact the therapeutic efficacy of novel medications intended to treat SUDs (Namba et al., 2021). Moreover, these comorbidities are experienced far too commonly among those living with SUDs for the field to overlook building them into standard preclinical SUD paradigms. The present findings underscore how motivation for cocaine and the neuroimmune milieu of cocaine seeking are particularly sensitive to these comorbidity interactions.

Adolescents and women may be particularly vulnerable to poorer treatment outcomes associated with comorbidities (Tomlinson et al., 2004; Zilberman et al., 2003). As well, with the success of ART in helping PLWH live longer, healthier lives, nearly 50% of PLWH are also aged 50 or older (U.S. Department of Health and Human Services, 2021). Thus, future preclinical research on treatment outcomes for comorbid HIV and SUDs should also consider age and sex. Indeed, this represents both a limitation and a future direction for the present work, which only examined the effects of gp120 exposure on cue-induced cocaine seeking in male subjects of the same age. Another limitation of the present work is that animals were exposed to only one HIV protein subchronically. However, one advantage of a protein approach such as this over transgenic models is temporal specificity over HIV protein exposure. Many people living with SUDs acquire HIV collaterally as a direct (e.g., injection drug use) or indirect result of drug use (e.g., risky sexual practices while under the influence of drugs). Thus, the

administration of HIV proteins into the CNS *after* a history of drug use is a distinct translational advantage over other preclinical models. Nevertheless, future studies could benefit from models such as the EcoHIV model, which can provide temporal specificity over direct administration of a chimeric virus construct that closely mimics HIV and successfully infects murine immune cells (Potash et al., 2005). Characterization of addiction-like behaviors and medications development using such models would be a major advancement in the preclinical study of vulnerable subpopulations of people living with SUDs.

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APPENDIX A

TABLES

Table 1

Percent Contribution of Variables to Principal Components for 5-Day Abstinence Rats

Variable	PC1	Variable	PC2
IFNγ	9.00004	**CXCL1**	13.0436
Eotaxin	7.90629	**Leptin**	11.3308
IL-18	6.92046	**IL-6**	10.7157
CX3CL1	6.63542	**IL-4**	8.6154
IL-1α	6.32284	**IL-5**	6.69141
CXCL2	5.74261	**CCL5**	5.7164
IL-5	5.60452	**G-CSF**	5.41249
IL-2	5.52269	**IL-18**	5.0256
G-CSF	5.5043	**IL-2**	4.76148
CXCL5	5.19406	**EGF**	4.00162
IL-4	5.11392	**CCL3**	3.93828
IL-17a	4.55692	**IL-10**	2.87424
CCL5	4.18523	**GM-CSF**	2.77672
IL-6	4.04935	**IFNγ**	2.62582
VEGF	3.90489	**TNFα**	2.49302
IL-13	3.86777	**IL-17a**	2.21524
IL-10	2.51655	**CXCL10**	1.77452
TNFα	2.36324	**IL-13**	1.38428
CXCL10	2.32309	**IL-1α**	1.21056
GM-CSF	0.86769	**VEGF**	1.07508
EGF	0.66649	**Eotaxin**	0.75798
CXCL1	0.63907	**CX3CL1**	0.7361
IL-1β	0.25212	**IL-1β**	0.40808
CCL3	0.24968	**CXCL2**	0.38433
Leptin	0.09078	**CXCL5**	0.03134

Table 2

Percent Contribution of Variables to Principal Components for 21-Day Abstinence Rats

Variable	PC1	Variable	PC2
CXCL5	7.73927	**CCL5**	28.0526
CXCL2	7.50417	**IL-18**	16.343
IL-2	7.28448	**IL-4**	13.01
CCL3	7.07789	**IL-5**	9.76514
IL-1α	6.94879	**CXCL10**	6.42146
IFNγ	6.90139	**Leptin**	6.17074
IL-13	6.57922	**IL-10**	4.26723
VEGF	6.03599	**CX3CL1**	3.35296
IL-6	5.91354	**GM-CSF**	2.567
GM-CSF	5.85225	**G-CSF**	2.27958
Eotaxin	5.35526	**TNFα**	1.72597
IL-17a	4.10767	**IL-1β**	1.18825
CX3CL1	3.89899	**IL-6**	1.18694
CXCL1	3.4708	**IFNγ**	0.88538
TNFα	2.73603	**IL-13**	0.82143
G-CSF	2.2635	**CXCL1**	0.62198
Leptin	2.23148	**IL-2**	0.60379
CXCL10	1.67075	**EGF**	0.25323
IL-4	1.65319	**CCL3**	0.24546
IL-10	1.37073	**IL-1α**	0.11553
IL-5	1.3549	**IL-17a**	0.0651
IL-1β	1.32058	**Eotaxin**	0.04445
CCL5	0.33805	**CXCL5**	0.01027
IL-18	0.24127	**CXCL2**	0.00254
EGF	0.14983	**VEGF**	1.2E-05

APPENDIX B

FIGURES

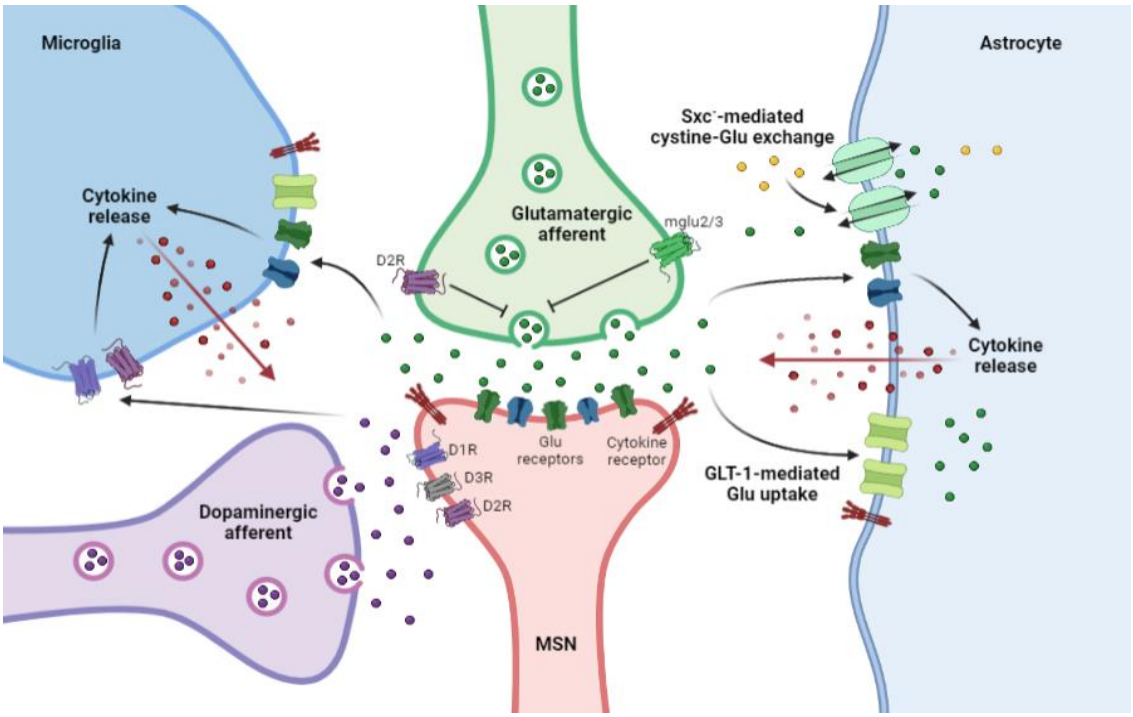


Figure 1. Immunomodulation of striatal dopamine-glutamate interactions. The heads of dendritic spines on GABAergic medium spiny neurons (MSNs) within the nucleus accumbens (NAc) form asymmetrical synapses with glutamatergic afferents, such as those from the prelimbic cortex (PrL), while the dendritic spine necks form symmetrical synapses with dopaminergic afferents from the ventral tegmental area (VTA). Postsynaptically, these spine heads express glutamate and dopamine receptors, which gate the physiological output of NAc MSNs. Such receptors include ionotropic glutamate receptors such as AMPA and NMDA receptors, and metabotropic dopamine receptors such as the dopamine D1, D2, and D3 receptors (metabotropic glutamate receptors not depicted here). D1 receptors are G_s-coupled receptors that stimulate adenylyl cyclase activity, while D2 and D3 receptors are both G_i-coupled receptors that inhibit adenylyl cyclase. However, D3Rs can couple with D1Rs to form a heteroreceptor complex that enhances the stimulatory effects of D1Rs on downstream adenylyl cyclase activity. Both astrocytes and microglia express glutamate transporters, such as GLT-1, which regulate extracellular levels of glutamate. The cystine-glutamate exchanger, system xc^- (Sxc^-), also regulates extracellular glutamate levels by exchanging extracellular cystine for intracellular glutamate. This glutamate provides tone to presynaptic mGlu2/3 autoreceptors, which inhibits the presynaptic release of glutamate. Presynaptic D2Rs also provide inhibitory tone over presynaptic glutamate release upon stimulation by dopamine. Glial cells also express glutamate receptors (e.g., NMDA and AMPA receptors), which can stimulate the release of cytokines and other neuroimmune factors.

These neuroimmune factors can bind to receptors on MSNs to modulate the physiological function of these cells and globally modulate the actions of dopamine and glutamate on MSN activity.

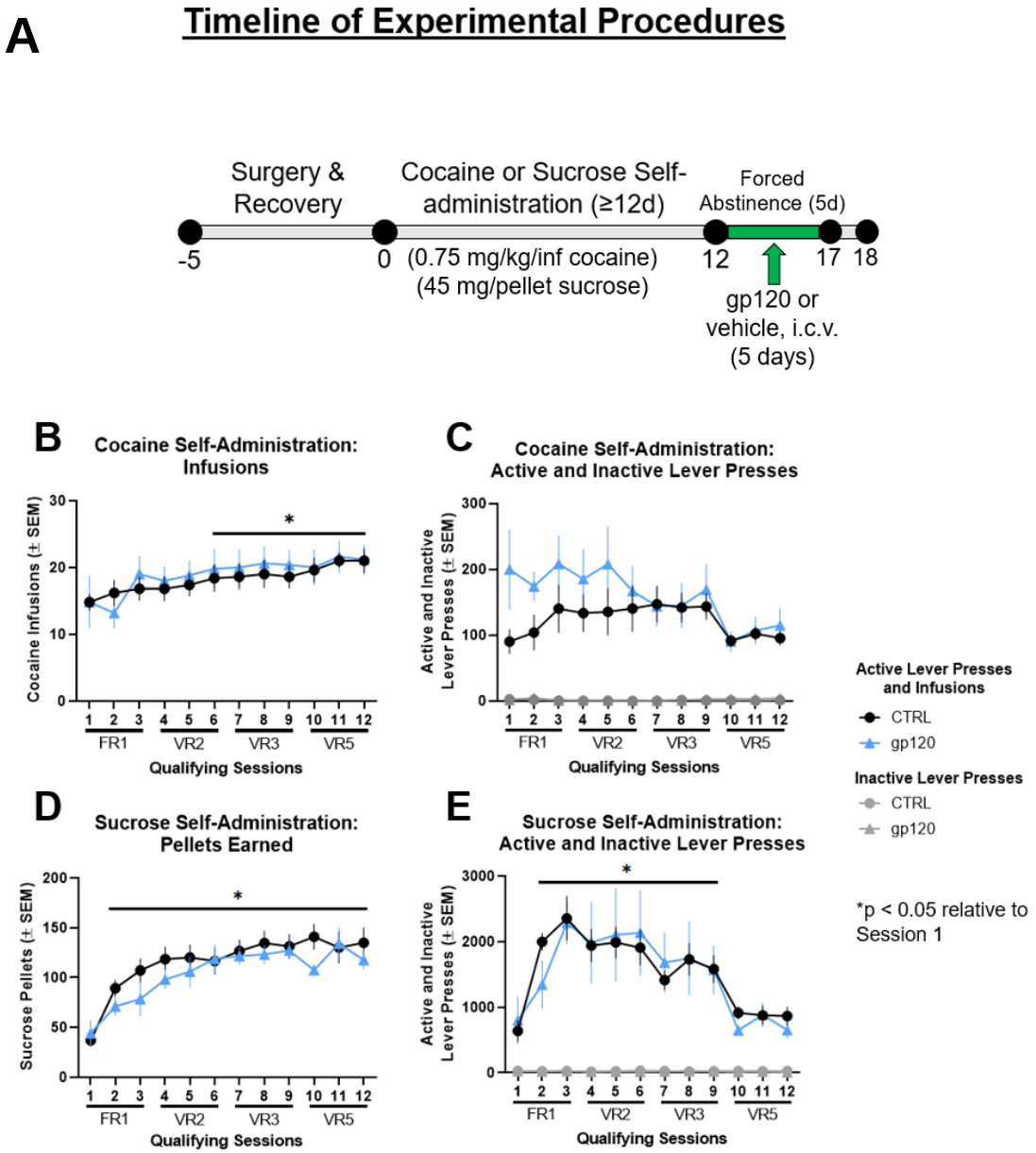


Figure 2. Cocaine and sucrose self-administration prior to gp120 exposure and 5-day abstinence. (A) Timeline of experimental procedures. (B) Cocaine infusions as well as (C) active and inactive lever presses earned for each qualifying session of cocaine self-administration did not differ significantly between the groups prior to their receiving of treatment. Likewise, (D) sucrose pellets as well as (E) active and inactive lever presses for each qualifying session of sucrose self-administration did not differ significantly between the groups prior to their receiving of treatment. *p < 0.05 relative to session 1, regardless of treatment group.

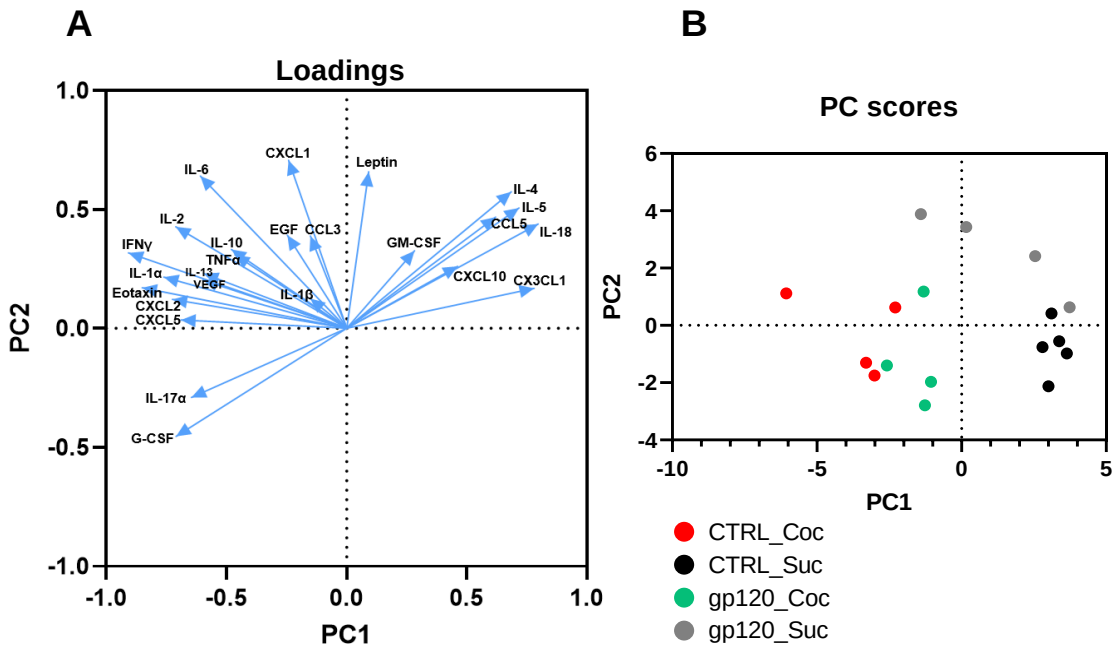


Figure 3. PCA of NAc core neuroimmune signaling after 5 days of cocaine or sucrose abstinence and subchronic i.c.v. gp120 or vehicle exposure. (A) The loading plot generated from PCA reveals the neuroimmune factors that load onto principal component 1 (PC1, x-axis) and principal component 2 (PC2, y-axis). The length of the arrows indicates the relative contribution of each neuroimmune factor to the two PCs. The first two PCs explain 51.94% of the variation in the dataset. (B) Individual PC scores for each animal show distinct clustering between sucrose (black and gray dots) and cocaine (red and green dots) abstinence conditions along PC1.

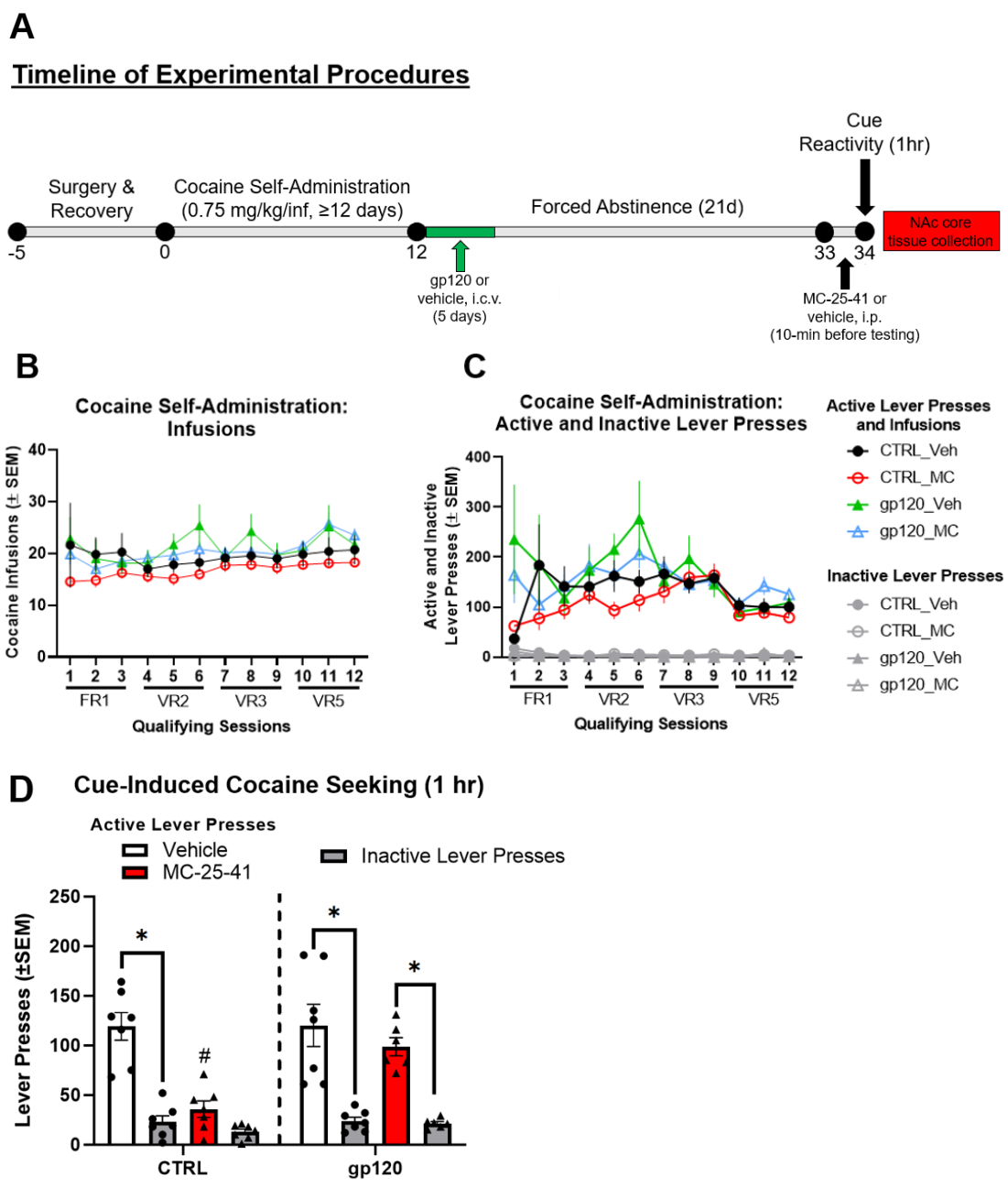


Figure 4. HIV gp120 exposure during early cocaine abstinence prevents the attenuation of cue-induced cocaine seeking by the D3R partial agonist MC-25-41. (A) Timeline of experimental procedures. (B) Cocaine infusions as well as (C) active and inactive lever presses did not differ between the groups during cocaine self-administration at each qualifying session prior to treatment administration and cue testing. (D) MC-25-41

significantly attenuated cue-induced cocaine seeking relative to vehicle treatment in control rats. However, MC-25-41 failed to attenuate cocaine seeking in rats treated with subchronic i.c.v. gp120 during the first 5 days of abstinence. *$p < 0.05$. $^{\#}p < 0.05$ relative to active lever presses of all other groups.

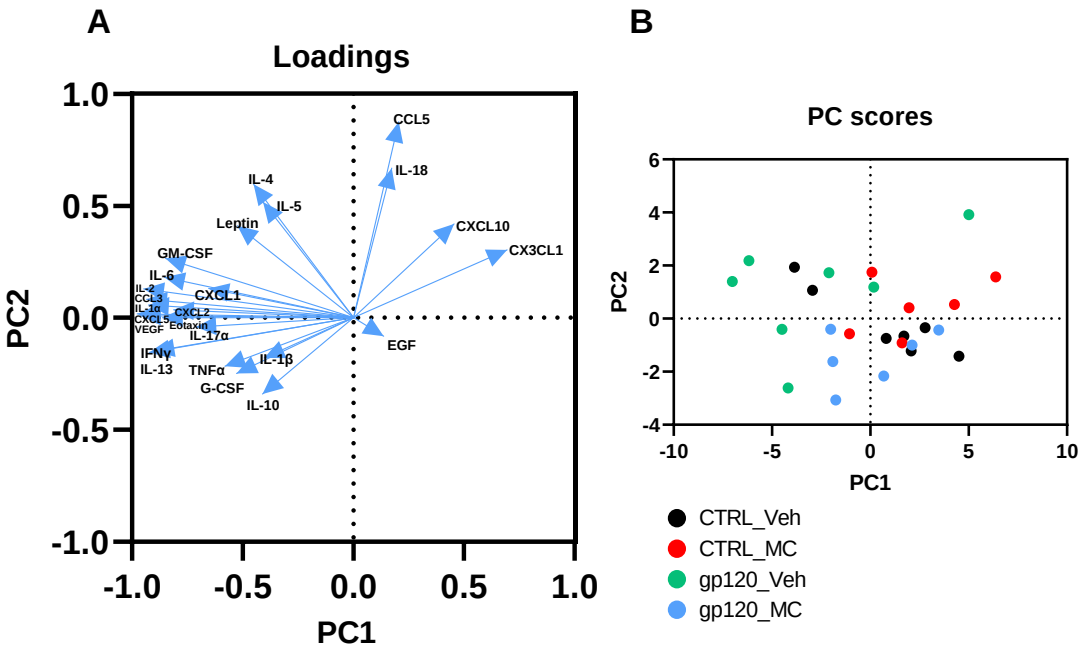
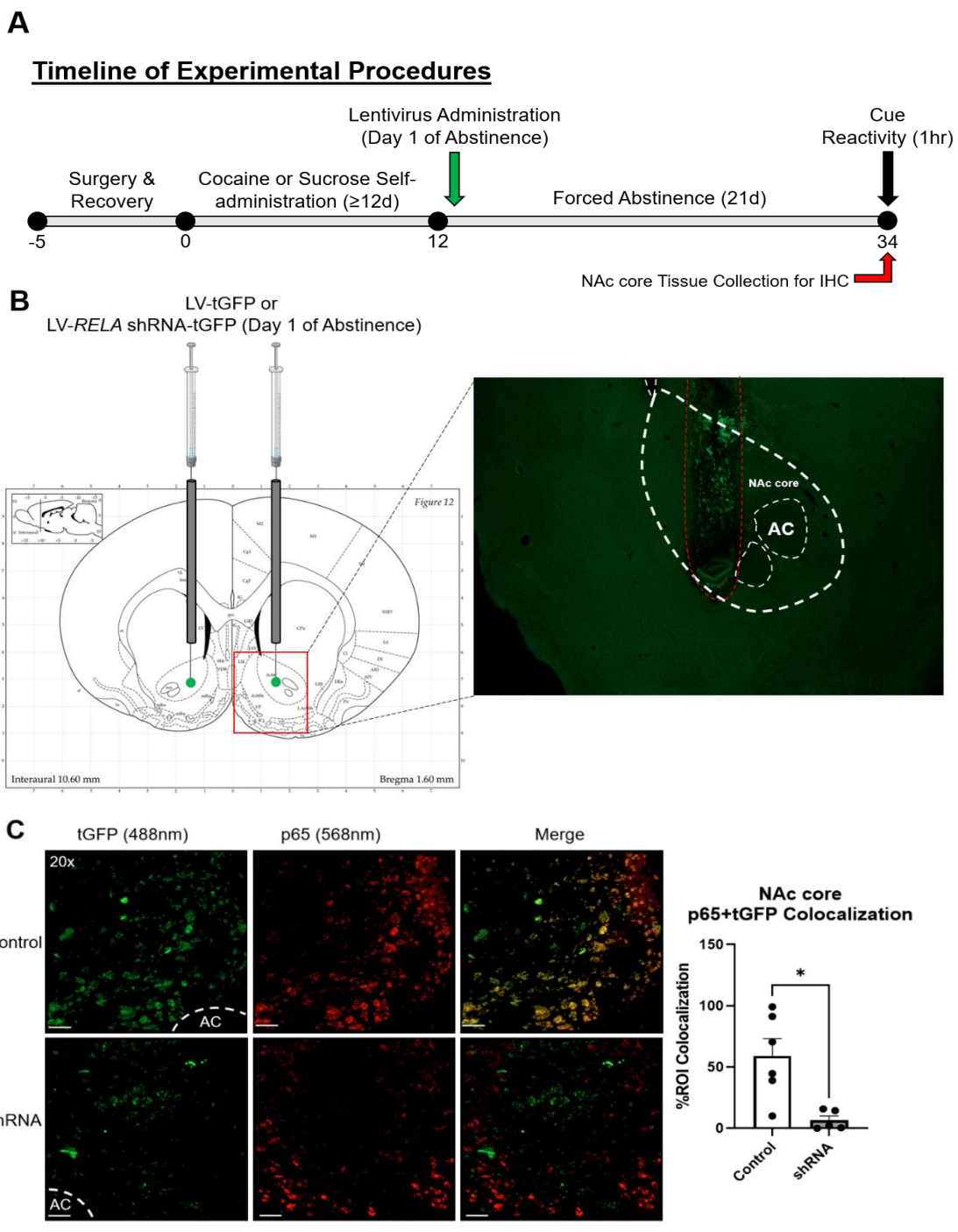


Figure 5. PCA of NAc core neuroimmune signaling after 21 days of cocaine abstinence, subchronic i.c.v. gp120 or vehicle exposure, and cue reactivity testing. (A) The loading plot generated from PCA reveals the neuroimmune factors that load onto principal component 1 (PC1, x-axis) and principal component 2 (PC2, y-axis). The length of the arrows indicates the relative contribution of each neuroimmune factor to the two PCs. The first two PCs explain 60.25% of the variation in the dataset. (B) Individual PC scores for each animal display clustering primarily between control (black and red dots) and gp120 (green and blue dots) groups along PC1, although MC-25-41-treated gp120 rats (blue dots) show less distinct clustering with vehicle-treated gp120 rats (green dots) along PC1.


A
Timeline of Experimental Procedures
Lentivirus Administration
(Day 1 of Abstinence)
Cue
Reactivity (1hr)
Surgery &
Recovery
Cocaine or Sucrose Self-
administration (≥12d)
Forced Abstinence (21d)
-5
0
12
34
NAc core Tissue Collection for IHC
B
LV-tGFP or
LV-RELA shRNA-tGFP (Day 1 of Abstinence)
Figure 12
Interaural 10.60 mm
Bregma 1.60 mm
NAc core
AC
C
tGFP (488nm)
p65 (568nm)
Merge
20x
Control
shRNA
AC
AC
NAc core
p65+tGFP Colocalization
%ROI Colocalization
150
100
50
0
*
Control
shRNA

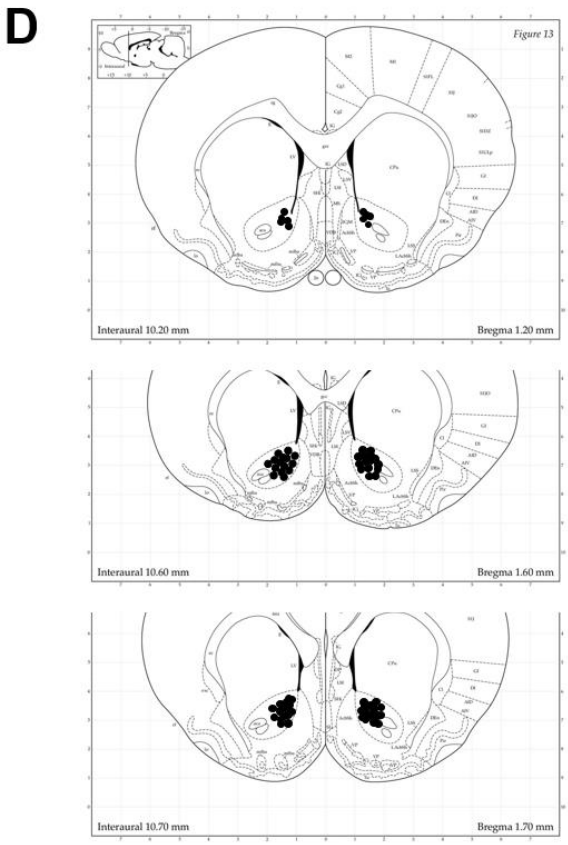


Figure 6. Lentiviral validation of p65 protein knockdown within the NAc core. pLKO.1-puro-CMV-TurboGFP™ positive control lentiviral particles ("Control" or "CTRL") or pLKO.1-puro-CMV-TurboGFP™-*RELA* shRNA lentiviral particles ("shRNA") were administered into the NAc core of cocaine- and sucrose-naïve rats. Rats were sacrificed after 21 days for quantitative immunohistochemical staining of p65. (A) Timeline of experimental procedures. (B) Representative coronal section of lentiviral expression of tGFP (488 nm) within the dorsomedial NAc core. Cannula track is outlined by the red dotted line. (C) Representative coronal sections containing the NAc core that are immunostained for p65. The dorsomedial NAc core was imaged at 20x magnification and colocalization of p65 (568 nm) and tGFP (488 nm) was quantified. Scale bars = 50 µm. The shRNA-expressing lentivirus significantly reduced colocalization of p65 and tGFP relative to the control vector, indicating a significant reduction in p65 expression in successfully transduced cells. n = 5-6/group. *$p < 0.05$. (D) Anatomical map of NAc core viral infusions among animals tested for cue-induced cocaine or sucrose seeking (N = 47). Animals with misplaced viral infusions or GFP expression within the NAcSh or the dorsal striatum were excluded from the study. AC = anterior commissure. Error bars = SEM.

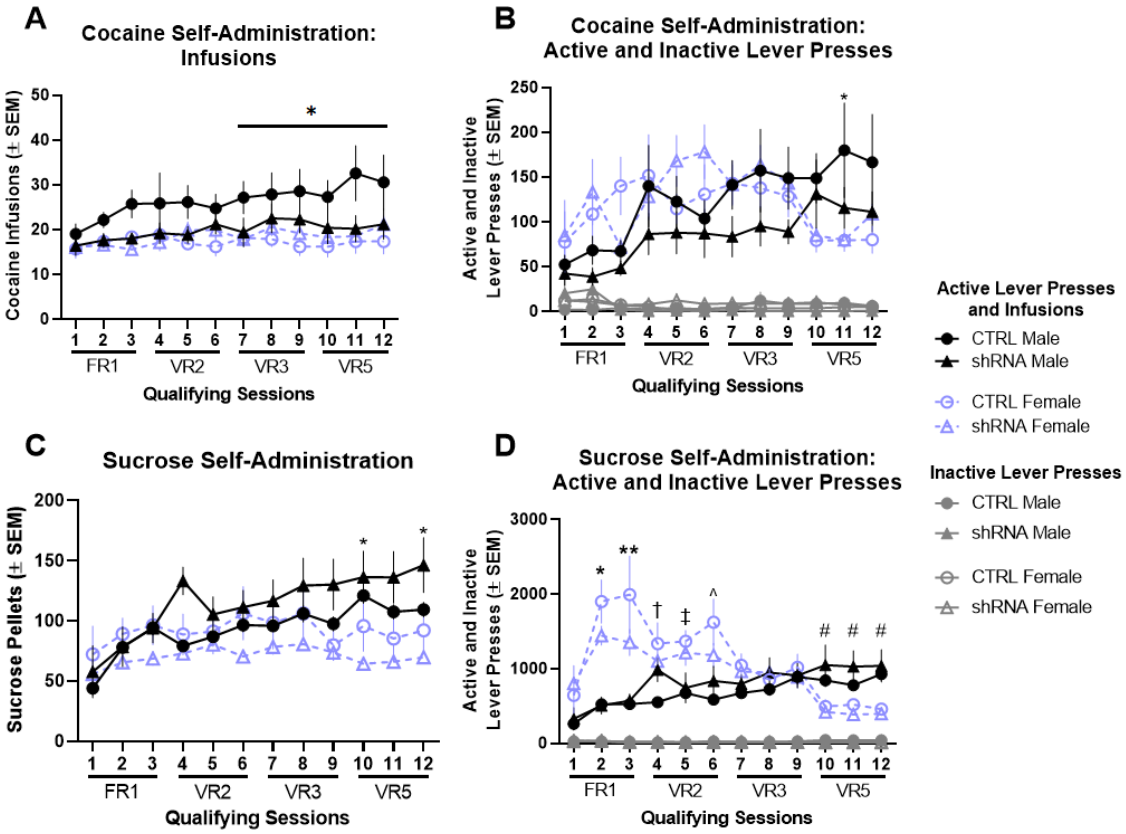


Figure 7. Cocaine and sucrose self-administration prior to lentivirus administration. Rats self-administered either cocaine or sucrose for a minimum of 12 days beginning on a FR-1 schedule of reinforcement and ending on a VR-5. (A) A *post hoc* Dunnett's test of the session main effect revealed a significant increase in cocaine infusions earned at sessions 7-12 compared to session 1 regardless of group (*$p < 0.05$). (B) A *post hoc* Tukey's test of the group-by-session interaction effect revealed CTRL-males had greater active lever presses than shRNA-females on session 11 (*$p < 0.05$). (C) A *post hoc* Tukey's test of the group-by-session interaction effect revealed shRNA-males earned more sucrose pellets on sessions 10 and 12 than shRNA-females (*$p < 0.05$). (D) Overall, females tended to exhibit greater active lever presses during early sucrose self-administration, whereas males exhibited greater active lever presses during late sucrose self-administration. A Tukey's multiple comparisons test revealed the following specific group differences across test sessions: *$p < 0.05$ comparing CTRL-male and shRNA-male each to CTRL-female and shRNA-female. **$p < 0.5$ comparing CTRL-male and shRNA-male each to CTRL-female and shRNA-female, as well as CTRL-female to shRNA-female. $^{\dagger}p < 0.05$ comparing CTRL-male to CTRL-female. $^{\ddagger}p < 0.05$ comparing CTRL-male and CTRL-shRNA to CTRL-female. ^$p < 0.05$ comparing CTRL-male to

CTRL-female and shRNA-female, as well as shRNA-male to CTRL-female. $^{\#}p < 0.05$ comparing shRNA-males to shRNA-females. n = 5-6/group. Error bars = SEM.

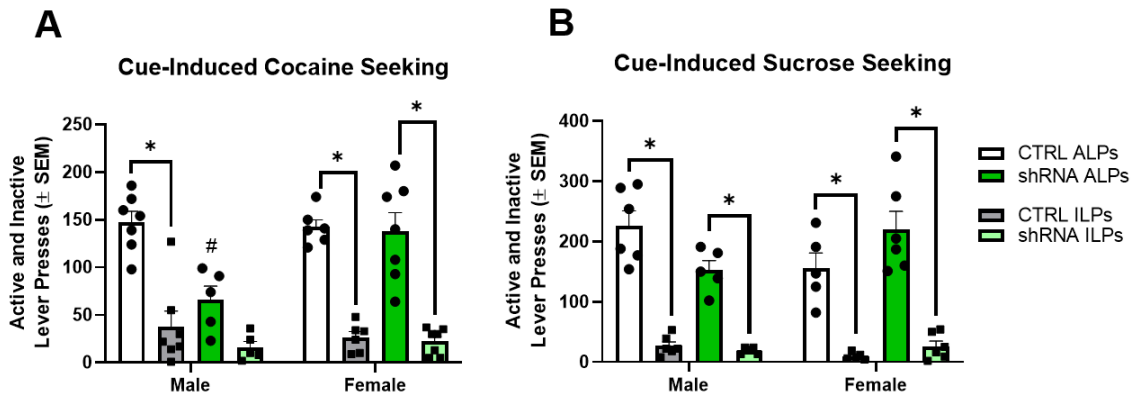


Figure 8. NAc core p65 knockdown inhibits cue-induced cocaine seeking in males. After lentivirus administration and 21 days of forced abstinence, rats were tested in a 1-hr cue-induced cocaine or sucrose seeking test. (A) shRNA-males exhibited significantly lower active lever presses compared to all other groups ($^{\#}p < 0.01$). All groups of rats except shRNA-males exhibited significantly greater active lever presses relative to inactive lever presses (*$p < 0.01$; n=5-7/group). (B) *Post hoc* tests revealed no significant differences in cue-induced cocaine and sucrose seeking across sex and virus type, however all groups exhibited significantly greater active lever presses relative to inactive lever presses (*$p < 0.01$; n=5-6/group). "ALPs" = active lever presses. ILPs = inactive lever presses. Error bars = SEM.

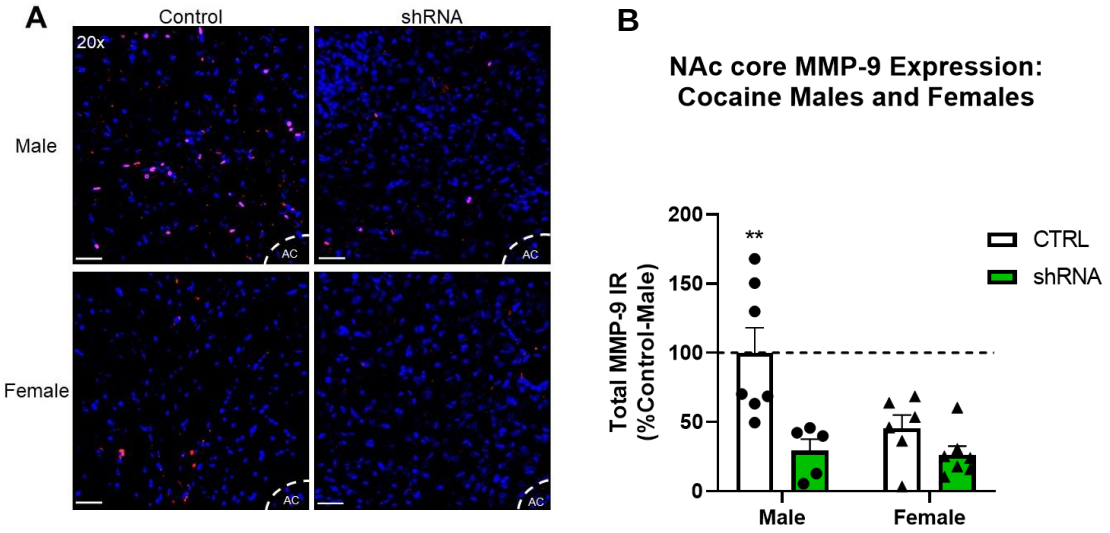


Figure 9. NAc core MMP-9 expression in virus-treated male and female cocaine rats measured after cue reactivity testing. Immediately after the cue-induced cocaine seeking test, rats were sacrificed for immunohistochemical analysis of MMP-9 expression within the NAc core. (A) Representative images of MMP-9 (568 nm) and DAPI (405 nm) immunoreactivity (IR) within the NAc core of male and female cocaine rats treated with control or shRNA lentivirus, imaged at 20x magnification. AC = anterior commissure. Scale bars = 50 µm. (B) CTRL-males exhibited greater NAc core MMP-9 expression than all other groups. n = 5-7/group. *p < 0.05. Error bars = SEM.

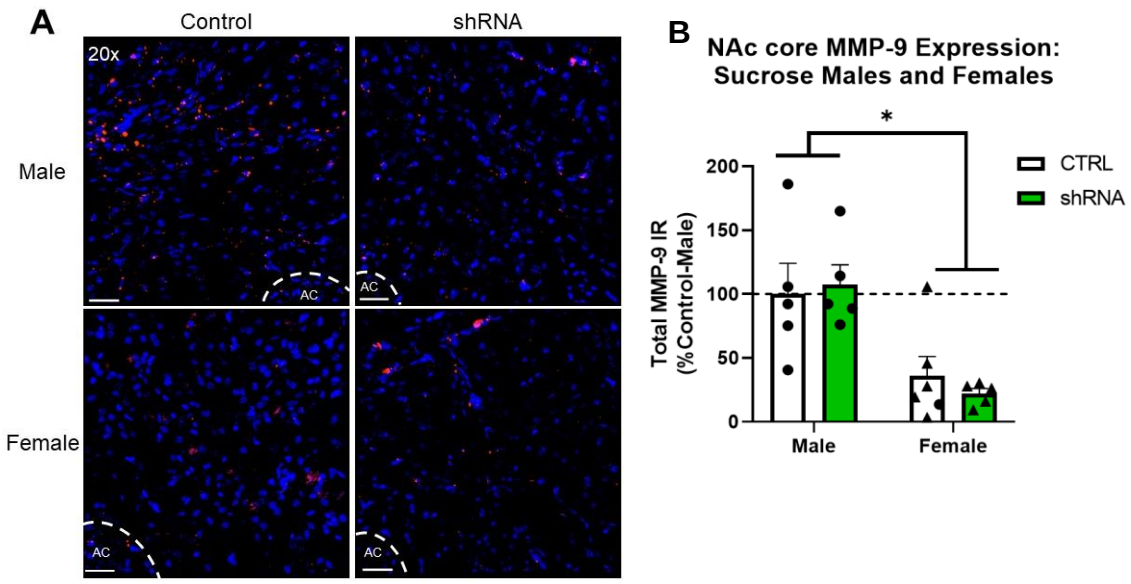


Figure 10. NAc core MMP-9 expression in virus-treated male and female sucrose rats measured after cue reactivity testing. Immediately after the cue-induced sucrose seeking test, rats were sacrificed for immunohistochemical analysis of MMP-9 expression within the NAc core. (A) Representative images of MMP-9 (568nm) and DAPI (405nm) immunoreactivity (IR) within the NAc core of male and female sucrose rats treated with control or shRNA lentivirus, imaged at 20x magnification. AC = anterior commissure. Scale bars = 50 µm. (B) A two-way ANOVA revealed a significant main effect of sex but no significant main effect of virus or sex-by-virus interaction. n = 5-6/group. *$p < 0.05$ for main effect of sex. Error bars = SEM.

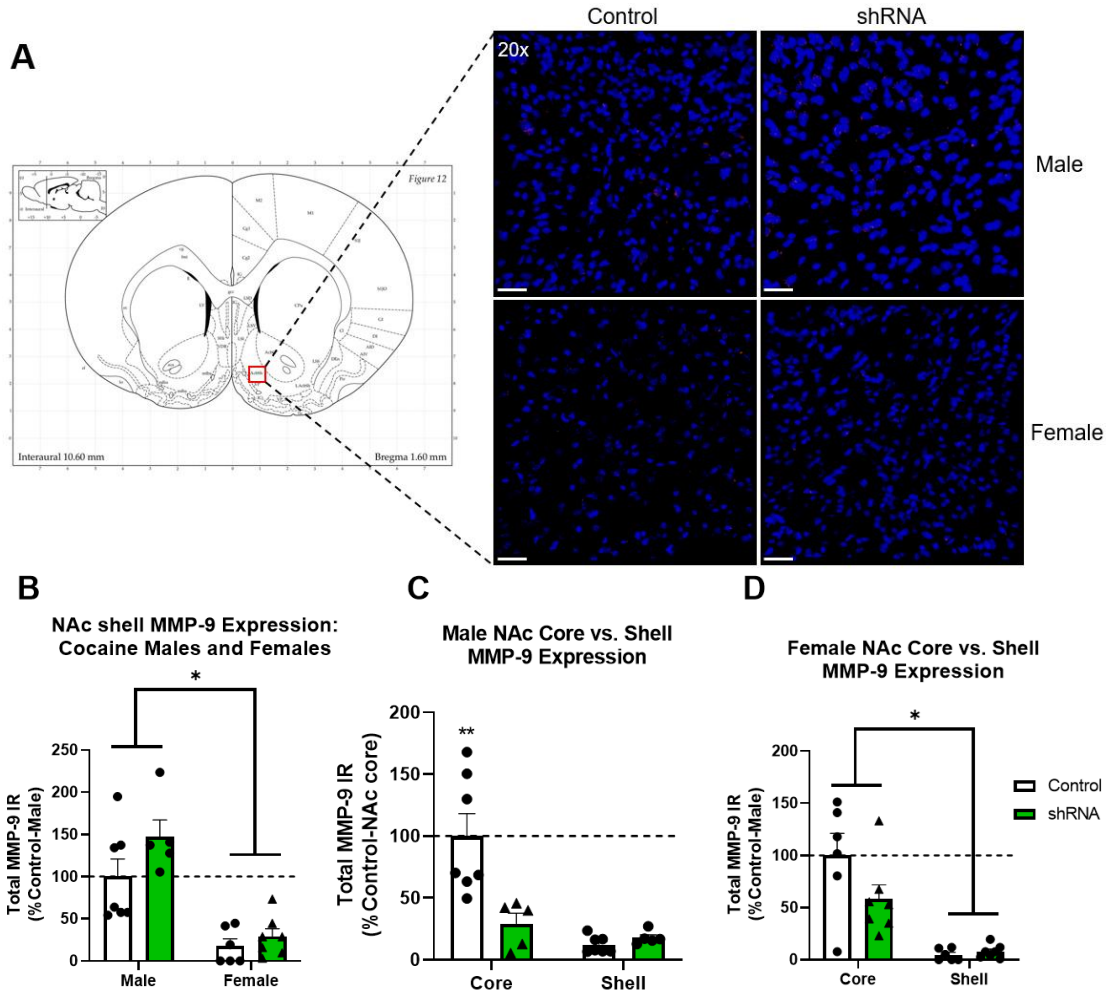


Figure 11. Male rats exhibit greater MMP-9 expression within the NAcSh. We examined MMP-9 expression within the NAcSh to 1) probe whether our observed sex difference within the NAc core is also observed within the NAcSh and 2) confirm our viral manipulation was not associated with changes in MMP-9 expression observed within the NAc core. (A) Anatomical location of NAcSh MMP-9 quantification and representative images of NAcSh MMP-9 (568nm) and DAPI (405nm) expression across virus type and sex. Scale bars = 50 µm. (B) Similar to the NAc core, males exhibited greater overall expression of MMP-9 compared to females. A two-way ANOVA revealed a significant main effect of sex, but no significant main effect of virus or virus-by-sex interaction. *$p < 0.05$ for main effect of sex. (C) Among male cocaine rats, shRNA treatment significantly reduced MMP-9 expression levels down to those observed within the NAcSh. **$p < 0.01$. (D) Female cocaine rats exhibited significantly greater MMP-9 expression within the

NAc core compared to the NAcSh. A two-way ANOVA revealed a significant main effect of brain region but no main effect of virus or virus-by-brain region interaction. *p < 0.05 for main effect of brain region. NAcSh MMP-9 expression was not quantified in sucrose animals because it was completely undetectable in all but 2 animals.

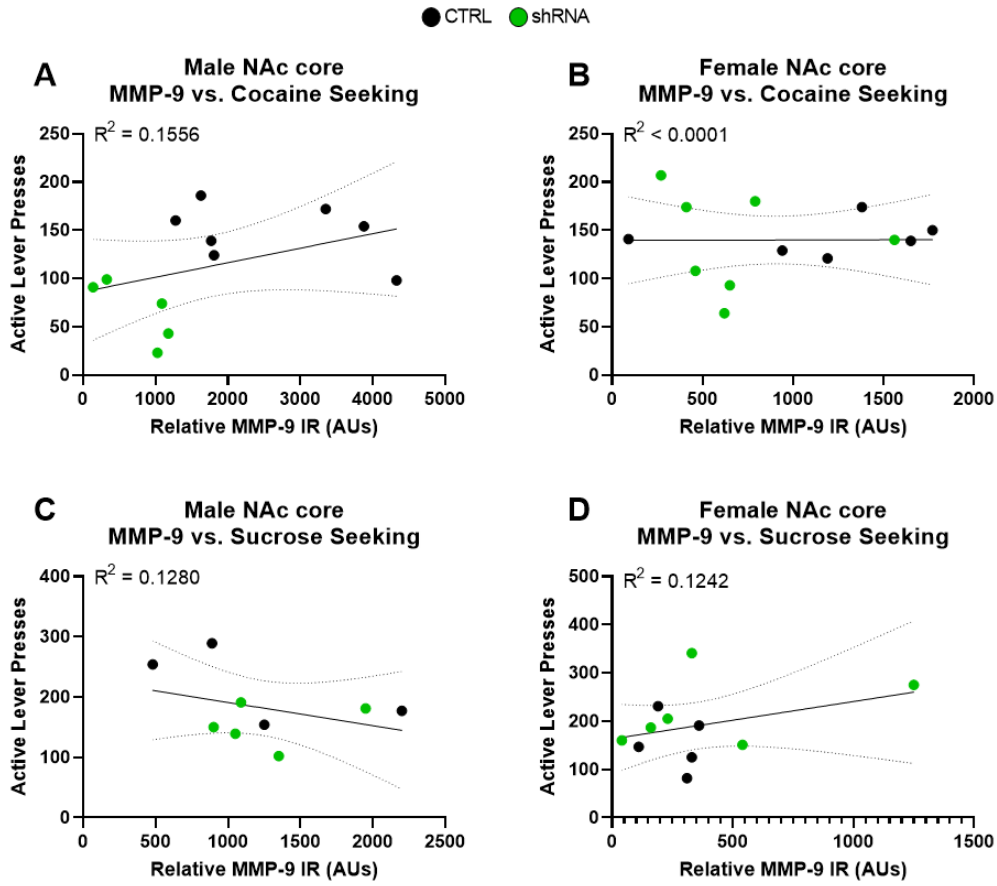


Figure 12. Total NAc core MMP-9 expression does not significantly correlate with cue-induced cocaine or sucrose seeking. Relative MMP-9 immunoreactivity within the NAc core did not significantly correlate with cue-induced cocaine seeking in both (A) males and (B) females. Likewise, relative MMP-9 immunoreactivity within the NAc core did not significantly correlate with cue-induced sucrose seeking in both (C) males and (D) females. Dotted lines = 95% confidence bands. AU = arbitrary units.

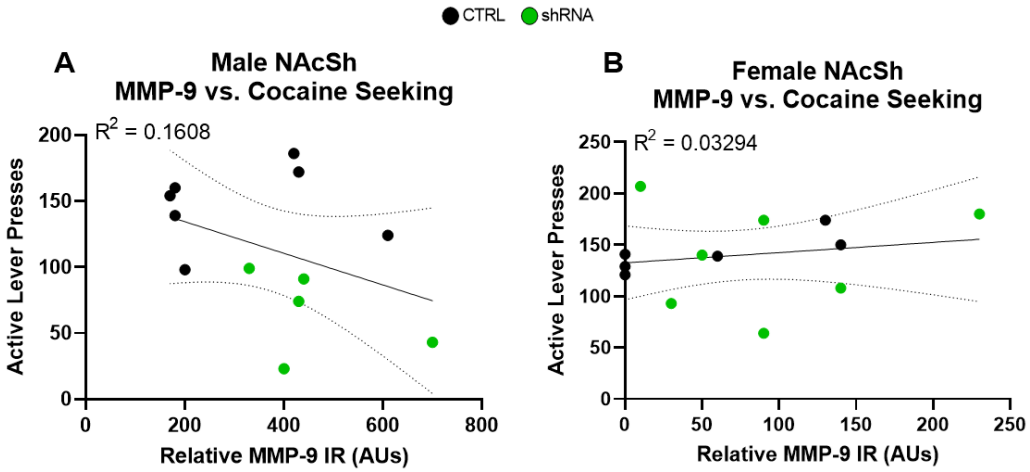


Figure 13. Total NAcSh MMP-9 expression does not significantly correlate with cue-induced cocaine seeking. Relative MMP-9 immunoreactivity within the NAcSh did not significantly correlate with cue-induced cocaine seeking in both (A) males and (B) females. Dotted lines = 95% confidence bands. AUs = arbitrary units.

APPENDIX C

ANIMAL SUBJECTS APPROVAL

Institutional Animal Care and Use Committee (IACUC)
Office of Research Integrity and Assurance
Arizona State University
660 South Mill Avenue, Suite 312
Tempe, Arizona 85287-6111
Phone: (480) 965-6788 *FAX:* (480) 965-7772

Animal Protocol Review

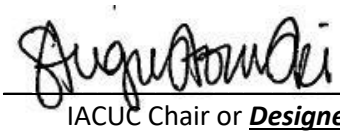
ASU Protocol Number:	**19-1679R**
Protocol Title:	**Serotonin involvement in drug addiction-related behavior**
Principal Investigator:	**Janet Neisewander**
Date of Action:	**11/1/2018**

The animal protocol review was considered by the Committee and the following decisions were made:

The protocol was approved.

If you have not already done so, documentation of Level III Training (i.e., procedure-specific training) will need to be provided to the IACUC office before participants can perform procedures independently. For more information on Level III requirements see https://researchintegrity.asu.edu/animals/training.

Total # of Animals:	**3130**		
Species:	**Rats**	**Unalleviated Pain/Distress:**	**No-1560**
Species:	**Mice**	**Unalleviated Pain/Distress:**	**No-1570**
Protocol Approval Period:	**11/1/2018 – 10/31/2021**		
Sponsor:	**National Institute of Drug Abuse**		
ASU Proposal/Award #:	**AWD00027587**		
Title:	**Neural Mechanisms of Drug Seeking**		

Signature:  Date: 11/2/2018

IACUC Chair or ***Designee***

Cc: IACUC Office
IACUC Chair

Institutional Animal Care and Use Committee (IACUC)
Office of Research Integrity and Assurance
Arizona State University
660 South Mill Avenue, Suite 312
Tempe, Arizona 85287-6111
Phone: (480) 965-6788 *FAX:* (480) 965-7772

Animal Protocol Review

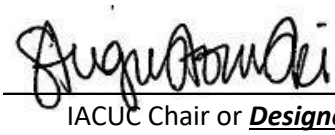
ASU Protocol Number:	**19-1679R RFC 17**
Protocol Title:	**Serotonin involvement in drug addiction-related behavior**
Principal Investigator:	**Janet Neisewander**
Date of Action:	**7/25/2019**

The animal protocol review was considered by the Committee and the following decisions were made:

Request for changes was approved to add 276 rats and additional procedures to the protocol.

If you have not already done so, documentation of Level III Training (i.e., procedure-specific training) will need to be provided to the IACUC office before participants can perform procedures independently. For more information on Level III requirements see https://researchintegrity.asu.edu/animals/training.

Total # of Animals:	**5164**	
Species:	**Mice**	**Unalleviated Pain/Distress: No-1570**
Species:	**Rats**	**Unalleviated Pain/Distress: No-3594**
Protocol Approval Period:	**11/1/2018 – 10/31/2021**	
Sponsor:	**National Institute of Drug Abuse**	
ASU Proposal/Award #:	**AWD00027587; FP00017154**	
Title:	**Neural Mechanisms of Drug Seeking; circ1Homer1 regulates synaptic plasticity and cocaine-seeking**	

Signature:  for C. Shalley Date: 7/29/2019

IACUC Chair or ***Designee***

Cc: IACUC Office
IACUC Chair

Institutional Animal Care and Use Committee (IACUC)

Office of Research Integrity and Assurance
Arizona State University
660 South Mill Avenue, Suite
312 Tempe, Arizona 85287-
6111
Phone: (480) 965-6788 *FAX:* (480) 965-7772

Animal Protocol Review

ASU Protocol Number: **19-1679R RFC 39**
Protocol Title: **Serotonin involvement in drug addiction-related behavior**
Principal Investigator: **Janet Neisewander**
Date of Action: **11/4/2020**

The animal protocol review was considered by the Committee and the following decisions were made:

The request for changes was approved by Designated Review to modify existing procedures on the protocol.

NOTE: If you have not already done so, documentation of Level III Training (i.e., procedure-specific training) will need to be provided to the IACUC office before participants can perform procedures without supervision. For more information on Level III requirements see https://researchintegrity.asu.edu/animals/training, or contact Research Support Services within DACT at dactrss@asu.edu.

Additional requirements:

☐ This protocol requires that DACT provide supervision for the first time a procedure is conducted. Contact dactrss@asu.edu to schedule.

☐ This protocol indicates that there are surgical procedures. A surgical checklist may be required to be submitted to Research Support Services within DACT (dactrss@asu.edu), prior to starting surgeries. ☐ Other requirements:

Total # of Animals:	**5388**	
Species:	**Rats**	**Unalleviated Pain/Distress: No - 3818**
Species:	**Mice**	**Unalleviated Pain/Distress: No - 1570**

Protocol Approval Period: **11/1/2018 – 10/31/2021**

Sponsor: **Multiple – See protocol**
ASU Proposal/Award #: **Multiple – See protocol**
Title: **Multiple – See protocol**

Signature: Nicole Shepherd for N. Henderson

Date: 11/23/2020 IACUC Chair or **Designee**

Cc: IACUC Office
IACUC Chair

APPENDIX D

CURRICULUM VITAE

MARK NAMBA
CURRICULUM VITAE

550 E. Orange St., ISTB1 RM 446, Tempe, AZ, 85281 | (321)-848-7628 | mnamba@asu.edu

EDUCATION

Arizona State University — In Progress
Ph.D. in Neuroscience

Arizona State University — May 2019
M.A. in Psychology, Behavioral Neuroscience

University of Florida — May 2016
B.S. in Psychology, *cum laude*

Eastern Florida State College — May 2013
Associate of Arts degree

POSITIONS HELD & RELATED EXPERIENCE

Graduate Research Associate — 2019 –22
Arizona State University, School of Life Sciences
Worked under the mentorship of Drs. Janet Neisewander and Foster Olive studying the role of neuroimmune signaling as well as HIV/SUD intersections within the mesolimbic reward pathway in an animal model of cocaine addiction and relapse.

Currently working under the mentorship of Drs. Janet Neisewander and Foster Olive studying the role of neuroimmune signaling as well as HIV/SUD intersections within the mesolimbic reward pathway in an animal model of cocaine addiction and relapse.

Graduate Research Assistant — 2016 – 19
Arizona State University, Department of Psychology
Worked under the supervision of Dr. Cassandra Gipson-Reichardt studying the role of extracellular glutamate transport and neuroimmune signaling within the mesolimbic reward pathway in an animal model of nicotine addiction and relapse.

Associate Editor — 2015 – 16
Journal of Young Investigators
Served as a peer review editor for an undergraduate research journal, where I critiqued undergraduate research manuscripts prior to publication.

Research Assistant
University of Florida 2014 – 16
Assisted Dr. Lori Knackstedt and Dr. Marek Schwendt with the coding and recording of stress-related behaviors in rats for future analysis of post-traumatic stress disorder (PTSD) traits, as well as performing and administering drug self-administration procedures, animal surgical procedures, and behavioral tests.

ACCOMPLISHMENTS AND AWARDS

ASPET Washington Fellows Program – Guide	2022
ASPET Washington Fellow	2020-21
ARCS Foundation Scholar Award (J. Kenneth Seward ARCS Scholar, $8,500/yr)	2019, 2021
Neuroscience Scholars Program Professional Development Award	2019
Society for Neuroscience "Neuroscience Scholars Program" Associate membership	2018
Cold Spring Harbor Laboratories Cellular Biology of Addiction course acceptance	2018
ASU Graduate and Professional Student Association Outstanding Research Award	2018
ASU Graduate and Professional Student Association Travel Award	2017-2019
Diversity Scholars Travel Award or the Society for Neuroscience annual meeting	2017
NIDA Travel Award for National Hispanic Science Network Conference 2017	2017
ASU College of Liberal Arts & Sciences Graduate Excellence Award	2017-2021
Diversity Scholars Travel Award for the Society for Neuroscience annual meeting	2016
NIDA Diversity Supplement Award (R00 DA036569-03S1, $21,049)	2016
ASU Department of Psychology Research Excellence Award	2016
University of Florida University Scholar's Award	2015
University of Florida Dean's List	2013, 2014

PEER-REVIEWED PUBLICATIONS

[#] = corresponding author(s)

Bechard, A., LaCrosse, A., **Namba, M.D.**, Jackson, B., #Knackstedt, L.A. (2018). Impairments in reversal learning following short access to cocaine self-administration. *Drug and Alcohol Dependence, 192*, 239-244.

Namba, M.D., Tomek, S.E., Olive, M.F., Beckmann, J.S., & #Gipson, C.D. (2018). The Winding Road to Relapse: Forging a New Understanding of Cue-Induced Reinstatement Models and their Associated Neural Mechanisms. *Frontiers Behavioral Neuroscience*, 12:17. doi: 10.3389/fnbeh.2018.00017.

Schwendt, M., Shallcross, J., Hadad, N., **Namba, M.D.**, Hiller, H., Wu, L., Krause, E.G., & #Knackstedt, L.A. (2018). A novel rat model of comorbid PTSD and addiction reveals intersections between stress susceptibility and enhanced cocaine seeking with a role for mGlu5 receptors. *Translational Psychiatry, 8*(1), 209.

Powell, G.L., Leyrer-Jackon, J.M., Goenaga, J.G., **Namba, M.D.**, Piña, J.A., Spencer, S.M., Stankevicuite, N., Schwartz, D., Allen, N.P., del Franco, A.P., McClure, E.A., Olive, M.F., & #Gipson, C.D. (2019). Chronic treatment with *N*-acetylcysteine decreases extinction responding and reduces cue-induced nicotine seeking. *Physiological Reports, 7*(1), e13958.

Goenaga, J.G., Powell, G.L., Leyrer-Jackson, J.M., Piña, J.A., Phan, S., Prakapenka, A.V., Koebele, S.V., **Namba, M.D.**, McClure, E.A., Bimonte-Nelson, H.A., & #Gipson C.D. (2019). N-acetylcysteine yields sex-specific efficacy for cue-induced reinstatement of nicotine seeking. *Addiction Biology*.

Powell, G.L., Cabrera-Brown, G., **Namba, M.D.**, Neisewander, J.L., Marusich, J.A., Beckmann, J.S., & #Gipson, C.D. (2019). Economic demand analysis of within-session dose-reduction during nicotine self-administration. *Drug and Alcohol Dependence, 201*, 188-196.

Namba, M.D., Kupchik, Y.M., Spencer, S.M., Garcia-Keller, C., Goenaga, J.G., Powell, G.L., Vicino, I.A., Hogue, I.B., & #Gipson, C.D. (2019). Accumbens neuroimmune signaling and dysregulation of astrocytic glutamate transport underlie conditioned nicotine seeking. *Addiction Biology, e12797*.

Powell, G.P., **Namba, M.D.**, Vannan, A., Bonadonna, J.P., Carlson, A., Mendoza, R., Chen, P.J., Luetdke, R.R., Blass, B.E., & #Neisewander, J.L. (2020). The Long-Acting D3 Partial Agonist MC-25-41 Attenuates Motivation for Cocaine in Sprague-Dawley Rats. *Biomolecules, 10*, 1076.

#**Namba, M.D.**, Leyrer-Jackson, J.M., Nagy, E.K., Olive, M.F., & #Neisewander, J.L. (2021). Neuroimmune Mechanisms as Novel Treatment Targets for Substance Use Disorders and Associated Comorbidities. *Front. Neurosci.*, *15*.

Buzhdygan, T., Rawls, S.M., Ramirez, S.H., **Namba, M.D.,** Bondy, E.O., Khatri, S.N., & #Gipson, C.D. (in prep). Troriluzole, analog of FDA-approved riluzole, reduces relapse to nicotine seeking in rats through a potential cytokine-based mechanism.

[#]**Namba, M.D.,** Phillips, M.N., [#]Neisewander, J.L., & Olive, M.F. (2022). Nuclear Factor Kappa B Signaling within the Rat Nucleus Accumbens Core Regulates Cue-Induced Cocaine Seeking Sex-Dependently. *Brain, Behavior, & Immunity*, 102, 252-265.

Namba, M.D., Phillips, M.N., [#]Olive, M.F., & [#]Neisewander, J.L. (in prep). Systemic Exposure to the HIV Protein gp120 Prevents the Inhibition of Cue-Induced Cocaine Seeking by the Novel Dopamine D3 Receptor Partial Agonist MC-25-41.

BOOK CHAPTERS

Piña, J., **Namba, M.D.**, Leyrer-Jackson, J.M., Cabrera-Brown, G., & Gipson C.D. (2018). Social influences on nicotine-related behaviors. In M.F. Olive & S.E. Tomek (Eds.), *Animal Models for Examining Social Influences on Drug Addiction* (pp. 1-32). Cambridge, MA: Elsevier.

Namba, M.D., Powell, G.L., Goenaga, J.G., del Franco, A.P., & Gipson, C.D. (2019). Brain gene expression in the context of nicotine reward: a focus on cholinergic genes. In V.R. Preedy (Ed.), *The Neuroscience of Nicotine: Mechanisms and Treatment* (pp. 321-328). London, UK: Academic Press.

Leyrer-Jackson, J.M., **Namba, M.D.**, Hood, L.E., & Olive, M.F. (in press). Using Western Blotting for the Evaluation of Metabotropic Glutamate Receptors Within the Nervous System: Procedures and Technical Considerations. In M.F. Olive, Burrows, B.T., & Jackson, J.M. (Eds.), *Neuromethods: Metabotropic Glutamate Receptor Technologies*, vol. 164 (chapter 8). New York City, NY: Springer US.

ABSTRACTS

Schwendt M., **Namba M.D.**, Frankowski J., Hiller H., Krause K., & Knackstedt L.A. Development and characterization of a novel animal model for co-morbid PTSD and cocaine addiction. (2015). Poster session presented at: Sunposium. 2nd biennial conference of the Max Planck Florida Institute for Neuroscience. Mar 30-31; Palm Beach Gardens, FL.

Namba M.D., Shallcross J., Hiller H., Krause K., Schwendt M., & Knackstedt L.A. Understanding the behavioral and neurobiological implications of PTSD and substance use disorder co-morbidity in an animal model. (2016). Poster presented at: University of Florida Undergraduate Research Symposium. 17th annual symposium of the University for Florida Center for Undergraduate Research. Mar 24; Gainesville, FL.

Namba M.D., Powell G.L., Goenaga J.G., del Franco A.P., McCallum J.J., & Gipson C.D. (2017). *N*-acetylcysteine inhibits cue-induced nicotine seeking through a GLT-1-dependent mechanism. Poster presented at: National Institute on Drug Abuse (NIDA) Diversity Supplement Workshop. May 05; Rockville, MD.

Namba M.D., Powell G.L., Goenaga J.G., del Franco A.P., McCallum J.J., & Gipson C.D. (2017). *N*-acetylcysteine attenuates cue-induced nicotine seeking through a GLT-1-dependent mechanism and decreases neuroinflammatory TNFα expression in the nucleus accumbens core. Poster presented at: National Hispanic Science Network annual meeting. October 04; Phoenix, AZ.

Namba M.D., Powell G.L., Goenaga J.G., del Franco A.P., McCallum J.J., & Gipson C.D. (2017). *N*-acetylcysteine inhibits cue-induced nicotine seeking through a glutamate transporter GLT-1-dependent mechanism and modulates neuroimmune signaling in the nucleus accumbens core. Poster presented at: Society for Neuroscience annual meeting. November 15; San Diego, CA.

Namba, M.D. & Gipson, C.D. Nuclear factor kappa B (NF-κB) signaling in the nucleus accumbens core mediates cue-induced nicotine seeking and modulates glutamate transporter 1 (GLT-1) expression. (2018). Poster presented at: American College of Neuropsychopharmacology. December 12; Hollywood, FL.

Namba, M.D., Vannan, A., Powell, G.L., Johnson, M., Guerrero, F., Mokbel, A.M.H., & Neisewander, J.L. (2019). The novel dopamine D3 receptor antagonist SWR-5 reduces motivation for cocaine. Poster presented at: Society for Neuroscience, October 19; Chicago, IL.

Namba, M.D., Vannan, A., Johnson, M., Olive, M.F., & Neisewander, J.L. Nuclear Factor Kappa B Signaling Within the Rat Nucleus Accumbens Core Modulates Cue-Motivated Reward-Seeking Behavior. (2020). Poster presented at: American College of Neuropsychopharmacology. December 8; Virtual.

Namba, M.D., Phillips, M.N., Chen, P.J., Blass, B.E., Olive, M.F., & Neisewander, J.L. (2021). Systemic Exposure to the HIV Protein gp120 Prevents the Inhibition of Cue-Induced Cocaine Seeking by the Novel Dopamine D3 Receptor Partial Agonist MC-25-41. Poster submitted to: American College of Neuropsychopharmacology. December 15; Virtual.

INVITED LECTURES AND ORAL PRESENTATIONS

Namba, M.D. (2018). Neuroimmunomodulation of Nicotine Relapse and Synaptic Plasticity. Oral presentation presented at: College on Problems of Drug Dependence. June 12; San Diego, CA.

Namba, M.D. (2019). Gut, Brain, and Nicotine Addiction: Nicotine Self-Administration is Associated with Changes in Gut Microbiota and Accumbens Pro-inflammatory Cytokines. Oral presentation presented at: Society for Research on Nicotine and Tobacco. February 22; San Francisco, CA.

Namba, M.D. (2019). Viral Vectors as Tools in Behavioral Neuroscience – Advancing Our Understanding of the Brain and Behavior. Guest lecture for NEU 591 (Biotechnology – Viruses as Tools). Arizona State University. April 17; Tempe, AZ.

Namba, M.D. (2020). Neuroimmune Mechanisms of HIV and Substance Use Disorders. Oral presentation presented at: Achievement Rewards for College Scientists (ARCS) Fall 2020 Virtual Scholar Showcase. October 15; Virtual.

Namba, M.D. (2020). Neuroimmune Regulation of Cocaine Motivation. Oral presentation presented at: 11th Annual ASU-BNI Neuroscience Research Symposium. February 04; Virtual.

Namba, M.D. (2022). Neuroimmune and Behavioral Intersections of HIV and CUDs. Oral presentation presented at: 12th Annual ASU-BNI Neuroscience Research Symposium. January 29; Phoenix, AZ.

EXTRAMURAL GRANT SUPPORT

F31 DA047072 – National Institute on Drug Abuse (PI). "Neuroimmunomodulation of Nicotine Relapse and Synaptic Plasticity" – $132,483.00 (06/01/2019 – 05/31/2022).

G2019100191831450 – Sigma XI Grants in Aid of Research (PI). "Behavioral and Neuroimmune Mechanisms of Comorbid HIV/AIDS and Cocaine Use Disorders" - $915.00 (01/01/2020 – 12/31/2020).

INTRAMURAL GRANT SUPPORT

Arizona State University (ASU)

Institute for Social Science Research Seed Grant (Co-PI) – $8,000.00 (05/08/2020 – 06/01/2021).

ASU Graduate Research Grant Program (PI) – $2,000.00 (03/21/21 – 05/31/21)

School of Life Sciences Graduate Student Support Grant Program (PI) - $1,976.58 (5/15/2021 – 5/15/2022)

TEACHING AND INSTRUCTION

Research Methods (PSY 290) – TA (2018)
Neuroanatomy (PSY 426) – TA (2019)

PROFESSIONAL SERVICE

Editorial Reviews of Manuscripts and Book Chapters
Stress (2020)
iScience (2021)
Departmental and University Service at Arizona State University
Member, Search Committee for Neuroscience Tenure-Track Faculty Member, School of Life Sciences, 2021
Member, Undergraduate Honors Thesis Committee, Megan Phillips, School of Life Sciences, 2021

COMMUNITY SERVICE AND OUTREACH

- Various on-campus outreach events through UF Psychology and Neuroscience clubs (e.g. Brain Awareness Week, NAMI Suicide Awareness events, etc.) (2013-2016).
- Volunteered with ASU Graduate Association for Interdisciplinary Neuroscience Students (GAINS) at the University of Arizona "Connect2STEM" event (01/2017).
- Volunteered with ASU Department of Psychology for ASU's "Night of the Open Door" (2017).
- Volunteered at Gavilan Peak School (grades K-8, New River, AZ) for their annual Science Expo (2017).
- Elected Secretary of ASU Graduate Association for Interdisciplinary Neuroscience Students (05/2017-05/2018).
- Presented a Q&A session to undergraduate neuroscience club about graduate school application process (2017).
- Helped organize the annual Brain & You Fair with GAINS (2018).
- Elected Treasurer of ASU Graduate Association for Interdisciplinary Neuroscience Students (05/2018 – 05/2019).
- Volunteered with ASU Graduate Association for Interdisciplinary Neuroscience Students at the University of Arizona "Connect2STEM" event (01/2019).
- Helped organize and curate an approved budget for the Brain & You Fair with GAINS (2019).
- Volunteered with GAINS at Kyrene de la Colina, where we displayed brain specimens for children to hold and manipulate (08/2019)
- Volunteer with Shot in the Dark, where I work the phone line to connect participants to organization services (06/2020 – 06/2021)

SCIENCE COMMUNICATION AND MEDIA COVERAGE

Holtyn, A. (2018). "Highlighting Members' Preclinical Research: Neuroinflammation as a novel mechanism
underlying nicotine relapse" *College on Problems of Drug Dependence*.
https://cpdd.org/highlighting-members-research-preclinical/

Namba, M.D. (2021). "How a new needle exchange law will help Arizona fight the opioid crisis". *The Arizona Republic*. https://www.azcentral.com/story/opinion/op-ed/2021/06/21/needle-exchange-law-help-arizona-fight-opioid-crisis/7721024002/

TECHNICAL SKILLS AND EXPERIENCE

Neurobiology: Immunohistochemistry, western blotting & gel electrophoresis, PCR, *in vivo* zymography, viral gene transfer, chemogenetics, drug preparations, chemical titration and dilution procedures, *in vivo* microinjections, spectrophotometry, and confocal microscopy.
Animal behavioral testing: T-maze, elevated plus maze, acoustic startle, predator scent stress exposure, and intravenous drug self-administration, behavioral economics.

Histology and tissue handling: Cryostat and vibratome tissue slicing, tissue mounting, and rat brain extraction and preservation (e.g. flash freezing in isopentane or paraformaldehyde fixation).
Animal handling: Rat handling, subcutaneous, intraperitoneal, and intramuscular injections, intravenous catheter maintenance, and pre-/post-operative rat care.
Surgical procedures: Transcardial perfusions, rat stereotaxic surgery, and rat jugular catheter surgery.
Computer: Essential knowledge of Microsoft Office Suite (i.e. Microsoft Excel, Word, etc.), MiniTab, SPSS, SAS, and GraphPad Prism statistical software, Noldus EthoVison XT animal tracking software, and MedAssociates (Med-PC) software.

CAREER DEVELOPMENT

Max Planck Florida Institute for Neuroscience Sunposium Conference (2015)
Society for Neuroscience Annual Meeting and NIDA/NIAAA Frontiers in Addiction Mini-Convention (2016-2019)
Arizona Alzheimer's Disease Research Center (ADRC) workshop on ethics and responsible conduct of research (2017)
Responsible Conduct of Research (RCR) Phase II training: "Authorship and Collaborative Research" (2017)
National Institute on Drug Abuse (NIDA) Diversity Supplement Workshop (2017)
Arizona Alzheimer's Disease Research Center (ADRC) workshop on ethical treatment of animal subjects (2018)
College on Problems of Drug Dependence Annual Meeting (2018)
American College of Neuropsychopharmacology Annual Meeting (2018)
Society for Research on Nicotine and Tobacco Annual Meeting (2019)
American College of Neuropsychopharmacology Annual Meeting (2020)
American College of Neuropsychopharmacology Annual Meeting (2021)

UNDERGRADUATE MENTEES

Armani del Franco – Ph.D. student at University of Minnesota, Department of Neuroscience
Joseph McCallum – MS in Science of Healthcare Delivery; Medical Scribe, Scottsdale Emergency Associates Ltd.
Gabriella Cabrera-Brown – Research Data Coordinator, Huntsman Cancer Institute, University of Utah
Michael Johnson – Master's student in the Neisewander lab
Megan Phillips – Pharm.D. student at Creighton University, School of Pharmacy and Health Professions

PROFESSIONAL AFFILIATIONS

ASU Graduate Association for Interdisciplinary Neuroscience Students (**GAINS**)
Society for Neuroscience (**SfN**)
American Society for Pharmacology and Experimental Therapeutics (**ASPET**)

American Psychological Association (**APA**)

PROFESSIONAL & ACADEMIC REFERENCES

- Dr. Lori A. Knackstedt
  - Associate Professor, University of Florida, Department of Psychology
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- Dr. Marek Schwendt
  - Assistant Professor, University of Florida, Department of Psychology
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- Dr. Janet L. Neisewander
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  - janet.neisewander@asu.edu
- Dr. M. Foster Olive
  - Professor, Arizona State University, Department of Psychology
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- Dr. Amber LaCrosse
  - Lab Director, Northern Michigan University, Upper Michigan Brain Tumor Center
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