Spring 2021

DREADDs Modulation of Operant Behavior in Male and Female HIV-1 Transgenic and F344/N rats

Jessica Illenberger

DREADDs Modulation of Operant Behavior in Male and Female HIV-1 Transgenic and F344/N rats

by

Jessica Illenberger

Bachelor of Science
University of South Carolina, 2015

Master of Arts
University of South Carolina, 2018

Submitted in Partial Fulfillment of the Requirements

For the Degree of Doctor of Philosophy in

Experimental Psychology

College of Arts and Sciences

University of South Carolina

2021

Accepted by:

Steven Harrod, Major Professor

Rosemarie Booze, Committee Member

Charles Mactutus, Committee Member

Eva Monsma, Committee Member

Tracey L Weldon, Interim Vice Provost and Dean of the Graduate School

Dedication

The current document is dedicated to my parents.

Acknowledgments

Thank you to my committee members and mentors for their guidance and advice throughout my career so far. Thank you to Kristen Kirchner, Victor Madormo, and Elizabeth Balog for their in assistance running these experiments. Thank you to the friends and family that supported me and lightened the weights I put on my shoulders. This work was supported in part by grants from NIH (National Institute on Drug Abuse, DA013137; Eunice Kennedy Shriver National Institute of Child Health and Human Development, HD043680; National Institute of Mental Health, MH106392; National Institutes of Neurological Disorders and Stroke, NS100624) and the interdisciplinary research training program supported by the University of South Carolina Behavioral-Biomedical Interface Program.

Abstract

The neurobiological processes which determine the choice between 2 (or more) reinforcers are unidentified despite the remarkable benefits which could result from better understanding or control of such processes. Most prominently, reducing choices to pursue drug over non-drug reinforcers could curtail the development or continuation of drug dependence. Likewise, increasing goal-directed behavior in single-schedule and choice settings may alleviate some of the consequences of apathy, a reduction in goal-directed behavior which can occur with neuropathologies including HIV-associated neurocognitive disorders. Dysregulation of the mesolimbic circuit, connecting the ventral tegmental area to the nucleus accumbens, has been implicated in both drug dependence and apathy and is thus a propitious target for the manipulation of reinforcer intake. After training animals to lever-press for sucrose and cocaine under single-schedule and choice (cocaine vs. sucrose) procedures, the current experiment utilized DREADDs (designer receptors exclusively activated by designer drugs) retroviral technique to bidirectionally manipulate the activity of designer human M3 muscarinic and kappa opioid receptors within the mesolimbic circuit. As hypothesized, biological sex influenced genotype-differences and choice behavior supporting that F344/N females and HIV-1 Tg females, respectively, may be more vulnerable to drug dependence and apathy than males within their given genotype. Significantly, mesolimbic stimulation reduced choice for cocaine over sucrose in F344/N females and males. Mesolimbic stimulation did not have a clear influence on HIV-1 Tg

animals' reinforcer intake in the choice procedure despite influencing sucrose intake in the single-schedule procedure. Besides informing hypotheses regarding the unknown neurobiological mechanisms which determine choice behaviors, the current choice procedure revealed biological sex and presence of the HIV-1 transgene as factors that influence the effect of mesolimbic stimulation on choice behavior, establishing the procedure as a valuable tool for identifying factors that may exacerbate resistance to treatments.

Table of Contents

List of Tables

List of Figures

List of Abbreviations

ANOVA ..... Analysis of Variance

C21 ..... Compound 21

cART ..... Combination Antiretroviral Therapies

CNO ..... Clozapine-N-oxide

DA ..... Dopamine

DAT ..... Dopamine Transporter

DREADDs ..... Designer Receptors Exclusively Activated by Designer Drugs

EC50 ..... Half Maximal Effective Concentration

FR(1) ..... Fixed-Ratio (1)

GFP ..... Green Fluorescent Protein

Glu ..... Glutamate

HAND ..... HIV-associated Neurocognitive Disorders

HIV-1 ..... Human Immunodeficiency Virus 1

hM3D(Gq) ..... Human M3 Muscarinic Receptor

IP ..... Intraperitoneal

IV ..... Intravenous

KORD ..... Kappa Opioid Receptor

MSNs ..... Medium Spiney Neurons

NAc ..... Nucleus Accumbens

PFC ..... Prefrontal Cortex

PR ..... Progressive-Ratio

RMANOVA ..... Repeated Measures Analysis of Variance

Sal B ..... Salvinorin B

SEM ..... Standard Error of the Mean

Tg ..... Transgenic

VTA ..... Ventral Tegmental Area

Chapter 1. Introduction

The mesolimbic circuit is reported to be involved in reinforcement processing and goal-directed behavior, but it is currently unclear if manipulation of this circuit can alter choice behavior. Altering choice behavior to reduce harmful choice-making and/or increase desired choice-making could significantly inform treatments for psychobehavioral pathologies characterized by dysregulated reinforcement processing. The current section will describe some of what is currently known about how the mesolimbic circuit is involved in altering certain types of choice behavior, how the mesolimbic circuit is altered by repeated drug exposures and apathy, and our hypotheses for the current experiment, which attempts to alter drug choice and reduce signs of apathy in F344/N and HIV-1 transgenic (Tg) male and female rats.

1.1. Goal-directed and choice behaviors involve mesolimbic circuitry

It has long been established that goal-directed behavior is mediated by instrumental and Pavlovian learning (Skinner, 1938; Rescorla & Solomon, 1967; Colwill & Rescorla, 1985a; Colwill & Rescorla, 1985b; Dickinson & Balleine, 1994). Positive reinforcement occurs when a subject completes an action that results in the presentation of a consequence, increasing the likelihood the action will occur again (Skinner, 1938). Unexpected consequences that promote preceding actions are termed 'reinforcers' and support neuronal learning to drive associations between the appetitive consequence and the stimuli that preceded it (Schultz et al., 1997) by eliciting phasic dopamine (DA) release

from the ventral tegmental area (VTA) to the nucleus accumbens (NAc), also known as the mesolimbic circuit, and activating downstream signaling systems to alter synaptic plasticity through stimulation of striatal D1 receptors and adenylate cyclases (Nicola et al., 2000; Yao et al., 2008). The motivational function of this DA response is also to mediate the incentive salience of reinforcers and, specifically, to sub-serve 'wanting' to obtain the reinforcer through by providing a neural representation of motivational value distinct from 'liking' (Berridge & Robinson, 1998; Robinson & Berridge, 1998). DA responses mediate the perceptual, as well as the motivational salience of reinforcers and with repeated experiences, phasic DA release begins to occur with presentation of the antecedent stimuli, even when the appetitive consequence itself does not occur, as a result of classical conditioning (Salamone et al., 1994; Schultz et al., 1997; Berridge & Robinson, 1998; Pessiglione et al., 2006; Rutledge et al., 2009; Schultz et al., 2016; Wise and McDevitt, 2018; Mohebi et al., 2019) allowing the initiation of preparatory actions prior to presentation of the reinforcers. While these processes have been established as those underlying goal-directed behavior, it is not yet clear how these processes are influenced and how choice is determined when subjects encounter the opportunity to choose between responding for more than one reinforcer.

Determining the neurobiology underlying choice is complicated by the possible scenarios under which choice behavior can occur. Outside of choices made between two reinforcers, choices between pursuing a reinforcer or avoiding a punisher are also common. Compared to a 'reinforcer' a punisher' indicates that the response will be less likely to occur as a result of the associated consequences (Skinner, 1938). Stimulating striosomes which receive input directly from the pregenual anterior cingulate cortex and caudal

orbitofrontal cortex have been reported to bias avoidance over approach behavior (Amemori et al., 2020). These striosomes also receive input from surrounding striatal interneurons which likely allow the activity of many regions to influence the excitation of striosomes and modulate action selection (Friedman et al., 2015). However, the activity of these striosomes seems to be selectively relevant to decisions that require a cost/benefit analysis as stimulation of these cells did not influence actions when decisions between two reinforcing options were offered. While serotonin activity has been implicated in the neurobiology underlying punishment-induced inhibition of behavior (Crockett et al., 2009), phasic DA is involved in action (e.g., go vs. no-go) selection (Guitart-Masip et al., 2014) and goal-directed action initiation and inhibition (Berridge & Robinson, 1998) and is thus also likely to be involved in choices to pursue a reinforcer or avoid a punisher.

DA activity is also likely to be involved in choices between 2 or more reinforcers. Stimulation of mesolimbic DA has been shown to reduce reward thresholds (Markou & Koob, 1992). Additionally, an experiment by Day et al., (2010) suggests that the mesolimbic DA response to a stimulus is reduced when the value of the associated reinforcer is decreased and may thus be involved in determining choices between the same reinforcer at different magnitudes, prices, and/or delays. Likewise, an experiment by Salamone et al., (1994) demonstrated that when given the choice between four food pellets or two, antagonizing accumbal DA activity does not prevent animals from choosing to respond for four pellets. However, if the amount of effort required to obtain four pellets is increased while the effort required to obtain two pellets remains unchanged, DA depletions substantially reduce the frequency in which animals choose four pellets (Salamone et al., 1994). Results from Cromwell et al. (2018) suggest that ventral striatum neurons promote

choice behavior by providing important information regarding the relative value of a reinforcer compared to other available options (e.g., raspberry vs. orange juice). Interestingly, the majority of ventral striatum neurons which responded to stimuli predicting a preferred reinforcer in a choice setting did not respond when the same stimuli were presented outside of a choice setting (Cromwell et al., 2018). It should also be noted that the activity of these neurons following presentation of the stimuli predicted which reinforcer was chosen (Cromwell et al., 2018). Collectively, while the role of DA and mesolimbic activity in driving goal-directed behavior and certain types of choice is compelling, it is still unknown if manipulating these mechanisms can also influence the choice between two different types of reinforcers. The neurobiology which determines the choice between two different reinforcer types is of interest for the current experiment as manipulating such choices may significantly improve clinical outcomes for individuals experiencing certain psychobehavioral pathologies characterized by dysregulations of reinforcement processing.

1.2. Mechanisms of drug choice shift and become biased with experience

Investigations into treatments and preventions of drug dependence make up one facet of research that has benefited from understanding the neurobiology underlying goal-directed behaviors and that would benefit immensely from a better understanding of that underlying choice between one or more reinforcers. An important feature of drug dependence is the persistent choice of drug over other available non-drug reinforcers (Robinson & Berridge, 2008; Ahmed et al., 2013). Thus, identifying the mechanisms which drive the choice of drugs over other reinforcers, rather than just drug-seeking when other reinforcers are not available, is critical to developing effective treatments for drug

dependence (Banks & Negus, 2017; Smith, 2020). Nevertheless, understanding and being able to compare how drug and non-drug reinforcers promote goal-directed behavior in single-schedule settings are critical first steps to identifying how choices between such reinforcers may be determined.

When an individual is initially exposed to cocaine, a euphoric affective response occurs quickly and reliably to promote reinforcement learning (Wise & Koob, 2014), and a supraphysiological DA response results in a neural representation of exaggerated salience (Schultz, 2016). Specifically, within one-minute following cocaine administration, 2 – 4-fold increases in extracellular DA and dose-dependent increases in DA uptake in the NAc have been recorded (Oleson et al., 2009). Additionally, cocaine's ability to block dopamine transporters (DAT; Ritz et al., 1987) enhances the effects of phasic DA release and thus promotes cocaine's incentive salience compared to other reinforcers which may elicit comparable concentrations of mesolimbic DA release. As a result, DA activation of D1-expressing medium spiny neurons (MSNs) of the NAc is increased in response to cocaine and promotes the formation of cocaine reward-context associations (Calipari et al., 2016). Likewise, stimuli that precede the onset of the drug effect (such as seeing, holding, or feeling drug paraphernalia or being in a context in which the drug is regularly taken) take on high incentive value and can contribute to the maintenance of drug-taking and promote relapse during times of abstinence (Robinson et al. 2018). Additionally, spine densities in the NAc core and shell following cocaine administration exhibit increases correlated with preference to be in the location with which cocaine is commonly paired (i.e. cocaine-induced place preference; Marie et al., 2012). The ability of cocaine and other drugs to promote learning may also be due to the discriminative effects of drugs, such as sensing of

autonomic activity or distortions in sensory processing, compared to non-drug reinforcers (Siegel, 2005).

It is important to note, however, that powerful demonstrations of associative learning also occur with other reinforcers, such as food in conditioned taste avoidance learning (Garcia et al., 1955). Reinforcement learning related to non-drug reinforcers also elicits phasic DA responses but depends on additional systems not required for cocaine reinforcement (Berridge et al., 1989; Berridge, 1996; Ahmed et al., 2013). Regarding food, taste-related sensory information is transmitted from receptors on the tongue to the hindbrain pons (Norgren & Pfaffmann, 1975), hypothalamus, and the brainstem (Kanoski, 2012) with sucrose "liking" driven by opioid peptide signaling throughout the hypothalamus, amygdala, NAc, and VTA (Berridge, 1996; Kanoski, 2012). DA depletion is thus associated with aphagia but not taste reactivity (Berridge et al., 1989). Understanding why or under what conditions drugs are initially chosen over non-drug reinforcers can inform treatment and prevention strategies to reduce the number of individuals that progress from acute drug use to drug dependence. However, repeated exposure to drugs of abuse is known to alter the fronto-striatal circuity, including the mesolimbic circuit, and therefore the mechanisms which determine choice are also likely to be dependent on drug experience.

A notable feature of drugs that separate them from other types of reinforcers is that the phasic DA-enhancing response which mediates incentive salience does not habituate, or decrease, with repeated drug exposures (Di Chiara, 1998). However, drug "liking," which is driven through opioid peptide signaling in the hypothalamus, does habituate with continued drug exposure. Thus, the acute effects of DA release discussed thus far, however,

only drive drug use during the early stages of drug dependence (Koob & Le Moal, 2001). Notably, the sensitivity of MSNs in the NAc to DA release from the VTA is dependent on input from regions related to stress (amygdala), memory (hippocampus), and decision making (prefrontal cortex; PFC) and these connections are critical for reward-association learning (Floresco, 2015; Wise and McDevitt, 2018). Specifically, increased drug-seeking and the development of drug withdrawal symptoms are thought to be mediated by neural adaptations that occur in response to repeatedly escalated DA activity (Wise & Koob, 2014). System adaptations neutralize the hedonic effects of the drug both by mechanisms of metabolic tolerance and by the influence of 'anti-reward' systems (Koob & Le Moal, 2008) emerging as opponent processes (Solomon & Corbit, 1974) which drive the emergence of negative affective states leading to maintenance of drug-taking according to a negative reinforcement contingency. While positive reinforcement signifies that a consequence is *added or presented* following a response, 'negative' reinforcement signifies that a consequence is *removed or prevented* following a response (Skinner, 1938). Thus, drug-seeking maintained on a negative reinforcement contingency suggests that the expected removal of withdrawal and/or craving symptoms, rather than the expected euphoric effects of drugs, is what primarily drives drug-seeking.

The emergence of drug-use driven by negative rather than positive reinforcement likely shifts the neurobiological determinants of drug choice and the literature supports that individuals exposed to repeated cocaine exhibit changes to, or even deficits in, components of reinforcement processing. Reduced feedback to the NAc from areas of the PFC contributes to reduced inhibitory control over responses (Jentsch & Taylor, 1999; Volkow & Fowler, 2000) and there is evidence that individuals with a history of cocaine abuse

display reduced modulation of reward prediction error signals, as measured by electroencephalogram feedback negativity, in response to an unpredicted loss compared to a predicted loss (Parvaz et al., 2015). Further, some suggest that adaptions that occur in response to repeated drug exposures cause drug use to shift from being mediated by ventral to dorsal striatal circuits and a parallel shift from drug use being voluntary to more habitual or 'compulsive' (Wolffgramm & Heyne, 1995; Vanderschuren & Everitt, 2004; Everitt & Robbins, 2005 & 2013). While drug-dependent individuals may exhibit choice for drug over non-drug reinforcers under many conditions, it is currently unclear if drug-seeking by these individuals is indeed "compulsive" or "involuntary" in that drug-seeking is invariant and is chosen under all conditions where drug is available. More so, it is possible that the effects of repeated drug use may themselves be insufficient to elicit irreversible 'compulsive' or 'involuntary' drug-seeking, but that some comorbidities may make certain individuals more susceptible to compulsive patterns of drug use (Robinson & Berridge, 2003; Heyman, 2009).

Of note, reports within the current literature stress the significance of including of sex as a biological factor in assessing the choice between sucrose and cocaine. Clinically, women begin using cocaine at an earlier age, exhibit faster escalation to addiction, and display a more severe addiction relative to males (Kosten et al., 1993; Becker, 2016). In animal models, female rats are more sensitive to cocaine reward (Lynch & Carroll, 1999; Becker, 2016), have been shown to earn more cocaine reinforcers under single-schedule testing conditions (Jackson et al., 2006), and more likely than males to display choice for cocaine over food (Kerstetter et al., 2012; Perry et al., 2013). Additionally, both striatal D1 receptor binding and dopamine transporter affinity for cocaine have been reported to

change with animals' estrous cycle (Levesque et al., 1989; Calipari et al., 2017). Females may also be more sensitive to reinforcement from nonpharmacological signals (i.e., reward-associated stimuli) available in both sucrose- and cocaine-operant testing sessions and are likely to display greater intake of both rewards, compared to males (Chaudhri et al., 2005).

Identifying the neurobiological substrates of drug choice early and later in drug experience has significant implications for how drug dependence is treated. The role of the mesolimbic circuit in mediating goal-directed behavior and certain types of choice behavior make this circuit a likely mediator of the choice between two types of reinforcers. A primary aim of the current experiment is to determine if manipulation of mesolimbic circuit activity can alter choice behavior; particularly, the choice between a drug (cocaine) and non-drug (sucrose) reinforcer, which is significant to the understanding of the mechanisms of drug dependence. As described previously, a critical feature of drug dependence is the choice of a drug over non-drug reinforcers. Thus, if adding cocaine to the reinforcers available in an operant procedure reduces the choice of sucrose so that it is significantly lower than that of cocaine, it would be of significant clinical interest to determine if manipulation of the mesolimbic or other neural circuits can alter choice to reduce the choice of a drug over non-drug reinforcers. Likewise, continued research into how the choice between reinforcers is determined and therefore, how harmful choice behavior can be treated or prevented, can potentially benefit individuals experiencing other forms of dysregulated reinforcement processing such as internet addiction, obesity, or apathy.

1.3. Mechanisms of choice are likely disrupted in apathy related to HIV-1

Understanding the mechanisms of goal-directed and choice behavior, and whether neural circuit manipulation can be harnessed to encourage more desirable choices may also inform treatment strategies for psychobehavioral pathologies outside of addiction. Those interested in disruptions to reinforcement processing that occur with HIV-1 exposure, for example, could potentially benefit from understanding how to increase desirable goal-directed behaviors without increasing less desirable behaviors such as drug-taking. The advent of combination antiretroviral therapies (cART) has significantly improved clinical outcomes for individuals with HIV-1 (Teeraananchai et al. 2016). However, cART only successfully suppresses HIV-1 in the periphery, leaving the central nervous system (CNS) neurons and microglia vulnerable to HIV-1 viral proteins (Tat and gp120) and other products (Woods et al., 2009; Elbirt et al., 2015; McIntosh et al., 2015). Reductions in goal-directed behaviors pertinent to maintaining employment, medication adherence, and interpersonal functioning have been reported in persons living with HIV (Gorman et al., 2009; Cysique & Brew, 2019). Such symptoms characterize apathy, defined as a quantitative reduction of self-generated voluntary and purposeful (goal-directed) behavior. Apathy has been estimated to affect approximately 11-50% of HIV-1+ individuals (Cysique & Brew, 2019) (estimated at 30-60% by van Reekum et al., 2005), and is repeatedly associated with neurodegenerative diseases, age-related dementia, stroke damage, or other sources of disruption to the fronto-striatal circuit, which is targeted by HIV (van Reekum et al., 2005; Levy & Dubois 2006; Woods et al., 2009; McIntosh et al., 2015; Cysique & Brew, 2019; Illenberger et al., 2020). More so, apathy has been associated with lower mental and physical quality of life in HIV-1 individuals, independent of

depression, neurocognitive impairment, functional status, and current CD4 count (Elbirt et al., 2015; Kamat et al., 2016). It is therefore critical and to understand the neurobiological mechanisms of apathy in HIV-associated neurocognitive disorders to develop effective methods to treat apathy and continue to improve the livelihoods of patients living with HIV-1.

White matter damage and synaptodendritic injury within the fronto-striatal circuit are associated with alterations in motivation (i.e., apathy) in individuals with chronic exposure to HIV-1 (Castellon et al., 1998 & 2000; Cole et al., 2007; Woods et al., 2009; Heaton et al., 2010; Kuper et al., 2011; Kamat et al., 2012; Desplats et al., 2013; Du Plessis et al., 2014; Elbirt et al., 2015; Ipser et al., 2015; McIntosh et al., 2015; Walker and Brown, 2018; Cysique and Brew, 2019). In addition to damage to white matter tracts, human imaging (Wang et al., 2004; Chang et al., 2008; Risacher and Saykin, 2013), postmortem brain tissues (Sardar et al., 1996; Slivers et al., 2006; Kumar et al., 2011), cerebrospinal fluid levels (Berger et al., 1994; di Rocco et al., 2000), and reduced accumbal volume (Paul et al., 2005) support that dopamine is reduced in HIV+ individuals and DA dysregulation is correlated with cognitive deficits. However, the most forthcoming evidence of DA reductions being a primary mechanism of HAND may be that the neurocognitive symptomology observed in HIV+ individuals is commonly compared with that of Parkinson's disease (of those already cited: Sardar et al., 1996; Castellon et al., 1998 and 2000; di Rocco et al., 2000; Wang et al., 2004; Chang et al., 2008; Woods et al., 2009; Kumar et al., 2011; Kuper et al., 2011; Kamat et al., 2012 & 2014; Elbirt et al., 2015; Illenberger et al., 2020; also see: Dunlop et al., 1992; Berger and Nath, 1997; Lopez et al., 1999; Berger and Arendt, 2000; Valcour et al., 2008; Muller-Oehring et al., 2020).

Specifically, patients with HIV displayed reduced reaction time (Dunlop et al., 1992), hypoactivation of the PFC (Muller-Oehring et al., 2020), and scored significantly higher on the Unified Parkinson Disease Rating Scale (Valcour et al., 2008) compared to patients not exposed to HIV. Thus, while it has been suggested that increased DA may aid in HIV viral proliferation and immune dysfunction in the CNS (Matt & Gaskill et al., 2019; Nickoloff-Bybel et al., 2019), increases in DA in the HIV-exposed brain relative to controls is likely only relevant early on in HIV disease progression, prior to significant HIV-induced dysfunction in the fronto-striatal circuit associated with a hypodopaminergic state (Purohit et al., 2011; Illenberger et al., 2020). The functions of the fronto-striatal circuit in mediating goal-directed behaviors and observations of the behavioral effects of DA dysregulation in other populations suggest that DA dysregulation, in addition to altered circuit connectivity, likely play a large role in the appearance of HAND and apathy in HIV-1 individuals (Berger and Arendt, 2000; Illenberger et al., 2020).

Experiments utilizing the HIV-1 transgenic (Tg) rat, developed by Reid et al., (2001), have been central to elucidating some mechanisms by which chronic HIV-1 viral protein exposure alters the fronto-striatal circuit. Removal of gag and pol from the viral plasmid used to create the HIV-1 Tg rat prevents the replication of HIV. However, the expression of 7 of 9 HIV-1 genes permits encoding of viral proteins such as Tat, Rev, and gp120, providing a model of the fronto-striatal circuitry in the presence of chronic suppressed HIV replication (Reid et al., 2001). The fronto-striatal circuit of HIV-1 Tg rats exhibits signs of stress (Li et al., 2013; Pang et al., 2013; Yang et al., 2016; Shah et al., 2019) and dysregulation (Midde et al., 2011; Festa et al., 2015; Reid et al., 2016; Khodr et al., 2018) which appear to advance with age. Similar to what is observed in HIV-1+

individuals (Chang et al., 2008), the HIV-1 Tg rat exhibits a decrease in DA tone and altered DA reuptake (Sultana et al., 2010; Moran et al., 2012; McIntosh et al., 2015; Zhu et al., 2016; Javadi-Paydar et al., 2017; Sinharay et al., 2017; Denton et al., 2019; Goulding et al., 2019). HIV-1 Tg rats also exhibit a shift in spine morphology (Roscoe et al., 2014) with an increased relative frequency of stubby spines on more proximal branches of MSNs which receive DA afferents from the VTA and glutamate (Glu) afferents from the PFC (McLaurin et al., 2018a) supporting altered connectivity between these regions (Wayman et al., 2016; McLaurin et al., 2018b). As posited in the previous section (Chapter 1.2) behavioral symptoms of dysregulated reinforcement processing may be alleviated by targeting the activity of the mesolimbic circuit. Specifically, DA activity may serve as a prolific target for treatments of apathy in addition to drug dependence (as described in the previous section) as DA activity is an established target of HIV-1 viral protein exposure.

Behaviorally, HIV-1 Tg rats also display altered as well as reduced exploration of novel objects (Reid et al., 2016), social opportunities (Nemeth et al., 2014), motor performance and locomotor habituation (June et al., 2010; Moran et al., 2013; Nemeth et al., 2014; Reid et al., 2016), and goal-driven behavior in operant testing (Bertrand et al., 2018; Huynh et al., 2020; McLaurin et al., under review) compared to F344/N control rats. In an experiment by Bertrand et al., (2018), despite exhibiting similar sucrose taste preference in a 5-bottle test, ovariectomized HIV-1 Tg rats took significantly longer to acquire sucrose-maintained responding and displayed reduced response vigor for various (0-30% w/v) concentrations of sucrose under fixed and progressive ratio schedules of reinforcement. These results were supported by those of McLaurin et al., (under review) also in ovariectomized F344/N and HIV-1 Tg rats. When responding for intravenous (IV)

cocaine, however, Bertrand et al., (2018) reported that ovariectomized HIV-1 Tg rats displayed slower escalation of cocaine (1.0 mg/kg) intake and reduced response vigor and sensitivity to cocaine of various doses (0.01-1.0 mg/kg/infusion). In comparison, McLaurin et al., (under review) reported that ovariectomized HIV-1 Tg rats displayed faster escalation of cocaine (0.75 mg/kg) intake and a differential reinforcing efficacy across doses of cocaine compared to F344/N animals. Of interest, McLaurin et al., (under review) also report that dendritic spines on MSNs of the NAc from HIV-1 Tg animals exhibit a population shift towards more immature (e.g., stubby) spine types compared to F344/N animals. A more recent report from Huynh et al., (2020) supports that HIV-1 Tg male rats also exhibit reduced motivation to earn cocaine rewards compared to F344/N male rats. This is notable as biological sex has been shown to moderate the influence of HIV-1 genotype on neurobiological and behavioral outcomes such as accumbal MSN dendritic spine morphology (McLaurin et al., 2018a), histamine+ cell expression (Denton et al., 2019), neuroinflammation (Rowson et al., 2016), and performance in signal detection tasks (McLaurin et al., 2017), prepulse inhibition (McLaurin et al., 2018c), and locomotor tasks (Rowson et al., 2016). Importantly, McLaurin et al., (under review) also reported that treating ovariectomized HIV-1 Tg rats with S-equol, a phytoestrogen implicated as an efficacious therapeutic for HIV-1 associated neurocognitive impairments, shifted dendritic spines towards a more mature phenotype (e.g., thin) and alleviated some of the genotypic differences observed in sucrose- and cocaine-maintained responding.

Notably, HIV-1 Tg rats also exhibit motivational impairments when given the opportunity to earn both 5% (w/v) sucrose solution or 0.33 mg/kg/infusion IV cocaine rewards on concurrent fixed ratio (1) schedules of reinforcement. On the first day of

concurrent choice testing, ovariectomized F344/N rats earn more sucrose rewards but then decrease sucrose intake and increase cocaine intake to earn more cocaine rewards on the remaining days (up to 7 days). The change in choice behavior across testing days may indicate that the relative value of sucrose and cocaine to each other was changed. In stark contrast, ovariectomized HIV-1 Tg rats earn more sucrose rewards on the first day of testing but then decrease sucrose intake so that there was no significant difference between sucrose and cocaine intake on the remaining days (Bertrand et al., 2018). Thus, although the relative value of sucrose compared to cocaine appeared to decrease across sessions in both F344/N and HIV-1 Tg rats, HIV-1 Tg rats did not demonstrate an accompanying increase in cocaine value relative to sucrose. These results are especially interesting given that cocaine reinforcement is likely more dependent on mesolimbic DA activity than sucrose reinforcement, which is more dependent on opioid receptor activity, peripheral nervous system (e.g., digestive) activity, and a more expansive circuitry, including feedback from areas such as the hypothalamus and brainstem (Norgren & Pfaffmann, 1975; Berridge, 1996; Arbisi et al., 1999; Kelley et al., 2002; Kanoski, 2012; Ahmed et al., 2013).

In addition to traditional methods of assessing goal-directed behavior, concurrent schedules of reinforcement, or choice procedures, highlight differences in the way in which reinforcers interact. There is conflicting evidence in the literature suggesting that HIV-1 Tg animals exhibit reduced (Bertrand et al., 2018; Huynh et al., 2020) or enhanced (McLaurin et al., under review) responding for cocaine under single-schedule procedures. However, only Bertrand et al., (2018) reported cocaine vs. sucrose choice behavior, and this therefore may provide insight into the reinforcing properties of cocaine in HIV-1 + individuals. Choice procedures should therefore be included as a method to assess relative

reward value and reward-related processing in populations that are vulnerable to dysregulation of reinforcement processing and motivation; such as females who may be vulnerable to the dependence-forming effects of repeated drug exposures or such as the HIV+ population which may be vulnerable to apathy. Notably, concurrent choice procedures are easily adapted for testing across species (e.g., humans (Sanders, 1968), nonhuman primates (Ferster, 1957), rodents (Ferster and Skinner, 1957), pigeons (Ferster and Skinner, 1957) and should be tested to determine if they can identify motivation dysregulation early on in disease progression. Further, performance in concurrent choice procedures may provide a more precise method by which to evaluate apathy compared to self-reports on apathy scales which are commonly used to research apathy in HIV-infection but are also likely to be influenced by anosognosia (Cysique & Brew, 2019). Concurrent choice procedures and other assessments of goal-directed processing may therefore be critical to identifying at-risk individuals both with and without HIV-1 exposure prior to any significant neurocognitive decline. Genotypic differences in the choice procedure reported by Bertrand et al., (2018) emphasize that the procedure can be used to identify motivational alterations between populations of interest. It is possible that stimulation of the mesolimbic circuit may not influence choice behavior displayed by certain populations of interest (e.g., those exhibiting drug dependence or apathy) in the same way. A secondary aim of the current experiment is thus to determine if stimulating mesolimbic activity alters choice behavior in a sex-dependent or genotype-dependent manner. Specifically, if females exhibit greater drug choice than males or if HIV-1 Tg animals exhibit altered choice compared to F344/N animals, it is of significant interest to determine if mesolimbic

stimulation can alleviate symptoms of drug dependence or apathy to reduce such sex and genotype differences, respectively.

Chapter 1.4: Can mesolimbic stimulation alter drug choice and/or reduce apathy?

The current experiment aimed to determine if modulation of mesolimbic circuit activity alters concurrent choice behavior in F344/N and HIV-1 Tg rats. Subsequently, it was of interest to determine if manipulation of mesolimbic activity altered choice behavior in a manner that reduced signs of drug dependence or apathy, hypothesized to be observed, respectively, in females compared to males and in HIV-1 Tg animals compared to F344/N animals, according to the current literature. The contemporary chemogenetic approach, designer receptors exclusively activated by designer drugs (DREADDs; Zhu & Roth, 2015), was utilized according to the methods of Li et al., (2019) to selectively manipulate the activity of targeted neurons of the VTA which project to the NAc. The current methods thus provide enhanced selectivity of circuit modulation compared to pharmacologically-elicited activity. Specifically, adeno-associated viruses (AAVs) which provoke the expression of Cre and green fluorescent protein (GFP) (see Kaspar et al., 2002) were infused bilaterally into the NAc of each animal and either saline or adeno-associated viruses (AAVs) which Cre-dependently provoke the expression of an excitatory G protein-coupled human M3 muscarinic (hM3D(Gq)) (Thompson et al., 2018; Jendryka et al., 2019) and an inhibitory Gi-coupled kappa-opioid receptor (KORD) (Vardy et al., 2015) were infused bilaterally into the VTA. The current procedure allowed for bidirectional manipulation of populations of cells expressing both receptor types. Additionally, hM3D(Gq) and KORD receptors have separate selective ligands, Compound 21 (C21) and Salvinorin B (Sal B), respectively. Thus, excitatory and inhibitory ligands can be

administered sequentially prior to behavioral testing to determine if stimulating KORD receptors reverses the influence of stimulating hM3D(Gq) receptors (Vardy et al., 2015).

Stimulation of hM3D(Gq) receptors with C21 (Thompson et al., 2018; Jendryka et al., 2019) has been shown to significantly influence the activity of cells in regions infused with hM3D(Gq)-evoking AAVs. Our laboratory previously reported an increase in the locomotor response to novelty selectively in F344/N rats expressing DREADDs hM3D(Gq) receptors in the same circuit targeted in the current experiment (Li et al., 2019). However, Li et al., (2019) utilized intraperitoneal (IP) administration of the hM3D(Gq) ligand clozapine-N-oxide (CNO) while the current experiment will utilize IV-administration of C21. Stimulation of DREADDs hM3D(Gq) with IP-administered CNO selectively in DA neurons of the VTA was reported by Mahler et al., (2019) to increase reinstatement of cocaine-seeking behavior when administered on its own and in addition to exposure to cocaine-paired cues, cocaine, or pharmacological stress. In contrast, application of 100 nM of the DREADDs KORD ligand, Sal B, to tissues infused with KORD-inducing AAVs led to robust and significant membrane potential hyperpolarization (Vardy et al., 2015). Likewise, Marchant et al., (2016) reported a significant decrease in spontaneous (e.g., following saline) and cocaine-induced locomotor activity when rats expressing KORD in the VTA were administered Sal B subcutaneously (7.5 – 30 mg/kg). More so, in an experiment using DREADDs Gi protein-coupled human M4 muscarinic DREADDs receptors to inhibit DA cells of the VTA in mice, inhibition did not significantly alter cocaine-conditioned place preference or sucrose preference, but did extend extinction of cocaine-maintained responding and reduced motivation to work for sucrose rewards (Runegaard et al., 2018).

Regarding the current experiment, it was hypothesized that each of the 4 phenotypes represented in the current experiment (i.e., F344/N males, F344/N females, HIV-1 Tg males, and HIV-1 Tg females) process reward-related signals differently and may therefore display unique patterns of choice behavior relative to the other groups. It was hypothesized that females would exhibit greater intake of cocaine over sucrose than males in concurrent choice procedures and HIV-1 Tg animals would exhibit apathy, as evidenced by disrupted choice behavior, compared to F344/N animals. Stimulation of hM3D(Gq) receptors on cells of the VTA projecting to the NAc was hypothesized to alter goal-directed behavior in F344/N rats during exposure to the choice procedure. It was of particular interest to determine if observed changes to the choice procedure reduce the choice of cocaine over sucrose and/or if choice is altered sex-dependently. The current experiment also aimed to support and expand on the results of Bertrand et al. (2018). It was hypothesized that, similar to that described by Bertand et al. (2018), HIV-1 Tg rats would display reduced motivation to earn sucrose and cocaine under single-schedule and concurrent-schedule operant procedures compared to F344/N rats. It was thus of interest to determine if mesolimbic stimulation could alter choice behavior in HIV-1 Tg rats, and in particular, if observed changes reduced genotypic differences in choice behavior compared to F344/N rats. Genotypic differences in the ability to alter reward intake following DREADDs stimulation with C21 were hypothesized and may suggest altered circuit composition/organization such as greater competition between DA inputs from the VTA and inputs of other neurotransmitter (e.g., Glu-ergic or GABAergic) types or of different origins (e.g., local NAc interneurons or the PFC) in mediating goal-directed behavior. Amongst the neural systems involved in determining goal-directed behavior, the signals

which are dominant enough to overcome opposing signals and determine functional and/or behavioral outcomes may differ depending on the pre-existing state of the neural systems. The hypothesized differences in the choice between sucrose and cocaine or in the ability to change choice across phenotypes would suggest underlying differences in fronto-striatal circuit function, and more so, suggest that HIV-1 exposure and/or biological sex are important factors to consider in the development of treatments for neurobehavioral pathologies driven by fronto-striatal dysfunction (e.g. apathy and/or addiction)

Chapter 2. Methods

2.1. Animals

Adult male (n = 20) and female (n = 20) F344/N rats were ordered from Envigo laboratories to match male (n = 20) and female (n = 20) HIV-1 Tg rats from our laboratory's breeding colony (AUP: 2382). The colony room in which animals were housed throughout the experiment was maintained at approximately 20 ± 2°C, 50 ± 10% relative humidity with a 12-hour light/12-hour dark cycle (lights on at 7:00h and lights off at 19:00h). Food (Pro-Lab Rat, Mouse, Hamster Chow #3000) and water were available in home cages *ad libitum* throughout the experiment unless otherwise stated.

2.2. Testing Apparatus

Operant chambers (ENV-008; Med-Associates, St. Albans, VT) had stainless steel front and back panels, metal grid floors, and polycarbonate sides and tops and were housed within sound-attenuating enclosures. The front panel of the chamber houses a 5 cm × 5 cm receptacle with an infrared sensor used to detect head entries and an opening that allows a recessed 0.01 cc dipper cup controlled by Med-PC computer interface software to deliver sucrose solution following a successful response on the active levers located on either side of the receptacle. An inactive lever was located on the wall opposite the receptacle and responses on this lever produced no consequence. A 28-V house light is located above the

inactive lever and were illuminated throughout testing sessions except for 20 s following successful responses during cocaine-maintained responding and choice trials.

2.3. Sucrose-Maintained Responding

To acclimate animals to sucrose and minimize agoraphobia prior to any behavioral testing, approximately 20 (10/animal) 45 mg sucrose pellets were placed into home cages daily for approximately 12 days before beginning testing. To begin sucrose-maintained response training, animals were placed in operant chambers and were required to poke their nose into the receptacle from which sucrose would be delivered to begin the session. Once this occurred, dippers raised at a variable interval schedule of reinforcement for the duration of the 50-minute testing session, providing 4 seconds of access to a 5% (w/v) sucrose solution. To aid the animal's habituation to the testing chamber prior to autoshaping, this procedure was repeated on the second day of training. Autoshaping began on the third day of testing. Autoshaping sessions lasted up to 42 minutes during which the animal could press either of the active levers to receive access to 5% (w/v) sucrose reinforcer on a fixed-ratio (1) (FR(1)) schedule of reinforcement. In addition, a reinforcer was automatically delivered every 10 minutes to encourage animals to approach the receptacle. Autoshaping sessions terminated early if animals reached 120 reinforcers. As per the methods of Bertrand et al., (2018) and McLaurin et al., (under review), animals were required to reach a minimum of 60 reinforcers for 3 consecutive days to move on from autoshaping. While, on average, animals completed autoshaping criteria in approximately 12 days, a number of animals failed to meet criteria after 60 days of autoshaping training and were, therefore, water restricted for up to 18 hours prior to daily testing to increase responding. It should be noted that the current procedure does differ

from that of Bertrand et al., (2018) and McLaurin et al., (under review) in that animals in the current experiment were not water restricted until at least 60 days of training had passed without meeting criteria. This was done to provide a measure of stimulus-reinforcement learning that was not altered by experimentally enhancing the salience of the reinforcer. If animals were water restricted after 60 days of training, *ad libitum* water access was provided again once animals met criteria under water-restricted conditions. Animals continued autoshaping testing under non-restricted conditions until criteria were met to ensure that proficiency in the task was demonstrated before comparing the for reinforcing efficacy and response vigor of various sucrose and/or cocaine doses across groups.

After meeting autoshaping criteria, animals were placed on an FR(1) schedule of reinforcement. In this FR(1) portion of the experiment, no reinforcers were delivered non-contingently and there was no maximum number of reinforcers that could be earned within the 60-minute sessions. Animals remained in FR(1) training until meeting the criteria of at least 60 reinforcers for 3 consecutive days.

2.4. Sucrose Dose-Response

To determine if the response vigor, sensitivity to, or reinforcing efficacy of sucrose differed between phenotype, animals' motivation to press for sucrose solutions of various concentrations (0, 1, 3, 5, 10, and 30%) was tested on progressive ratio (PR) and then FR(1) schedules of reinforcement according to the methods described in Bertrand et al., (2018) and McLaurin et al., (under review). Doses were tested every other day according to a Latin-square design, except for water (0% w/v sucrose solution) which was tested after all other doses to prevent extinction of responding. Each dose was tested only once under a PR schedule of reinforcement and once under a FR(1) schedule of reinforcement. PR

sessions lasted a maximum of 120 minutes and were terminated early if no reinforcer was earned within 60 minutes. In addition, animals were returned to the FR(1) schedule of reinforcement on days that fell between dose responses testing to prevent extinction of responding following low doses. Once again, water-restriction was avoided in the current experiment to provide an unobstructed measure of sucrose intake. Most animals had met autoshaping and FR(1) criteria with a training dose of 5% (w/v) sucrose solution and therefore this was the dose used during "maintenance days" between dose-response testing. However, animals that had not completed autoshaping of operant responding to earn 5% sucrose after 110 days were trained to earn sucrose solution of an increased (10% w/v) concentration (F344/N Males: n = 3; HIV-1 Tg Males: n = 6; HIV-1 Tg Females: n = 2). After 175 days, animals that had still not completed sucrose testing were trained to earn a 26% (w/v) sucrose solution (HIV-1 Tg Males: n = 5; HIV-1 Tg Females: n = 1). The sucrose concentration which each animal was trained on upon meeting autoshaping criteria was the dose used during FR(1) "maintenance days" between sucrose dose-response testing. After completing dose-response testing under both PR and FR(1) schedules of reinforcement, animals participated in FR(1) sessions on their training dose of sucrose (5, 10, or 25% w/v) at least twice a week until their cage mate also completed testing and were thus ready for surgeries.

2.5. Surgeries

Following testing of sucrose-maintained responding, each animal underwent stereotaxic surgery to infuse a DREADDs viral vector into the NAc and DREADDs viral vector or vehicle (saline) into the VTA according to the methods described by Li et al., (2019). Animals were anesthetized with 3-5% inhalant sevoflurane (Abbot Laboratories,

North Chicago, IL) and maintained at 2-3% sevoflurane throughout IV catheter implantation immediately following stereotaxic surgery. For infusion of DREADD viral vectors, each animal was placed in a stereotaxic apparatus (Model 900; Kopf Instruments, Tujunga, CA) where the skull was exposed and small 0.4 mm holes were drilled bilaterally at 0.5 mm lateral, 1.2 mm rostral to Bregma for infusions (7mm depth) of AAV-CMV-GFP/Cre into the NAc and at 1mm lateral, 5mm caudal to Bregma for infusions (8mm depth) of DREADD vectors or saline into the VTA.

IV catheterizations and post-surgical treatment were performed according to the method described in Bertrand et al., (2018) and McLaurin et al., (under review). A sterile IV catheter was implanted into the right jugular vein and the dorsal portion of the catheter was affixed to an acrylic pedestal embedded with mesh which rested directly below the skin on the dorsal surface of the animal, below and between the shoulder blades.

Subcutaneous butorphanol (1.0 mg/kg Dolorex) and 1% gentamicin (0.2 mL IV) were administered immediately after surgery to provide analgesia and prevent infection, respectively. Animals were monitored in a heat-regulated chamber until recovery from anesthesia. Animals were given 4 days to recover from surgeries before training cocaine-maintained responding. 1% gentamicin (0.2 mL) was administered to each animal IV daily for 10 days following surgery and a 1 mg/kg IV injection of 1 mg/kg solution containing 2.5% heparin and 1% gentamicin was administered IV daily.

2.6. Viral Vectors

Viral vectors AAV-CMV-GFP/Cre (serotype 9; Plasmid #49056), pAAV-hSyn-DIO-hM3D(Gq)-mCherry (serotype 2; Plasmid #44361), and pAAV-hSyn-dF-HA-

KORD-IRES-mCitrine (serotype 8; Plasmid #65417) were ordered from Addgene (Watertown, MA). Each animal received a bilateral infusion of approximately 4 μL (2 μL/hemisphere) of AAV-CMV-GFP/Cre into the NAc. Animals that received DREADDs infusions (n=48) had approximately 2 μL of pAAV-hSyn-DIO-hM3D(Gq)-mCherry and 2 μL pAAV-hSyn-dF-HA-KORD-IRES-mCitrine infused bilaterally into the VTA. Vehicle of the same volume was infused into the VTA of sham animals (n = 32). The GFP (green), mCherry (red), and mCitrine (yellow) tags allowed for ex vivo verification of vector-induced protein expression following behavioral testing. The hSyn promotor on DREADD vectors ensured hM3D(Gq) and KORD receptor expression only in neurons while double inverted coding sequences allowed for targeted receptor expression selectively in cells of the VTA which project to cells of the NAc expressing Cre. This procedure was adapted from Li et al., (2019) which suggested that when DREADDs viral vectors which elicit expression of Cre and hM3d(Gq) receptors were infused into the stereotaxic coordinates proposed here, expression of Cre occurs within the NAc and expression of hM3D(Gq) occurs within the posterior ventral tegmental area, primarily made up of DA cells.

2.7. Drugs

Cocaine hydrochloride (Sigma-Aldrich Pharmaceuticals, St. Louis, MO) was weighed as the salt and dissolved in saline (0.9%; Hospira, Inc. Lake Forest, IL). All solutions were prepared fresh before the start of each session to prevent significant hydrolysis of cocaine.

The hM3D(Gq) ligand, Compound 21 (C21), was obtained from HelloBio (Princeton, NJ; Cat #: HB6124) and weighed as the salt and dissolved in saline at the start of each needed testing day. The KORD DREADDs agonist, Sal B, was obtained from

Cayman Chemical (Ann Arbor, MI; Cat #:23582) and prepared according to the manufacturer's instructions at the start of each needed testing day. Notably, the proposed experiment will be the first of our knowledge to report the effects of DREADDs ligands administered via the IV rather than the IP route of administration. IV administration should increase the bioavailability of the ligand within the brain and this occurs at a faster rate than IP administration. Additionally, because animals were receiving other IV injections through their IV catheters before each session, administering the ligand via the IV route rather than the IP route likely alleviated unnecessary stress due to the use of a route of administration to which animals were less habituated.

2.8. Cocaine-Maintained Responding

After 4 days of recovering from surgery, animals began training to respond for IV cocaine infusions based on the methods of Morgan et al., (2006). Lever-pressing was reinforced with a 0.2 mg/kg infusion of cocaine as reported by McLaurin et al., (under review). Specifically, once animals successfully earned their first cocaine infusion, they were tested for 5 days (1 hour-long session/day) on an FR(1) schedule of reinforcement. These FR(1) sessions, like FR(1) sessions with sucrose as the reinforcer, did not have a programmed maximum number of responses that could be made. Throughout the remainder of the experiment, each IV cocaine reinforcer was followed by a 20-second "time out" period in which the house light turned out and active levers remained retracted to prevent the animal from responding for either sucrose or cocaine.

After 5 days of cocaine-maintained responding on an FR(1) schedule, animals were switched to a PR schedule for at least 7 days to allow for escalation of cocaine-reinforced

responding. During PR sessions, the dose of IV cocaine which animals earned was increased to 0.75 mg/kg/infusion based on the results of McLaurin et al., (under review).

2.9. Sucrose Responding in the Presence of hM3D(Gq) Stimulation

To determine if activating the hM3D(Gq) receptor via injection with IV C21 altered the magnitude of responding when sucrose was the sole reinforcer, sucrose-maintained responding was tested on an FR(1) schedule of reinforcement following IV injections of 0.01, 0.03, 0.10 mg/kg C21. Following 2 days of testing sucrose maintained-responding after 1.0 mg/kg saline, doses of C21 were tested in an ascending fashion with maintenance days, in which animals were injected with 1 mg/kg IV saline, between testing days. The number of sucrose reinforcers was summed into 6, 7-minute bins to evaluate how responding changes throughout the session, as the timecourse within which IV C21 can influence behavior is currently unclear.

2.10. Choice

Next, to determine F344/N and HIV-1 Tg male's and female's choice between sucrose and cocaine prior to DREADDs activation, animals were tested in 7 concurrent schedule sessions. Balanced across animals, responses on the left or right active lever were reinforced with 4 seconds of access to a 5% (w/v) sucrose solution while responses on the other lever were reinforced with a 0.2 mg/kg infusion of IV cocaine. Following 7 days of choice between sucrose and cocaine, each animal's choice between sucrose and saline and then between water and saline were tested to verify that animals can discriminate between the presence/absence of reinforcers rather than just responding for the reinforcement provided by reinforcer-related cues. All sessions for the remainder of the experiment lasted

a total of 1 hour and had no programmed maximum number of responses. The number of sucrose and cocaine reinforcers earned was summed into 12, 5-minute bins to evaluate how responding changes throughout the session.

2.11. Choice in the Presence of hM3D(Gq) Stimulation

Following the establishment of choice behavior following IV saline, C21 was administered prior to choice sessions to determine if activation of the hM3D(Gq) receptor with C21 could switch which reinforcer is chosen. Doses of 0.01, 0.03, 0.10, and 0.30 mg/kg IV C21 were tested in ascending order with the administration of IV saline every other day, between testing doses. Doses of C21 were adjusted from those active doses reported following IP administration by Jendryka et al., (2019).

2.12. Sal B Reversal of C21 Influence on Choice

Lastly, to determine if the effects of C21 on choice behavior can be bidirectionally manipulated, IV C21 injections were followed by an injection of 0.15 mg/kg IV Sal B (adjusted from Vardy et al., 2015). Based on the results of the C21 dose-response testing during sucrose-maintained and choice responding, all groups were injected with 0.10 mg/kg IV C21 except for F344/N females which were injected with 0.30 mg/kg C21. Due to the different timecourse of C21 and Sal B's actions, C21 was injected IV and then animals were returned to home cages for approximately 15 minutes before IV Sal B injection and initiation of choice sessions (Vardy et al., 2015; Jendryka et al., 2019).

2.13. Verification of Cannula Placement and DREADDs expression

Following behavioral testing, animals were sacrificed, and brains were extracted to confirm that cannula tracts terminated in the NAc and VTA (sham: n=16; DREADDs:

n=16) and to confirm the presence of GFP expression in cells of the NAc and the absence or presence of expression of GFP, mCherry, and mCitrine in the VTA (sham: n=16; DREADDs: n=32). According to the methods of Li et al., (2019), all animals were deeply anesthetized with sevoflurane and transcardially perfused with ~100ml of 100mM PBS followed directly by ~150 ml of chilled 4% paraformaldehyde buffered in PBS. Brains were post-fixed in 4% chilled paraformaldehyde and then sectioned in 100 µm-thick coronal slices, mounted on Superfrost Plus microscope slides, (FisherScientific # 12-550-15), and covered with microscope cover glass (FisherScientific #12-544-D) using Cytoseal XYL mounting medium (ThermoScientific #8312-4).

Images were taken with a Nikon TE-2000E confocal microscope utilizing Nikon's EZ-C1 software (version 3.81b). Expression of mCherry and mCitrine was hypothesized to be present selectively within the posterior VTA, as reported of mCherry expression by Li et al., (2019).

2.14. Statistical Analyses

Statistical analyses were conducted in SPSS Statistics 26 (IBM) and slope parameters for lines fitted to raw behavioral data were conducted in GraphPad Prism 9.0.0 (121). SAS Studio 3.8/University Edition was used to conduct post hoc analyses with censored data (described in Chapter 3.5.). An alpha level of 0.05 was used and effect sizes (partial eta squared) are reported with statistically significant findings. Averages are presented as Mean ± Standard Error of the Mean (SEM). A priori hypotheses regarding the function of within-subjects effects guided orthogonal within-subjects' comparisons. When the effect of a DREADDs ligand was considered, simple comparisons between each dose and saline were made. Age at the time of behavioral testing was included as a random effect

variable in all applicable analyses conducted within SPSS. Bonferroni corrections were utilized for multiple comparisons.

First, linear regression was used to determine if the rate at which each group (F344/N male, F344/N female, HIV-1 Tg male, HIV-1 Tg female) met autoshaping criteria ($\geq$ 60 reinforcers on 3 consecutive days) across the first 60 days of testing was best represented with a single line, lines averaging across biological sex, lines averaging across genotype, or different lines for each group. Slopes (β_1) of the best lines fits were compared to test the hypothesis that the rate at which HIV-1 Tg rats would meet autoshaping criteria at a significantly slower rate than F344/N rats and males would meet criteria at a significantly slower rate than females.

Similarly, linear regression was used to determine if the rate at which animals met FR(1) criteria ($\geq$ 60 reinforcers on 3 consecutive days) was significantly influenced by genotype or biological sex. We hypothesized that once animals met autoshaping criteria, the rate at which each group met FR(1) criteria would not be significantly different. A genotype × sex × day mixed-models analysis of variance (ANOVA) was also used to determine if the number of sucrose reinforcers earned by each group in the first 5 days of FR(1) testing was significantly different. It was hypothesized HIV-1 Tg animals would earn significantly fewer sucrose reinforcers than F344/N animals and males would earn significantly fewer sucrose reinforcers than females. The number of sucrose reinforcers earned across the first 5 days of FR(1) testing was not expected to significantly change across day.

A genotype × sex × sucrose dose mixed-models ANOVA was used to test the hypotheses that, across doses, HIV-1 Tg rats would earn significantly fewer sucrose

reinforcers than F344/N rats and males would earn significantly fewer sucrose reinforcers than females when tested under both PR and FR(1) schedules of reinforcement. It was also hypothesized that the number of reinforcers earned would increase with sucrose dose, as reported under the PR schedule of reinforcement by McLaurin et al., (under review). Sigmoidal curves were fit to these data to test the hypotheses that the half-maximal effective concentration (EC50) under FR(1) and PR testing would not significantly differ across the 4 phenotypes, as suggested by the results of Bertrand et al., (2018).

The average number of cocaine reinforcers earned across the first 5 days of FR(1) testing was examined with a genotype × sex × day mixed-models ANOVA to test the hypotheses that, HIV-1 Tg animals would earn more cocaine reinforcers than F344/N animals, as reported by McLaurin et al., (under review) when ovariectomized, rather than intact, animals were tested under with the same dose of IV cocaine. We also hypothesized that, according to the literature, females would earn more cocaine reinforcers than males.

Linear regression was used to determine if cocaine intake across 7 PR test days had a positive slope (suggesting an escalation of cocaine intake) and if the slope was different for each of the 4 groups. Once again, according to the results reported by McLaurin et al., (under review) it was hypothesized that the rate at which HIV-1 Tg escalate cocaine intake would be significantly greater than that of F344/N rats, and the rate at which males escalate cocaine intake would be significantly lower than that of females, however, all groups would exhibit a positive slope.

A genotype × sex × VTA infusion × C21 dose × time mixed-models ANOVA was used to determine if administration of 0.01, 0.03, or 0.10 mg/kg IV C21 significantly altered the intake of sucrose reinforcers under single-schedule FR(1) testing. While the

shape of the function of dose on sucrose intake was not hypothesized a priori, orthogonal contrasts for within-subjects effects were nevertheless consulted as the shape of the C21 dose-effect is of interest. When statistically significant within-subjects' effects of C21 dose were detected, the nature of these effects was explored further with post hoc simple comparisons between the number of sucrose reinforcers earned following saline and the tested doses of C21. Similar to what was expected in other tasks, we hypothesized that F344/N animals would generally earn more sucrose reinforcers than HIV-1 Tg animals and females would generally earn more sucrose reinforcers than males. When compared to sucrose intake following administration of saline, we hypothesized that C21 would significantly increase the number of sucrose reinforcers earned across the session only in animals that received DREADDs infusions into the VTA, reducing genotypic differences but not sex differences observed following saline.

A genotype × sex × day × reinforcer mixed-models ANOVA was used to assess animals' intake of sucrose and cocaine when both are available. According to the results of Bertrand et al., (2018), HIV-1 Tg animals were expected to earn significantly fewer sucrose and cocaine reinforcers than F344/N animals, and F344/N females were expected to earn significantly more cocaine reinforcers than F344/N males, while sex differences were expected to be diminished in HIV-1 Tg rats. Furthermore, F344/N females were only expected to earn significantly more cocaine reinforcers on later days of testing, after initially earning more sucrose reinforcers while HIV-1 Tg animals were expected to exhibit decreased intake of both reinforcers with day.

To test if animals altered choice behavior when cocaine and sucrose are replaced by saline and water, respectively, a genotype × sex × choice condition × reinforcer mixed-

models ANOVA was used. A significant choice condition × reinforcer interaction was hypothesized, with animals across genotype, and sex, decreasing responding for “cocaine” when cocaine is replaced with saline; and similarly decreasing responding for “sucrose” when sucrose solution is replaced with water.

A VTA infusion × C21 dose × time × reinforcer mixed-models ANOVA was conducted in animals of each group to test the hypothesis that hM3DG(q) stimulation alters choice behavior specifically in animals that received infusions of DREADDs into the VTA. We hypothesized that administration of C21 would decrease the choice of cocaine in F344/N animals and increase reinforcer intake to reduce signs of apathy in HIV-1 Tg animals. Likewise, because we hypothesized that genotypic differences would be observed in choice behavior following saline administration, a genotype × C21 dose × time × reinforcer mixed-models ANOVA was conducted specifically in males and/or females, respectively, which had received DREADDS infusions into the VTA. Similarly, because we hypothesized that sex differences would be observed in choice behavior following saline administration, a sex × C21 dose × time × reinforcer mixed-models ANOVA was conducted specifically in F344/N and/or HIV-1 Tg animals, respectively, which had received DREADDS infusions into the VTA.

C21 doses in each group which were determined to alter choice behavior selectively in DREADDs animals of each group were then compared in an injection × time × reinforcer mixed-models ANOVA to determine if the influence on choice behavior was reversed with the additional administration of the DREADDs inhibitory ligand, Sal B. Thus, it was hypothesized that Sal B would increase genotypic and sex differences compared to following C21, but not compared to following saline.

Chapter 3. Results

3.1. Sucrose-Maintained Responding

Straight lines were fit by least squares method to determine if each group (F344/N males, F344/N females, HIV-1 Tg males, HIV-1 Tg females) met autoshaping criteria (earning ≥ 60 reinforcers for 3 consecutive days) at significantly different rates within the first 60 days of testing. Lines fit according to genotype and biological sex, respectively, supported the hypotheses that F344/N animals acquired autoshaping faster (0.7026 ± 0.02675) than HIV-1 Tg animals (0.3265 ± 0.009751) [genotype: $F(1,116) = 174.6$, $p \leq 0.0001$] and that females (0.6198 ± 0.02574) acquired autoshaping faster than males (0.4092 ± 0.01117) [sex: $F(1,116) = 56.33$, $p \leq 0.0001$]. However, fitting data according to both genotype and biological sex revealed that each group acquired autoshaping at a significantly different rate from all other groups [$F(3,221) = 212$, $p \leq 0.001$], as shown in Figure 3.1. F344/N females acquired autoshaping most rapidly (0.4656 ± 0.0196), followed by F344/N males (0.3228 ± 0.0096), HIV-1 Tg females (0.2400 ± 0.0083), and then HIV-1 Tg males acquiring at the slowest rate (0.0864 ± 0.0033). To ensure data collected was uncensored, no animals were removed from the experiment due to an inability to meet criteria within a designated time period, although water restriction procedures were introduced, as described above. The last animal to reach autoshaping criteria was an HIV-1 Tg male that took a total of 181 days, in comparison to the average animal which met that criteria in approximately 38 days (± 2.647).

Once animals met autoshaping criteria, the schedule by which they earned sucrose was changed to an FR(1) so that the noncontingent reinforcer provided every 10 minutes

during autoshaping training was no longer provided. Straight lines were fit to data to determine if the rate at which each group met FR(1) acquisition criteria (earning ≥ 60 reinforcers for 3 consecutive days) was significantly different. As shown in Figure 3.2. (A), F344/N females met that criteria at the fastest rate (3.567 ± 0.1.420) under an FR(1) schedule [$F(3,180) = 5.334$, $p \leq 0.0015$], while the rate of FR(1) acquisition was not significantly different in the remaining groups (0.3516 ± 0.01161). A genotype × sex × day mixed-models ANOVA was used to examine the average number of sucrose reinforcers earned over the first 5 days of FR(1) testing. While the analysis indicated there was a significant linear effect of day [$F(1,65) = 7.139$, $p \leq 0.01$, partial$\eta^2 = 0.099$], pairwise comparisons did not reveal significant differences between any pairs of days. The analysis did reveal significant main effects of genotype [$F(1,65) = 28.721$, $p \leq 0.001$, partial$\eta^2 = 0.306$] and sex [$F(1,65) = 12.632$, $p \leq 0.001$, partial$\eta^2 = 0.163$] indicating that F344/N animals earned significantly more sucrose reinforcers (141.765 ± 7.372) than HIV-1 Tg animals (83.881 ± 7.836) (see Figure 3.2 (B)) and females earned significantly more reinforcers (132.359 ± 7.322) than males (93.287 ± 8.014) (see Figure 3.2 (C)). Similar to what was observed with autoshaping, the average animal met FR(1) criteria in an average of approximately 12 days (± 2.34). The last animal to meet FR(1) criteria was an HIV-1 Tg male that took a total of 118 days and this was a separate animal than that which took the longest to meet autoshaping criteria.

3.2. Sucrose Dose Response

After animals exhibited sucrose-maintained responding by meeting autoshaping and FR(1) criteria of at least 60 reinforcers earned across 3 consecutive days, we tested animals responding for various concentrations (0, 1, 3, 5, 10, and 30% w/v) of sucrose

solution on a PR schedule of reinforcement. A genotype × sex × sucrose dose mixed-models ANOVA indicated that, when averaged across doses, F344/N rats displayed higher response rates (7.595 ± 0.185) than HIV-1 Tg rats (6.749 ± 0.183) [genotype: $F(1,74) = 10.470$, $p \leq 0.002$, partial$\eta^2 = 0.124$] and female rats displayed higher response rates (7.470 ± 0.188) than male rats (6.874 ± 0.185) [sex: $F(1,74) = 4.965$, $p \leq 0.002$, partial$\eta^2 = 0.124$]. Interestingly, there was a significant sex × sucrose dose interaction [linear: $F(5,375) = 3.758$, $p \leq 0.002$, partial$\eta^2 = 0.048$] revealing that when tested on a PR schedule, female rats (9.204 ± 0.320) only responded significantly more than male rats (7.467 ± 0.316) for the 30% sucrose solution. Sigmoidal curves were fit to the data from males and females and revealed that the EC50 for each group was not significantly different (4.805 ± 0.7812). However, linear fits are displayed in Figure 3.3 (A) as the analysis supported the results of McLaurin et al., (under review) in that sucrose intake increased linearly across dose.

Next, responding for various sucrose concentrations (0, 1, 3, 5, 10, and 30% w/v) was tested on a FR(1) schedule of reinforcement. A genotype × sex × sucrose dose mixed-models ANOVA indicated that responding for sucrose followed a quadratic pattern across dose [$F(1,74) = 8.541$, $p \leq 0.005$, partial$\eta^2 = 0.103$]. Once again, F344/N rats (99.751 ± 3.9159) responded significantly more than HIV-1 Tg rats (82.872 ± 3.966) [genotype: $F(1,74) = 9.095$, $p \leq 0.004$, partial$\eta^2 = 0.109$] and female rats responded significantly more (101.703 ± 4.015) than male rats (80.919 ± 3.964) [sex: $F(1,74) = 13.139$, $p \leq 0.001$, partial$\eta^2 = 0.151$] when collapsed across sucrose dose. However, similar to what was observed under the PR schedule of reinforcement, a significant sex × sucrose dose interaction [quadratic: $F(1,74) = 18.974$, $p \leq 0.001$, partial$\eta^2 = 0.204$] indicated that females earned significantly more sucrose reinforcers than males when responding on a FR(1)

schedule of reinforcement for all tested concentrations of sucrose (0, 1, 3, 5, and 10%) except for 30% concentration. Sigmoidal dose-response curves fit to data from males and females revealed that the EC50 was not significantly different (3.288 ± 0.3314) and these data with quadratic fits across dose (according to the results of our analysis) are shown in Figure 3.3 (B).

3.3. Cocaine-Maintained Responding

After recovering from catheter implantation and DREADD viral vector infusions for 4 days, all animals began training to maintain lever responding for 0.2 mg/kg IV infusions of cocaine on a FR(1) schedule of reinforcement. The genotype × sex × day mixed-models ANOVA indicated that when infusions of 0.2 mg/kg IV cocaine were reinforced on a FR(1) schedule of reinforcement, there was no statistically significant effect of day, as hypothesized. Averaged across days, animals earned approximately 3.1466 ± 0.3094 mg/kg (15.373 ± 1.547 infusions) IV cocaine during each 1-hour cocaine-maintained responding session. In contrast to what was hypothesized and to what was observed by McLaurin et al., (under review), F344/N animals earned significantly more cocaine infusions (18.511 ± 2.240 infusions; 3.7 ± 0.448 mg/kg) than HIV-1 Tg animals (12.235 ± 2.151 infusions; 2.447 ± 0.4302 mg/kg) when collapsed across sex. However, the analysis did not support a statistically significant main effect of sex or a genotype × sex interaction.

After 5 days of responding for cocaine on a FR-1 schedule of reinforcement, animals were placed on a PR schedule of reinforcement to earn 0.75 mg/kg IV infusions of cocaine. This procedure was expected to elicit escalation of cocaine responding, which is thought to indicate sensitization to the salience of cocaine and may be an indicator of

addiction-like behavior (Morgan et al., 2006). It should, however, be noted that PR sessions were only continued up to 7 days, rather than 14 days as reported by Bertrand et al., (2018) and McLaurin et al., (under review), as this was shown to be sufficient to observe significant escalation of cocaine intake. The genotype × sex × day mixed-models ANOVA indicated that F344/N animals continued to earn more cocaine infusions (10.342 ± 0.843; 7.7565 ± 0.63225 mg/kg) than HIV-1 Tg animals (6.250 ± 0.821; 4.6875 ± 0.61575 mg/kg) when averaged across day [$F(1,67) = 12.039$, $p \leq 0.001$, partial$\eta^2 = 0.152$]. A genotype × day interaction [linear: $F(1,67) = 8.445$, $p \leq 0.005$, partial$\eta^2 = 0.112$] revealed that F344/N rats earned more reinforcers than HIV-1 Tg rats during all sessions except the second session, and genotypic differences in the mean number of infusions earned generally increased with each session after the second. F344/N animals increased their intake of cocaine from 7.279 ± 0.678 infusions (5.45925 ± 0.5085 mg/kg) on the first day of responding on a PR schedule of reinforcement, to 14.138 ± 1.154 infusions (10.6035 ± 0.8655 mg/kg) on the last (7^{th}) day. To compare, HIV-1 Tg animals increased their intake of cocaine from 4.987 ± 0.660 infusions (3.74025 ± 0.495 mg/kg) to 7.612 ± 1.124 infusions (5.709 ± 0.843 mg/kg) across the 7 days. According to the PR schedule of reinforcement, these increases in the number of cocaine infusions earned required animals to increase the number of lever presses completed from at least 15 to at least 77 lever-presses in F344/N animals and from at least 4 to at least 15 lever-presses in HIV-1 Tg animals. Figure 3.4. represents the average daily cocaine intake (mg/kg) across the 5 1-hour testing sessions on a FR(1) schedule of reinforcement and across 7 2-hour testing sessions on a PR schedule of reinforcement. Line fits to the data from F344/N and HIV-1 Tg rats while responding on a PR schedule of reinforcement supported that the slope of

each line was statistically significant from zero [F344/N: $F(1,5) = 143.6$, $p \leq 0.0001$; HIV-1 Tg: $F(1,5) = 33.26$, $p \leq 0.0022$] and, therefore, that animals indeed escalated their responding for cocaine regardless of genotype.

3.4. Sucrose Responding in the Presence of hM3D(Gq) Stimulation

To determine if stimulation of hM3DG(q) receptors with C21 administration alters reinforcer intake in a single-schedule procedure, sucrose intake following various doses (0.01, 0.03, 0.10 mg/kg) of IV C21 were compared to that following saline using a genotype × sex × VTA infusion × C21 dose × time mixed-models ANOVA. Orthogonal contrasts for within-subjects effects were consulted to determine the shape of the C21 dose-effect. Similar to the previous analyses, F344/N animals earned significantly more sucrose reinforcers (19.771 ± 1.398) than HIV-1 Tg animals (11.116 ± 1.488) [$F(1,56) = 17.979$, $p \leq 0.001$, partial$\eta^2 = 0.243$] and females earned significantly more sucrose reinforcers (19.343 ± 1.478) than males (11.544 ± 1.466) [$F(1,56) = 13.515$, $p \leq 0.001$, partial$\eta^2 = 0.194$]. Likewise, the pattern of sucrose intake across time was significantly influenced by genotype [linear genotype × time interaction: $F(1,56) = 24.350$, $p \leq 0.001$, partial$\eta^2 = 0.303$] and biological sex [linear sex × time interaction: $F(1,56) = 22.072$, $p \leq 0.001$, partial$\eta^2 = 0.283$]. Graphing the data revealed that F344/N and HIV-1 Tg animals exhibited significantly different intercepts (F344/N: 32.53 ± 1.526; HIV-1 Tg: 15.69 ± 1.617) and slopes (F344/N: -0.5209 ± 0.05599; HIV-1 Tg: -0.1868 ± 0.05932) of sucrose intake across time (see Figure 3.5. (A)), as did males (β_0: 16.16 ± 1.592; β_1: -0.1886 ± 0.05840) and females (β_0: 32.06 ± 1.613; β_1: -0.5191 ± 0.05916; see Figure 3.5 (B)). However, a significant genotype × sex interaction also revealed that genotype-dependent differences in sucrose responding were observed in females (F344/N: 27.810 ± 10.876; HIV: 10.876 ±

2.069) but not in males (F344/N: 11.733 ± 1.944; HIV: 11.356 ± 2.164) [$F(1,56) = 16.424$, $p \leq 0.001$, partial$\eta^2 = 0.227$]. When compared to the number of sucrose reinforcers earned following saline (sham: 16.131 ± 1.801; DREADDs: 13.667 ± 1.429), sham animals did not significantly change their intake of sucrose (15.396 ± 1.988), while animals that received DREADDs infusions into the VTA exhibited a significant increase in sucrose intake following the 0.01 mg/kg dose of C21 (17.279 ± 1.577) [quadratic VTA infusion × C21 dose interaction: $F(1,56) = 4.832$, $p \leq 0.032$, partial$\eta^2 = 0.079$] (Figure 3.6). A C21 dose × time × VTA infusion interaction revealed that sucrose intake by animals that received infusions of DREADDs into the VTA was significantly increased between minutes 21-28 of sessions following administration of 0.01 (17.048 ± 1.953) or 0.03 (17.200 ± 2.389) mg/kg IV C21, compared to the session following saline administration (9.882 ± 1.836) [quadratic-linear VTA infusion × C21 dose × time interaction: $F(1,56) = 6.927$, $p \leq 0.011$, partial$\eta^2 = 0.110$]. Furthermore, a significant genotype × sex × VTA infusion × C21 dose × time interaction [linear-cubic: $F(1,56) = 6.986$, $p \leq 0.011$, partial$\eta^2 = 0.111$] revealed that each group (F344/N males, F344/N females, HIV-1 Tg males, and HIV-1 Tg females) exhibited a unique response to IV C21 administration. Compared to that observed following saline administration, the 0.03 mg/kg dose of IV C21 increased sucrose intake in DREADDs F344/N males between minutes 21-28 (C21: 17.013 ± 4.520; saline: 4.994 ± 3.475; see Figure 3.7. (A)) whereas the 0.01 mg/kg dose of IV C21 significantly increased sucrose intake in DREADDs HIV-1 Tg males between minutes 7-14 (C21: 16.017 ± 4.469; saline: 6.569 ± 4.280; see Figure 3.7. (B)). In DREADDs F344/N females, the 0.01 and 0.03 mg/kg doses of IV C21, respectively, increased sucrose intake between minutes 21-28 (0.01 mg/kg C21: 27.655 ± 3.733; saline: 18.202 ± 3.510) and

decreased sucrose intake between minutes 0-7 (0.03 mg/kg C21: 32.507 ± 3.911; saline: 43.616 ± 4.207; see Figure 3.7. (C)). In HIV-1 Tg females, the 0.01 mg/kg dose of IV C21 significantly decreased intake of sucrose between minutes 0-7 (C21: 13.735 ± 4.330; saline: 20.221 ± 4.419; see Figure 3.7. (D)). Thus, the same doses that appeared to increase sucrose intake in F344/N and HIV-1 Tg males, respectively, between minutes 21-28 also appeared to decrease sucrose intake in F344/N and HIV-1 females, respectively, between minutes 0-7. The genotype × sex × VTA infusion × C21 dose × time interaction also revealed that the only influences of C21 administration on sucrose intake in sham animals was observed in F344/N females (data not shown). Compared to sucrose intake following saline administration, the 0.01 mg/kg dose of IV C21 significantly increased sham F344/N females' sucrose intake between minutes 0-7 of the session (C21: 51.875 ± 5.543; saline: 40.858 ± 5.657). The 0.03 mg/kg dose significantly increased sham F344/N females' sucrose intake between minutes 0-7 (C21: 53.204 ± 5.260; saline: 40.858 ± 5.657) but decreased their sucrose intake between minutes 35-42 (C21: 3.883 ± 5.174; saline: 22.672 ± 6.090). It should be noted that the analysis also revealed a significant genotype × sex × VTA infusion × time interaction and a significant genotype × sex × C21 dose × time interaction, however, these were not explored further (with post hoc comparisons) as intake of sucrose across dose or VTA infusion conditions, respectively, are not of interest to the current hypotheses.

3.5. Choice

A genotype × sex × day × reinforcer mixed-models ANOVA was used to assess choice behavior over 7 testing sessions. In light of the results of Bertrand et al., (2018) in ovariectomized F344/N and HIV-1 Tg rats, we hypothesized that F344/N females would

increase their intake of cocaine and decrease their intake of sucrose across days while HIV-1 Tg females were expected to decrease intake of both sucrose and cocaine across days. We also hypothesized that F344/N females would exhibit greater intake of cocaine on later days of testing and greater intake of sucrose on earlier days of testing compared to F344/N males. Orthogonal within-subjects' contrasts were thus consulted to determine if animals exhibited the hypothesized quadratic or linear patterns of reinforcer intake. The analysis revealed that when collapsed across the 7 testing days, there were no significant main effects genotype or sex and no significant genotype × sex interaction. A sex × day × reinforcer interaction [order 5-linear: $F(1,63) = 4.121$, $p \leq 0.047$, partial$\eta^2 = 0.061$] suggested that, when collapsed across genotype, the number of cocaine and sucrose reinforcers earned by females on each day were closer than the number of cocaine and sucrose reinforcers earned by males (data not shown). However, pairwise comparisons revealed that the number of cocaine and sucrose reinforcers earned by both males and females were statistically similar on each of the 7 testing days. Additionally, a significant genotype × sex × day interaction [cubic: $F(1,63) = 6.170$, $p \leq 0.016$, partial$\eta^2 = 0.089$] revealed that, when average across reinforcer type, F344/N females earned significantly more reinforcers than HIV-1 Tg females during the 1st (F344/N: 32.800 ± 3.970; HIV-1 Tg: 16.115 ± 4.142), 3rd (F344/N: 29.391 ± 4.568; HIV-1 Tg: 15.465 ± 4.765), 5th (F344/N: 30.226 ± 4.439; HIV-1 Tg: 16.711 ± 4.631), and 6th (F344/N: 32.082 ± 4.543; HIV-1 Tg: 16.320 ± 4.739) days. Similar to what was observed in the previous tasks, no significant genotype differences were observed in the average number of reinforcers earned by F344/N and HIV-1 Tg males during any of the 7 testing sessions (data not shown). More so, a genotype × sex × day × reinforcer interaction was statistically significant [cubic-linear:

$F(1,63) = 9.844$, $p \leq 0.003$, partial$\eta^2 = 0.135$] (see Figure 3.8.). F344/N males earned a statistically similar number of cocaine and sucrose reinforcers during each session (Figure 3.8. (A)) while HIV-1 Tg males earned significantly more sucrose than cocaine reinforcers during the 5th (coc: 8.175 ± 4.239; suc: 30.123 ± 8.526) and 6th (coc: 7.962 ± 4.798; suc: 30.812 ± 8.336) sessions (Figure 3.8 (B)). Comparatively, F344/N females generally increased their intake of cocaine across session, earning significantly more cocaine than sucrose reinforcers during the 3rd session (coc: 39.920 ± 5.811; suc: 18.682 ± 7.843) (Figure 3.8. (C)) while HIV-1 Tg females earned a statistically similar number of cocaine and sucrose reinforcers during each session (Figure 3.8. (D)). The percent of total reinforcers earned by each group which were cocaine reinforcers are listed by the first 7 days of choice testing in Table 3.1.

Next, a genotype × sex × choice condition × reinforcer mixed-models ANOVA was used to determine if animals appropriately altered their responding for "cocaine" or "sucrose" when the reinforcers were replaced with saline or H2O, respectively. The analysis revealed a significant main effect of genotype [$F(1,63) = 11.960$, $p \leq 0.001$, partial$\eta^2 = 0.160$] and a genotype × sex interaction [$F(1,63) = 4.296$, $p \leq 0.042$, partial$\eta^2 = 0.064$] supporting that while F344/N animals (26.191 ± 2.407) generally earned more reinforcers than HIV-1 Tg animals (14.201 ± 2.485), this genotype difference was not statistically significant in males (F344/N: 19.452 ± 3.370; HIV-1 Tg: 14.621 ± 3.400) as was observed in females (F344/N: 32.930 ± 3.528; HIV-1 Tg: 13.7 81 ± 3.680). The analysis also revealed a significant genotype × sex × choice condition interaction [quadratic: $F(1,63) = 5.962$, $p \leq 0.017$, partial$\eta^2 = 0.086$], a significant genotype × choice condition × reinforcer interaction [quadratic-linear: $F(1,63) = 4.814$, $p \leq 0.032$, partialη^2

= 0.071], and a significant genotype × sex × choice condition × reinforcer interaction [quadratic-linear: $F(1,63) = 4.047$, $p \leq 0.049$, partial$\eta^2 = 0.060$]. Compared to the 7th day of choice testing, all 4 groups significantly reduced the average number of reinforcers earned when sucrose was replaced with H2O but not when cocaine was replaced with saline. However, when considering the number of cocaine/saline and sucrose/H20 reinforcers earned separately, F344/N animals, but not HIV-1 Tg animals, displayed a significant reduction in the number of "cocaine" reinforcers earned when cocaine was replaced with saline (F344/N::7th day choice: 32.772 ± 3.966; cocaine replacement: 24.947 ± 3.436). Of note, F344/N animals, but not HIV-1 Tg animals, also significantly increased their intake of sucrose when cocaine was replaced with saline (F344/N::7th day choice: 22.926 ± 5.932; cocaine replacement: 35.724 ± 5.705). When cocaine was once again made available and sucrose was replaced with H2O, F344/N animals displayed a reduction in sucrose intake that was statistically significant when compared to that observed when cocaine was replaced with saline (sucrose replacement: 15.450 ± 2.805), but not compared to the 7th day of choice testing. Comparatively, HIV-1 Tg animals significantly decreased their intake of sucrose compared to that on the 7th day of choice testing (7th day choice: 21.296 ± 6.123; sucrose replacement: 10.019 ± 2.896) and compared to when cocaine was replaced with saline (cocaine replacement: 20.103 ± 5.889). When examining the 4 groups separately, F344/N males significantly reduced their intake of "cocaine" when cocaine was replaced with saline (7th day choice: 21.296 ± 6.123; cocaine replacement: 10.019 ± 2.896) but the reduction in sucrose intake when sucrose was replaced with H2O was not statistically significant (Figure 3.9 (A)). F344/N females significantly increased their intake of sucrose when cocaine was replaced with saline but responding for

cocaine/"cocaine" was relatively inflexible to choice condition (Figure 3.9. (C)). HIV-1 Tg males (Figure 3.9 (B)) and females (Figure 3.9. (D)) failed to significantly alter their response levels with changes to choice condition.

3.6. Choice in the Presence of hM3D(Gq) Stimulation

Because genotype × sex relationships were generally consistent throughout the tasks thus far, a VTA infusion × C21 dose × time × reinforcer mixed-models ANOVA was conducted on data from each of the 4 groups separately to determine if activating hM3D(Gq) receptors with various C21 doses (0.01, 0.03, 0.10. 0.30 mg/kg) significantly influence choice behavior compared to that observed following saline. Once again, while the shape of the function of C21 dose on choice behavior was not hypothesized a priori, orthogonal contrasts for within-subjects' effects were nevertheless consulted as the shape of the C21 dose-effect is of interest. The percent of total reinforcers which were cocaine reinforcers earned by DREADDs animals within each group following saline, C21 administration, and C21 + Sal B administration are listed in Table 3.2.

In F344/N males, a significant main effect of time [cubic: $F(1,15) = 4.755$, $p \leq 0.046$, partial$\eta^2 = 0.241$] revealed that animals generally increased their average intake of cocaine and sucrose reinforcers until the last 5 minutes of the session. The analysis revealed a significant VTA infusion × C21 dose interaction [quadratic: $F(1,15) = 6.105$, $p \leq 0.026$, partial$\eta^2 = 0.289$] suggesting that all of the tested doses of C21 significantly increased the average number of reinforcers earned per 5 minutes by 11 DREADDs F344/N males, but not in 7 sham F344/N males (Figure 3.10). The analysis also revealed significant VTA infusion × C21 dose × time [order 4-quadratic: $F(1,15) = 5.103$, $p \leq 0.039$, partial$\eta^2 = 0.254$], VTA infusion × dose × reinforcer [order 4-linear: $F(1,15) = 4.749$, $p \leq 0.046$,

partialη^2 = 0.240], and VTA infusion × time × reinforcer [linear-linear: $F(1,15) = 4.856$, $p \leq 0.044$, partialη^2 = 0.245] interactions. However, post hoc comparisons were not used to follow up these interactions as the VTA infusion × C21 dose × time × reinforcer interaction was also statistically significant [linear-order 4-linear: $F(1,15) = 4.954$, $p \leq 0.042$, partialη^2 = 0.248]. F344/N males which received DREADDs infusions into the VTA did not earn a statistically different number of cocaine and sucrose reinforcers at any of times tested (every 5 minutes) within the hour following saline administration (Figure 3.11 (A)). However, these same animals earned significantly more sucrose reinforcers than cocaine reinforcers in the first 5 minutes following administration of each dose (0.01, 0.03, 0.10, 0.30 mg/kg) of C21 tested. The number of sucrose reinforcers earned were also significantly greater than the number of cocaine reinforcers earned for an additional 20 minutes (between minutes 25-30, 35-45, and 50-55) following administration of the 0.03 mg/kg dose of C21 (Figure 3.11 (B)). These results suggest that administration of C21 and assumed activation of hM3D(Gq) designer receptors enhanced the number of sucrose reinforcers earned compared to sucrose so that this difference became statistically significant when it was previously not. However, these results in animals that received DREADDs infusions into the VTA should also be compared to that observed in animals that received vehicle infusions into the VTA. Sham F344/N males earned significantly more cocaine reinforcers than sucrose reinforcers between minutes 15-20 and 30-40 following saline administration but did not exhibit a difference in cocaine and sucrose responding following any of the tested doses of C21.

When first and second order polynomials were fit to cocaine and sucrose intake data the conclusions of the mixed-models ANOVA in DREADDs but not sham animals

were supported. DREADDs F344/N males intake of cocaine and sucrose over the hour following saline administration were best represented by the same, instead of different, linear functions [H_0 = same function: $F(2, 260) = 1.014$, $p \leq 0.3642$] (Figure 3.11 (A)). Following administration of 0.03 mg/kg C21, intake of cocaine was better fit to a linear function than a quadratic function [H_0 = linear: $F(1, 129) = 0.1263$, $p \leq 0.7229$] whereas sucrose was better represented by a quadratic function [H_0 = linear: $F(1, 129) = 7.865$, $p \leq 0.0058$] (Figure 3.11 (B)). According with the results of the mixed-models ANOVA, sham F344/N males intake of cocaine and sucrose over the hour following saline administration were best represented by different linear functions [H_0 = same function: $F(2, 164) = 10.50$, $p \leq 0.0001$]. However, in contrast to the results of the mixed-models ANOVA, sham F344/N males intake of cocaine and sucrose over the hour following administration of 0.03 mg/kg C21 were also best represented by different linear functions [H_0 = same function: $F(2, 164) = 3.212$, $p \leq 0.0428$] (data not shown).

In HIV-1 Tg males, a VTA infusion × C21 dose × time × reinforcer mixed-models ANOVA did not reveal any statistically significant effects suggesting that sham animals (n=7) behaved differently from DREADDs animals (n=11) or that hM3D(Gq) stimulation in DREADDs animals significantly altered choice behavior.

In F344/N females, a VTA infusion × C21 dose × time × reinforcer mixed-models ANOVA revealed a significant main effect of time [quadratic: $F(1,15) = 6.185$, $p \leq 0.029$, partial$\eta^2 = 0.340$] indicating that F344/N females exhibited a similar pattern of average reinforcer intake across time to that reported in F344/N males. A C21 dose × time interaction [linear-cubic: $F(1,15) = 4.837$, $p \leq 0.044$, partial$\eta^2 = 0.244$] suggested that F344/N females earned significantly fewer reinforcers between minutes 50-55 of the

session following the 0.10 dose of C21 when compared to that following saline administration. A main effect of VTA infusion [$F(1,15) = 6.185$, $p \leq 0.029$, partial$\eta^2 = 0.340$] suggested that sham animals (n=7) earned a greater average number of reinforcers per 5 minutes (3.714 ± 0.445) than DREADDs animal (n=10; 2.369 ± 0.372) when collapsed across doses of C21. Additionally, a VTA infusion × time × reinforcer interaction [$F(1,15) = 6.281$, $p \leq 0.024$, partial$\eta^2 = 0.295$] suggested that, when collapsed across doses of C21, sham F344/N females earned significantly more sucrose reinforcers than cocaine reinforcers between minutes 5-10 and 25-30 of the sessions whereas DREADDs F344/N females did not exhibit significantly different levels of cocaine and sucrose intake. According with our hypotheses, a VTA infusion × C21 dose interaction [cubic: $F(1,15) = 4.675$, $p \leq 0.047$, partial$\eta^2 = 0.238$] revealed that only F344/N females that received DREADDs infusions into the VTA exhibited a significant decrease in the average number of reinforcers earned per 5 minutes following the 0.10 mg/kg dose of C21 (1.263 ± 0.404) compared to following saline (2.771 ± 0.543) while F344/N females that received vehicle infusions showed no change in intake (Figure 3.12.).

In HIV-1 Tg females, a VTA infusion × C21 dose × time × reinforcer mixed-models ANOVA revealed a significant VTA infusion × time interaction [linear: $F(1,12) = 6.185$, $p \leq 0.029$, partial$\eta^2 = 0.340$] however, post hoc pairwise comparisons did not reveal significant differences between the average number of reinforcers earned during any of the 12 5-minute periods when averaged across C21 dose. The analysis also revealed a significant C21 dose × time interaction [cubic-order 4: $F(1,12) = 5.646$, $p \leq 0.035$, partial$\eta^2 = 0.320$] and a VTA infusion × C21 dose × time interaction [cubic-linear: $F(1,12) = 5.726$, $p \leq 0.034$, partial$\eta^2 = 0.323$]. When collapsed across VTA infusion, HIV-1 Tg females

increased the average number of reinforcers earned between minutes 15-25 following the 0.01 mg/kg dose, between minutes 20-45 following the 0.03 mg/kg dose, between minutes 25-35 following the 0.10 mg/kg dose, and between minutes 15-30 and 40-45 following the 0.30 mg/kg dose of C21. In HIV-1 Tg females that received DREADDs infusions into the VTA (n=9), the 0.03 mg/kg dose significantly increased the average number of reinforcers earned between minutes 20-25 and 40-45 of the session and the 0.30 mg/kg dose did so between minutes 40-45 and 55-60. However, effects of C21 dose were also observed in HIV-1 Tg females that had received infusions of vehicle into the VTA (n=6). Compared to that observed following saline administration, the average number of reinforcers earned was significantly increased between minutes 0-5, 30-40, and 50-55 following the 0.01 mg/kg dose, between minutes 45-50 following the 0.03 mg/kg dose, between minutes 25-30 following the 0.10 mg/kg dose, and between minutes 5-10, 15-20, and 25-30 following the 0.30 mg/kg dose of C21.

When average reinforcer intake by DREADDs HIV-1 Tg females in the hour following administration of saline, 0.03 mg/kg C21, or 0.30 mg/kg C21 was plotted and fit to straight lines, nonlinear regressions suggested that intake across time followed a linear rather than quadradic function (H_0 = first order polynomial) [saline: $F(1, 105) = 0.2167$, $p \leq 0.6425$; 0.03: $F(1, 105) = 2.818$, $p \leq 0.0962$; $F(1, 105) = 0.00001$, $p \leq 0.9969$] and supported that intake following each dose was best represented by a different linear function [H_0 = same function: $F(4, 318) = 5.022$, $p \leq 0.0006$] (Figure 3.13 (A)). Nonlinear regressions in GraphPad Prism supported that average reinforcer intake by sham animals following each dose was best represented by a different linear function [H_0 = same function: $F(8, 350) = 1.980$, $p \leq 0.0481$]. However, post hoc comparisons revealed that

average intake following each of the C21 doses tested, not including saline, did not follow significantly different linear patterns across time [H_0 = same function: $F(6, 280) = 0.2972$, $p \leq 0.9380$] (Figure 3.13 (B)).

We had hypothesized a priori that genotypic differences would be observed in choice behavior following saline administration and our results thus far suggest reliable genotype differences between F344/N and HIV-1 Tg females. Planned comparisons within a genotype × C21 dose × time × reinforcer mixed-models ANOVA specifically in females which had received DREADDS infusions into the VTA were conducted to determine if hM3D(Gq) stimulation made HIV-1 Tg females' choice behavior more similar to that observed in F344/N females following saline administration. Compared to DREADDs F344/N females following saline administration, DREADDs HIV-1 Tg animals following saline administration exhibited reduced cocaine intake for approximately 35 minutes (between minutes 0-10, 20-30, and 40-55) and reduced sucrose intake for the first 15 minutes of the hour-long session (Figure 3.14. (A)). All except for the 0.01 mg/kg dose of C21 decreased genotypic differences in cocaine intake and the 0.01 mg/kg dose actually increased genotypic differences in cocaine intake. When DREADDs HIV-1 Tg females were administered C21, they exhibited reduced cocaine intake for 45 minutes of the session following the 0.01 mg/kg dose (between minutes 0-30, 35-45, and 50-55), 10 minutes following the 0.03 mg/kg dose (between minutes 0-5, and 50-55), 25 minutes following the 0.10 mg/kg dose (between minutes 0-5, 20-30, 40-45, and 50-55), and 20 minutes following the 0.30 mg/kg dose (between minutes 0-20) compared to DREADDs F344/N females following saline administration. All tested doses of C21 reduced genotypic differences in sucrose intake except for the 0.30 mg/kg dose and all genotypic differences

in sucrose intake were observed at the start of the session. DREADDs HIV-1 Tg females displayed reduced sucrose intake during the first 15 minutes of the test session when administered either saline or 0.30 mg/kg C21 and compared to DREADDs F344/N females administered saline. No genotypic differences in sucrose intake were observed when DREADDs HIV-1 Tg females were administered 0.10 mg/kg C21 and compared to DREADDs F344/N females administered saline. Genotypic differences in sucrose intake were observed in the first 5 minutes of the sessions when DREADDs HIV-1 Tg females were administered 0.01 or 0.03 mg/kg C21. Thus, although the 0.10 mg/kg dose was most effective at reducing genotype differences in sucrose intake, the 0.03 mg/kg dose of C21 appeared to be most effective at reducing genotypic differences in cocaine intake observed between females following saline administration and was also effective at reducing differences in sucrose intake (see Figure 3.14. (B)). It should be noted, however, that while genotype differences were reduced in females, this was accomplished by increasing both cocaine and sucrose intake, highlighting that stimulation of the mesolimbic circuit may not reduce the choice of drug to serve as an effective treatment strategy for dysregulated choice in all populations.

We had also hypothesized prior to running this experiment that the choice procedure would reveal sex-dependent patterns of simultaneous cocaine and sucrose intake. Likewise, our results from the choice procedure and single-schedule procedures have revealed consistent sex differences between F344/N males and females, whereas sex differences were observed less frequently between HIV-1 Tg males and females. Thus, planned comparisons within a sex × C21 dose × time × reinforcer mixed-models ANOVA specifically in F344/N animals which had received DREADDS infusions into the VTA

were conducted to determine if hM3D(Gq) stimulation made F344/N females' choice behavior more similar to that observed in DREADDs F344/N males following saline administration. Following saline administration, DREADDs F344/N males earned significantly fewer cocaine reinforcers then DREADDs F344/N females for approximately 50 minutes of the hour-long session (between minutes 0-30, 35-40 and 45-60) and earned significantly more sucrose during the first 15 minutes of the session (Figure 3.15 (A)). Sex differences in sucrose intake were reduced from being observed for approximately 15 minutes of the session following saline to approximately 10 minutes of the sessions following C21 administration to DREADDs F344/N females. Of note, when cocaine intake by DREADDS F344/N males was compared to that by DREADDs F344/N females following the 0.03 mg/kg dose of C21, sex differences were apparent during more of the hour-long session (approximately 55 minutes) than when DREADDs F344/N females were administered saline. In contrast, administering the 0.01 and 0.10, doses of C21 to DREADDs F344/N females reduced sex differences compared to that observed following saline administration. Sex differences were observed for approximately 45 minutes following administration of 0.01 mg/kg C21 to DREADDs F344/N females and for approximately 15 minutes following administration of 0.03 mg/kg C21. Administration of the 0.30 mg/kg dose of C21 to F344/N females did not appear to reduce sex differences in cocaine intake, as sex differences in cocaine intake were significant for approximately 50 minutes of the session following either saline or 0.30 mg/kg C21 when compared to that in DREADDs F344/N males following saline. Thus, administration of the 0.10 mg/kg dose of C21 to DREADDs F344/N females appeared to be most effective at altering choice

behavior so that it more closely resembled that of DREADDs F344/N males following administration of saline (see Figure 3.15. (B)).

In addition to these analyses which were planned a priori, the analysis was repeated on censored data, from which session data was removed if the animal failed to meet the criteria of at least 2 of each reinforcer type. A VTA infusion × C21 dose × reinforcer mixed-model analysis was conducted on data from each group within SAS Studio 3.8 to prevent listwise deletion of any animal that did not meet the criteria within at least 1 session but less than all of the sessions. The code used is presented in Appendix A. The factor "time" was also removed so the outcome variable was the total number of each reinforcer earned within an hour, rather than the average number of each reinforcer earned per 5 minutes.

10% (20 of 200 intake values) of the data from F344/N males was missing prior to censoring the data, compared to 31% (62 of 200) after censoring the data. The analysis on censored data suggested that DREADDS F344/N males earned significantly more (11.1105 ± 17.0088) reinforcers than sham F344/N males [$F(1,59) = 4.72$, $p \leq 0.0339$] when averaged across doses of C21. The analysis also revealed a significant VTA infusion × C21 dose × reinforcer interaction [$F(5,59) = 3.28$, $p \leq 0.0111$] suggesting that, in DREADDS F344/N males, the number of sucrose reinforcers earned was significantly greater than the number of cocaine reinforcers earned following the 0.03 mg/kg and 0.30 mg/kg dose of C21, while there was no statistically significant difference in cocaine and sucrose responding following saline. Specifically, while the number of sucrose reinforcers earned following saline administration was approximately 16.25 (± 22.8716; $p \leq 0.4802$) greater than the number of cocaine reinforcer earned, the differences between sucrose and cocaine

reinforcers earned following 0.03 and 0.30 mg/kg C21 were approximately 50.2667 (± 16.7031; $p \leq 0.0038$) and 40.3111 (± 18.0414; $p \leq 0.0293$), respectively.

Similar to what was observed in F344/N males, only 10% of HIV-1 Tg males' data was missing prior to censoring. After removing data from sessions when HIV-1 Tg males did not meet choice criteria, only 49% of the data remained. The VTA infusion × C21 dose × reinforcer mixed-model analysis on the remaining data supported the analysis conducted on uncensored data in that statistically significant effects of VTA infusion and/or C21 dose were not supported.

Prior to censoring the data from F344/N females, 15% of the data was missing. Censoring the data from F344/N females only increased the percent missing to 18%. The analysis revealed a significant VTA infusion × C21 dose × reinforcer interaction [$F(5,72) = 5.22$, $p \leq 0.0004$] suggesting that, opposite to that observed in DREADDs F344/N males, DREADDs F344/N females earned significantly more cocaine than sucrose reinforcers following saline administration but not following administration of 0.10 mg/kg C21. Following saline administration, DREADDs F344/N females earned approximately 51.3 (± 23.3535; $p \leq 0.0313$) more cocaine reinforcers than sucrose reinforcers whereas the difference between the number of cocaine and sucrose reinforcers earned following the 0.10 mg/kg dose was approximately 48.25 (± 24.5272; $p \leq 0.0530$) reinforcers. These results support the analysis on the uncensored data in that the primary influence of C21 was observed following the 0.10 mg/kg dose of C21. However, the presence of a VTA infusion × C21 dose × reinforcer interaction and not of a VTA infusion × C21 dose interaction as was observed in the uncensored data suggests that C21 in DREADDs F344/N

females may have different influences on cocaine and sucrose intake during the choice procedure.

25% of the intake data from HIV-1 Tg females was missing prior to censoring. Only 35% of the intake data remained after censoring data from HIV-1 Tg females and the mixed model was unable to converge to provide reliable estimates.

To determine if the ability to alter choice behavior with ligand administration and assumed hM3D(Gq) activation in F344/N animals was dependent choice behavior prior to ligand administration, VTA infusion × C21 dose mixed model analyses were conducted separately in males and females with the percent of cocaine choice earned following saline included as a covariate. SAS Studio 3.8 code for this analysis is also included in Appendix A. The analysis in neither F344/N males or females supported that the ability to alter choice behavior was dependent on predominance of cocaine choice prior to DREADDs activation.

It is also of interest to determine if the number of rats that chose for cocaine at least 50% of their total session responses differed between each of the 4 groups. Binary logistic regressions were run in SPSS to determine if the number of "high drug responders" following administration of saline or any of the tested doses of C21 (0.01, 0.03, 0.10, 0.30 mg/kg) was dependent on sex or genotype. The analyses revealed that a maximum of 13% (Nagelkerke R^2) of the variance in the likelihood of being a "high drug responder" was explained by sex and genotype together. None of the Chi-square (χ^2) models were statistically significant, but the model explaining the most variance was fit to data from the session following saline administration [$\chi^2(2,52) = 4.406$, $p \leq 0.110$]. However, of the variance that was explained, a significant proportion was explained by biological sex following the 0.03 (β: 1.353 ± 0.587, $p \leq 0.021$) and 0.10 (β: 1.168 ± 0.588, $p \leq 0.047$)

mg/kg doses of C21. Females were 1.879 or 1.793 times more likely to be 'high drug responders" than not following the 0.03 mg/kg or 0.10 mg/kg doses of C21, respectively. In comparison, males were only 0.486 or 0.558 times more likely than not to earn at least 50% cocaine during when given the opportunity to choose between 0.2 mg/kg IV cocaine or 5% sucrose solution following the 0.03 and 0.10 mg/kg doses of C21, respectively.

3.7. Sal B Reversal of C21 Influence on Choice

To determine if injection of the KORD ligand Sal B would reverse C21-induced effects on choice behavior, choice of DREADDs animals of each group were compared following saline, C21, and C21 + Sal B. Reversal of C21 effects choice with the additional administration of Sal B would suggest that these effects are likely to be due to DREADDs-mediated changes in CNS activity, rather than due to off-target effects of IV C21. Thus, the 0.03 mg/kg dose of C21 was injected prior to 0.15 mg/kg IV Sal B in F344/N males to determine if Sal B administration would reverse the effect of 0.03 mg/kg IV C21. Likewise, the 0.10 mg/kg dose of IV C21 was injected prior to 0.15 mg/kg IV Sal B to reduce reinforcer intake in DREADDs F344/N females. These analyses were not run in HIV-1 Tg animals given fewer observed effects of C21 were apparent. Specific within-subjects' contrasts were utilized to test the hypothesis that the effect of injection would take on a primarily quadratic pattern, with the differences between intake following C21 + Sal B compared to following saline being blunted compared those differences between intake following C21 alone and saline. While we did not have specific a priori hypotheses regarding how the average number of sucrose and cocaine reinforcers per 5- minute "bin" would change across the 60-minute session, the orthogonal comparison providing the best fit (highest F-value) of the effects of time are also of interest and are therefore reported.

In DREADDs F344/N males, an injection × time × reinforcer repeated measures ANOVA (RMANOVA) revealed an injection interaction [quadratic: $F(1,10) = 16.913$, $p \leq 0.002$, partial$\eta^2 = 0.628$] supporting that adding an injection of Sal B following C21 (0.871 ± 0.209) blunted the increases in reinforcer intake per 5 minutes observed following administration of C21 alone (2.928 ± 0.529) and compared to following saline alone (0.420 ± 0.207). There was also a significant main effect of time [quadratic: $F(1,10) = 5.940$, $p \leq 0.035$, partial$\eta^2 = 0.373$] and an injection × time interaction [quadratic-quadratic: $F(1,10) = 16.913$, $p \leq 0.002$, partial$\eta^2 = 0.628$]. Compared to following saline, administration of 0.03 mg/kg C21 alone increased average reinforcer intake for all but the first 5 minutes of the hour-long session whereas reinforcer intake following administration of 0.03 mg/kg C21 and 0.15 mg/kg Sal B was only increased for 10 minutes of the session (between minutes 0-5 and 10-15) (Figure 3.16.). When these data were plotted in GraphPad Prism, nonlinear regressions revealed that average reinforcer intake following administration saline or C21 + Sal B followed a linear, rather than quadratic pattern as observed following administration of C21 alone (H_0 = first order polynomial) [saline: $F(1, 9) = 0.3448$, $p \leq 0.9942$; C21: $F(1, 9) = 11.69$, $p \leq 0.0076$]; C21 + Sal B: $F(1, 9) = 0.002683$, $p \leq 0.9598$]. While intake following C21 + Sal B was more similar to that following saline than intake following C21 alone was, intake following C21 + Sal B and following saline were still best represented by different linear functions [H_0 = same function, $F(2, 20) = 13.00$, $p \leq 0.002$] (see Figure 3.16).

In DREADDs F344/N females, the injection × time × reinforcer RMANOVA revealed a significant main effect of injection [quadratic: $F(1,9) = 8.730$, $p \leq 0.016$, partial$\eta^2 = 0.492$] supporting that the average number of reinforcers earned per 5 minutes

in the hour following administration of 0.10 mg/kg C21 and 0.15 mg/kg Sal B (2.162 ± 1.193) was closer to that observed following saline (2.771 ± 0.561) than was that observed following C21 alone (1.263 ± 0.344), although post hoc pairwise comparisons did not reveal significant differences between any of the injection conditions. There was also an injection × time interaction [linear-quadratic: $F(1,9) = 12.609$, $p \leq 0.006$, partial$\eta^2 = 0.584$] revealing that compared to following saline, administration of 0.10 mg/kg C21 alone decreased average reinforcer intake for approximately 25 minutes of the hour-long session (between 5-15, 25-25, and 50-55) whereas reinforcer intake following administration of 0.10 mg/kg C21 and 0.15 mg/kg Sal B was only decreased for 5 minutes of the session (between minutes 10-15) (Figure 3.17). Nonlinear regression in GraphPad Prism suggested that average reinforcer intake following saline, C21 alone, and C21 + Sal B was best represented by different functions [H_0 = same function, $F(4,354) = 9.150$, $p \leq 0.0001$]. However, post hoc comparisons revealed that intake following saline and following C21 + Sal B was better represented by a single linear fit than individual linear fits [H_0 = same function, $F(2,236) = 2.689$, $p \leq 0.070$] (see Figure 3.17).

3.8. Verification of Cannula Placement and DREADDs Expression

Cannula placement was verified in 28 brains and are represented in Figure 3.18. To confirm expression of GFP in the NAc and the expression of mCherry and mCitrine in the VTA, brains were sectioned into 100 µm coronal slices and imaged using a Nikon TE-2000E confocal microscope. DREADDs expression was examined in 3 sham and 12 DREADDs tissues. GFP was clearly expressed in the NAc, primarily in the NAc shell (Figure 3.18 (A)), and mCherry and mCitrine expression were clearly identified in the parabrachial nucleus of the posterior VTA (mostly posterior Bregma -5.3 mm) of

DREADDs animals (Figure 3.18 (B)), similar to the expression regions observed by Li et al., (2019). It should be noted that slices from the majority of animals exhibited fluorescent expression in regions between NAc and VTA such as the mediodorsal thalamus and the ventral pallidum. However, fluorescent signal in such regions was also observed in sham animals which only received infusion of the AAV-CMV-GFP/Cre, suggesting that designer receptor expression remained localized in cells of the VTA. Nevertheless, it is still not confirmed that only VTA cells projecting to the NAc, and not those projecting to the regions of the thalamus, displayed expression of designer receptors. Therefore, an aim for future research should be to replicate the currently described effects of mesolimbic stimulation on reinforcer intake in the single-schedule and choice contexts.

Table 3.1. Establishment of Drug Choice

Choice Day	**1**	**2**	**3**	**4**	**5**	**6**	**7**
F344/N Males	42	44	39	41	48	41	49
HIV-1 Tg Males	33	30	26	23	31*	37*	33
F344/N Females	39	63	68*	67	53	55	66
HIV-1 Tg Females	48	42	47	39	33	47	38

Values in table represent the percentage of responses which were reinforced with a 0.2 mg/kg IV cocaine infusion rather than 5% sucrose solution. Percentage values were converted from each group's average number of cocaine and sucrose reinforcers earned across each day's hour-long session. Values are rounded to the nearest percent. Each group's average number of cocaine and sucrose reinforcers earned across each day's hour-long session were determined by the genotype × sex × day × reinforcer mixed-models ANOVA described in Chapter 3.5. F344/N males and HIV-1 Tg females exhibited linear patterns of intake across 7 days which were not different for cocaine and sucrose. Although the current table does not suggest that drug-choice clearly increased or decreased across time, the genotype × sex × day × reinforcer mixed-models ANOVA did suggest that HIV-1 Tg males did display a linear pattern of cocaine intake across days which was shifted down compared to their linear pattern of sucrose intake. Additionally, F344/N females exhibited different quadratic functions of cocaine and sucrose intake across days. *: $p \leq 0.05$.

Table 3.2. Changes to Drug Choice with Mesolimbic Manipulation

Dose	**Saline**	**0.01 C21**	**0.03 C21**	**0.10 C21**	**0.30 C21**	**C21 + Sal B**
F344/N Males	50	49	26	40	44	37
HIV-1 Tg Males	18	26	10	34	32	25
F344/N Females	63	64	65	69	60	57
HIV-1 Tg Females	56	34	59	47	69	47

Values in table represent the percentage of responses which were reinforced with a 0.2 mg/kg IV cocaine infusion rather than 5% sucrose solution. Percentage values were converted from the average number of cocaine and sucrose reinforcers earned by DREADDS animals within each group across each day's hour-long session. The average number of cocaine and sucrose reinforcers earned across each day's hour-long session was determined by a C21 dose × reinforcer RMANOVA within DREADDS animals in each group. Values are rounded to the nearest percent. The total number of cocaine reinforcers earned was not significantly different from the total number of sucrose reinforcers earned by any of the groups following administration of saline, C21, or C21+Sal B. However, the analyses described in Chapter 3.5 which included the factor 'time' suggested that the 0.03 mg/kg dose of C21 changed the function of sucrose intake from a linear to quadratic pattern in DREADDs, but not sham, F344/N males. The analysis described in Chapter 3.5 also suggested that administration of 0.10 mg/kg altered average reinforcer intake, but not cocaine or sucrose intake selectively, in DREADDs F344/N females.

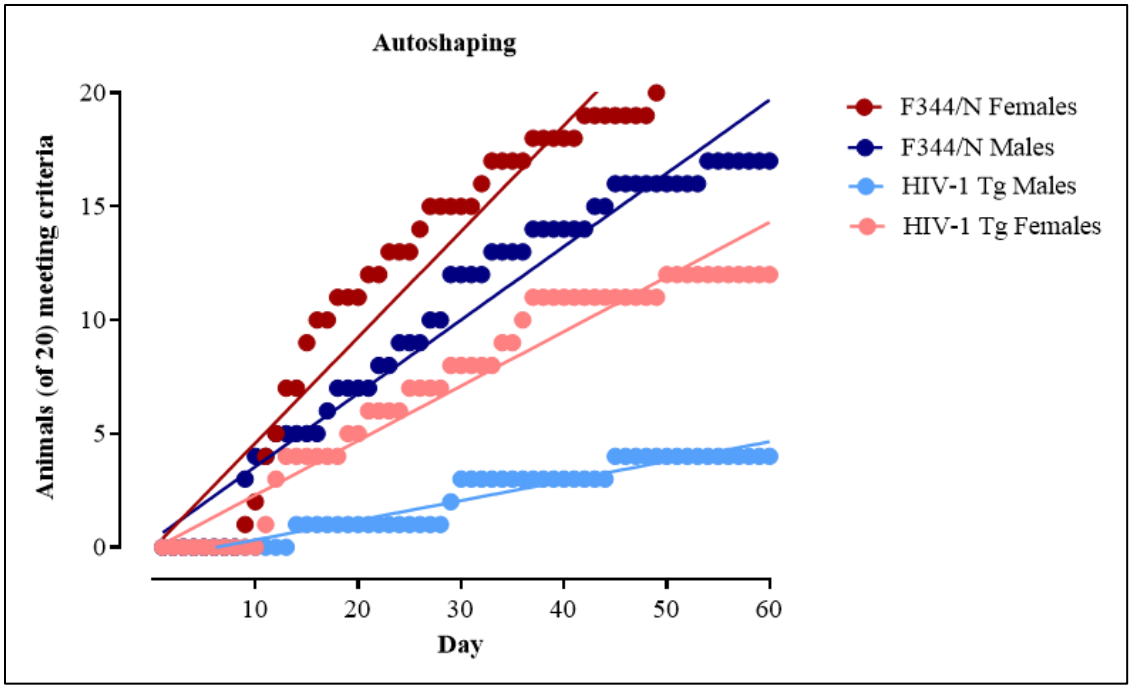


Figure 3.1. Sucrose Autoshaping Training. To ensure all animals had acquired stimulus-reinforcement learning, specifically that lever-pressing results in presentation of a sucrose reinforcer, a criteria of at least 60 earned reinforcers on at least 3 consecutive days was required before progressing to additional testing. During the 42-minute autoshaping training sessions, each lever press was reinforced with 4 seconds of access to a 5% sucrose solution and reinforcement independent of lever-pressing occurred every 10 minutes. Each of the four groups met autoshaping criteria at a significantly different rate. F344/N females acquired autoshaping at the fastest rate, with the last taking a total of 49 days and HIV-1 Tg males acquired autoshaping at the slowest rate, with the last taking a total of 181 days. It should also be noted that no animals were water restricted during the first 60 days of autoshaping training so that measures of stimulus-reinforcement learning were unobstructed.

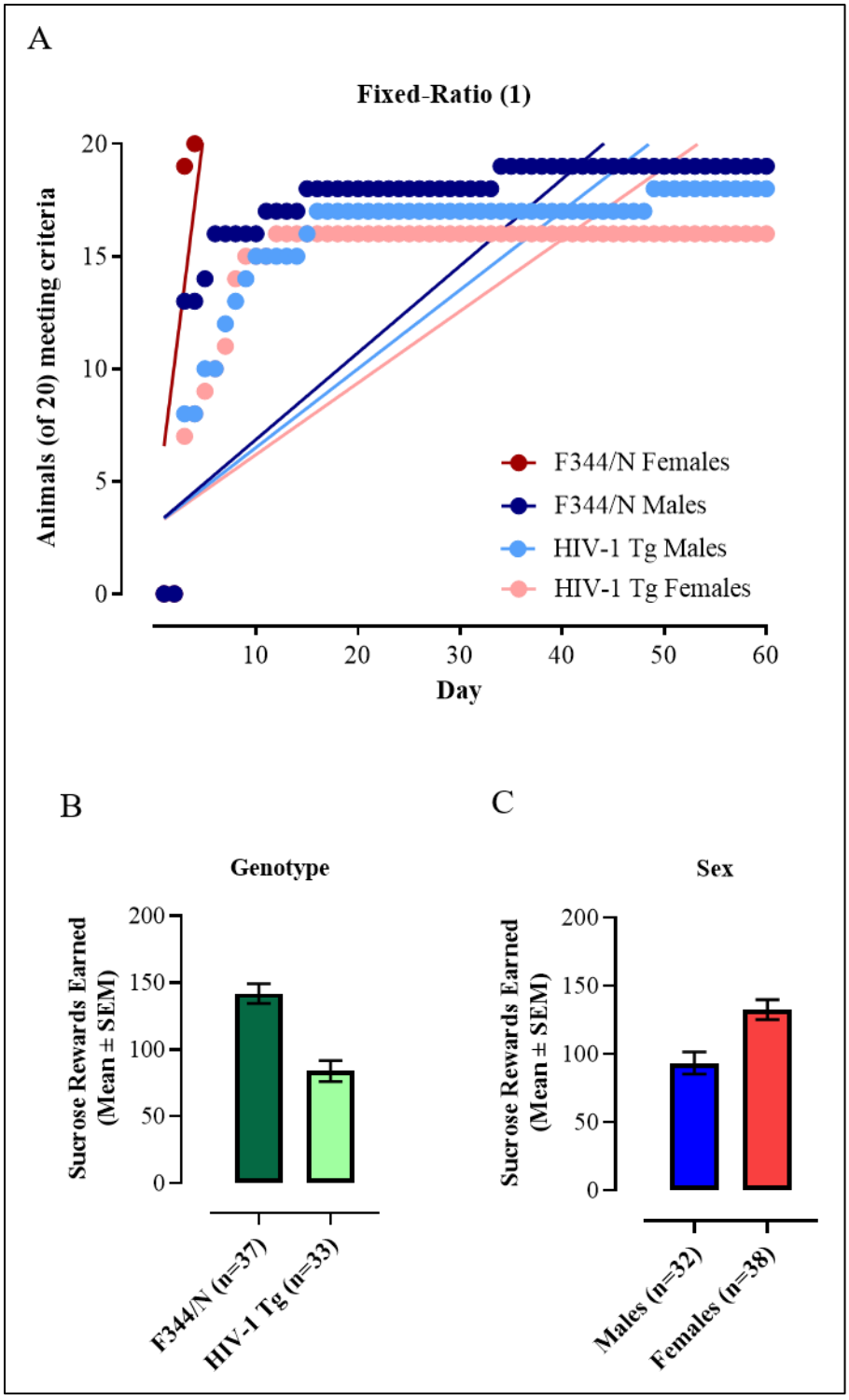


Figure 3.2. Sucrose Training on FR(1) Schedule of Reinforcement. After animals met autoshaping criteria, they were placed on a FR(1) schedule of reinforcement where each lever-press was reinforced with

4-second access to a 5% sucrose solution. Unlike autoshaping sessions, there were no non-contingent deliveries of sucrose reinforcers. Animals were required to meet criteria of at least 60 earned reinforcers on 3 consecutive days before progressing to additional training. F344/N females met FR(1) criteria at a faster rate than all other groups, which met criteria at a statistically similar rate. The number of sucrose reinforcers earned by F344/N animals was significantly greater than that earned by HIV-1 Tg animals and the number of sucrose reinforcers earned by females was significantly greater than that earned by males.

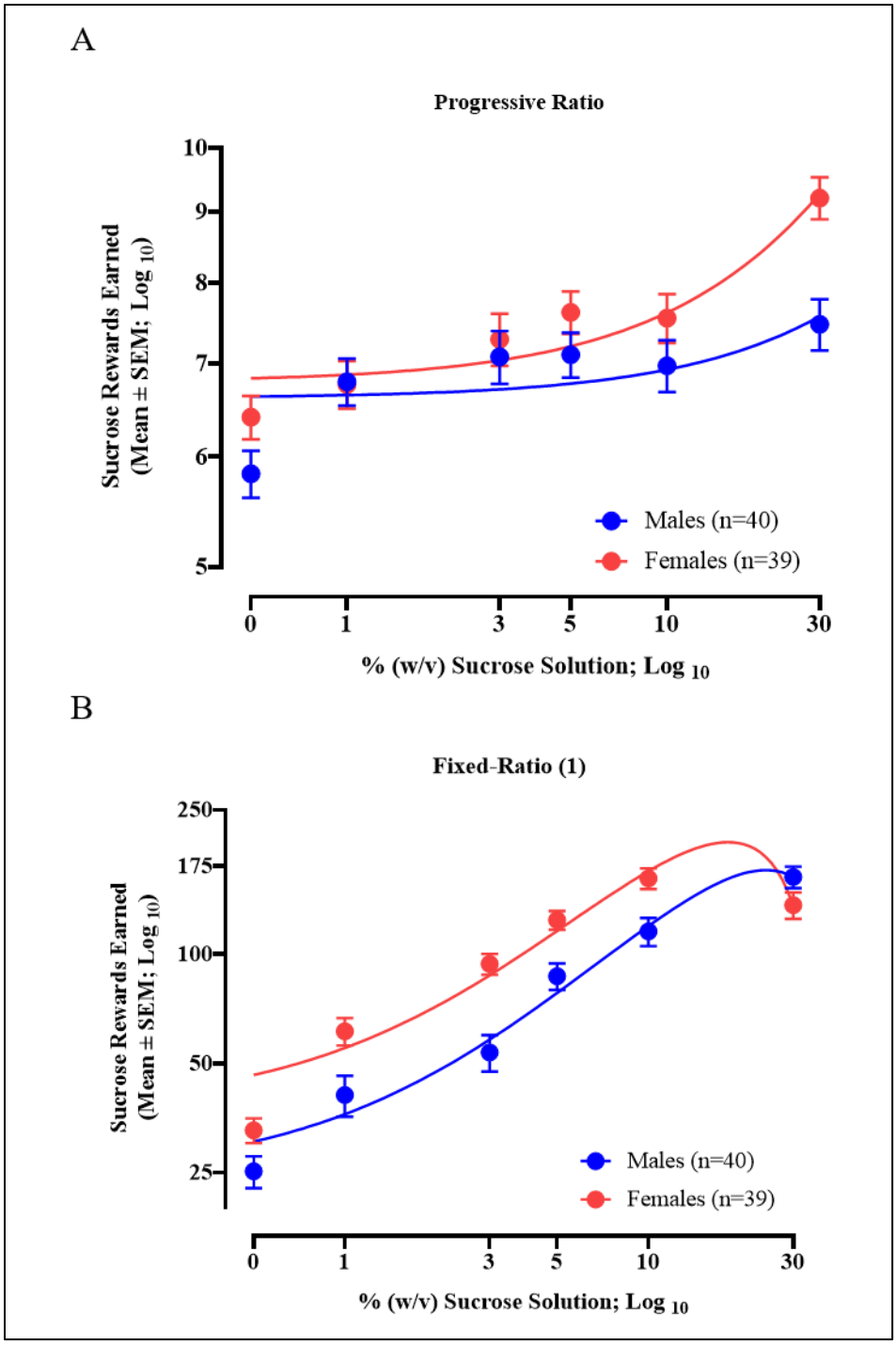


Figure 3.3. Sucrose Dose Response. After animals reached FR(1) criteria, responding for various concentrations of sucrose solution (0, 1, 3, 5, 10, and 30%) under a PR (A) and then a FR(1) (B) schedule of reinforcement. The genotype × sex × dose mixed-models ANOVAs did not reveal significant effects of genotype but did reveal significant sex × dose

interactions under both schedules. Under the PR schedule of reinforcement (A), female rats earned significantly more 30% sucrose reinforcers than males whereas, under the FR(1) schedule of reinforcement (B), females earned significantly more reinforcers of all sucrose doses (0, 1, 3, 5, and 10%) except for the 30% dose.

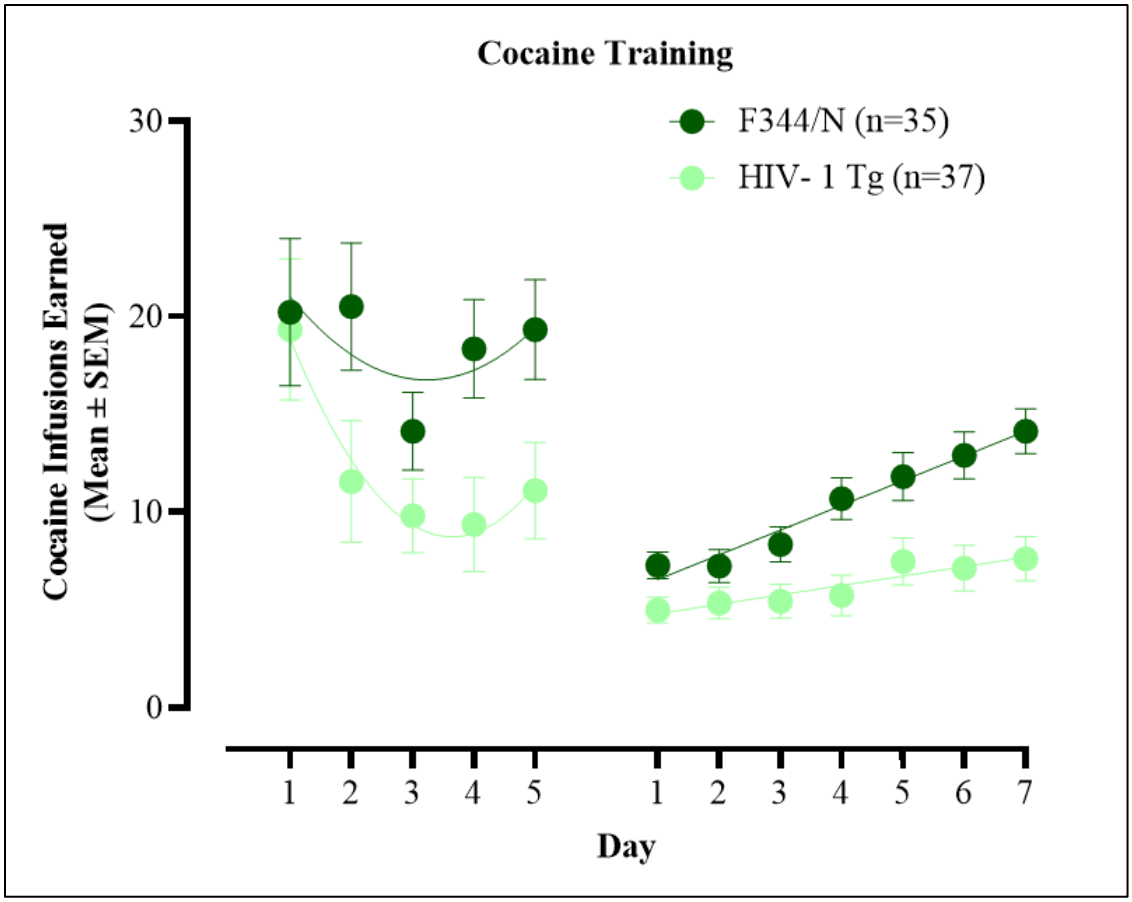


Figure 3.4. Cocaine-Maintained Responding. Once animals had recovered from surgeries, they were trained to lever-press for a low (0.2 mg/kg) dose of cocaine reinforcement over 5 days on an FR(1) schedule of reinforcement and for a higher (0.75 mg/kg) dose over 7 days on a PR schedule of reinforcement. This method was originally adapted from that of Morgan et al., (2006) which reported that this procedure elicited sensitization to the neural response to cocaine and, thus, escalation of cocaine intake. Indeed, both F344/N animals and HIV-1 Tg animals exhibited a positive slope in cocaine intake across the 7 days of testing on a PR schedule. This is within a shorter training period than that reported by Bertrand et al., (2018) or McLaurin et al., (under review). However, F344/N animals did earn significantly more cocaine reinforcers under the FR(1) schedule and escalated cocaine intake at a significantly greater rate under the PR schedule than HIV-1 Tg animals. The genotype differences shown in the current figure are collapsed across sex and are in contrast to those reported in ovariectomized animals by McLaurin et al., (under review) but agree with those reported in ovariectomized animals by Bertrand et al., (2018) which utilized higher doses (0.33 and 1.0 mg/kg infusion) of cocaine.

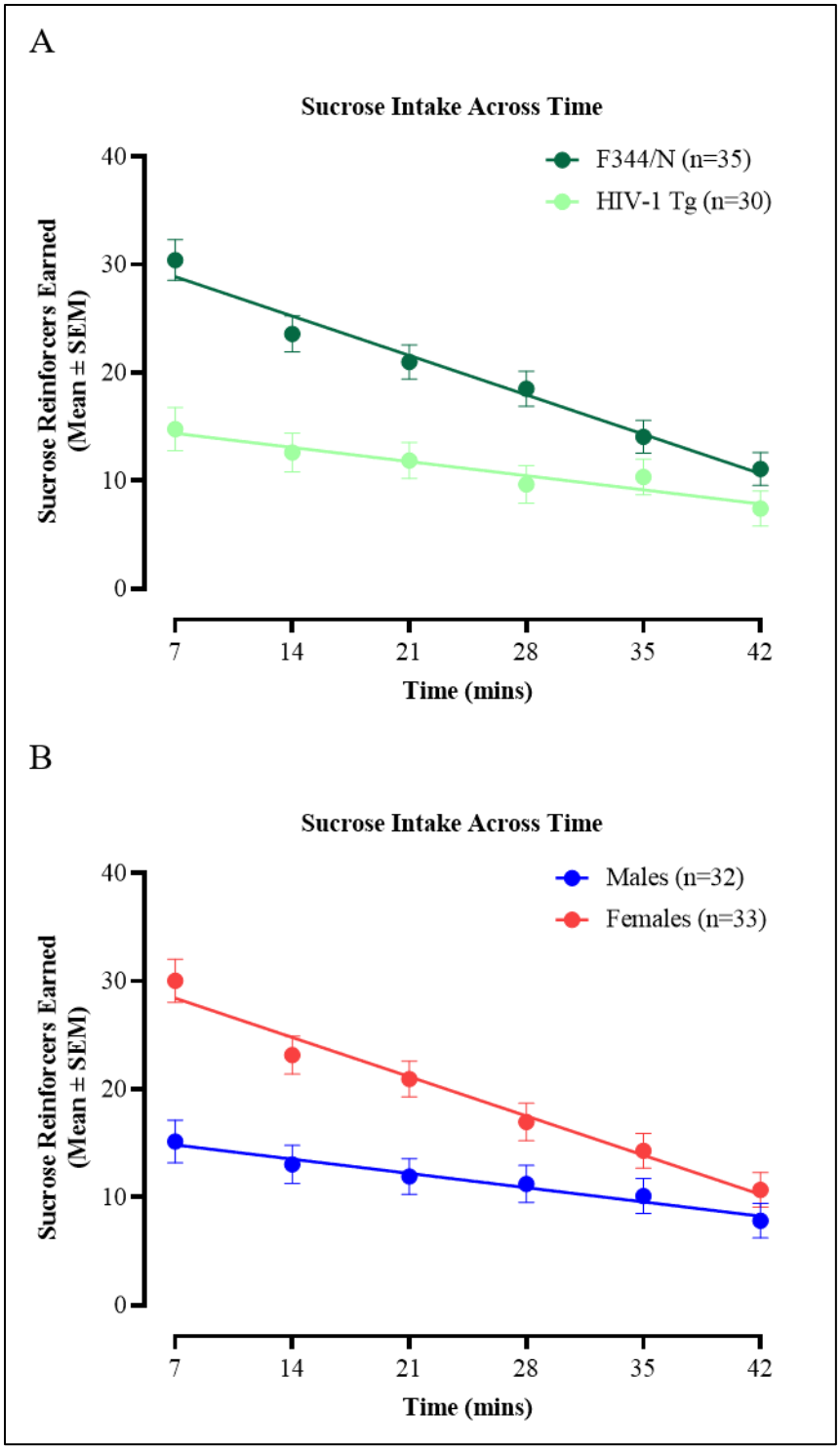


Figure 3.5. Sucrose Intake Across Time. Sucrose intake across time within 42-minute sessions was measured to determine if hM3DG(q) stimulation with C21 administration significantly altered sucrose intake selectively in DREADDs animals. The

genotype × sex × VTA infusion × C21 dose × time mixed-models ANOVA suggested that, when collapsed across VTA infusion and C21 dose, F344/N and HIV-1 Tg animals (A) exhibited significantly different patterns of intake across time, as did males and females (B). Lines fit to each genotype (A) or sex (B) exhibited significantly different y-intercepts and slopes but indicated that each group generally decreased sucrose intake across time within each session.

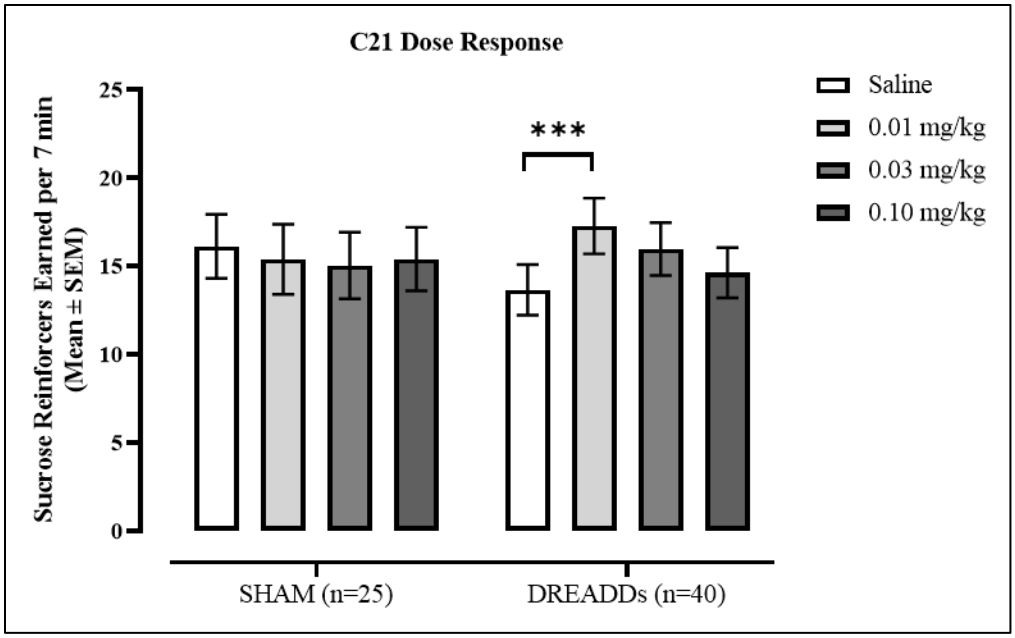


Figure 3.6. Sucrose Intake Across C21 Doses. Sucrose intake earned per 7-minute "bin" following saline was compared to that following 3 doses of C21 within DREADDs and sham animals to determine if hM3DG(q) stimulation significantly alters sucrose intake. When collapsed across genotype and sex, the genotype × sex × VTA infusion × C21 dose × time mixed-models ANOVA suggested that the 0.01 mg/kg dose of C21 significantly increased sucrose intake selectively in DREADDs animals. ***: $p \leq 0.001$.

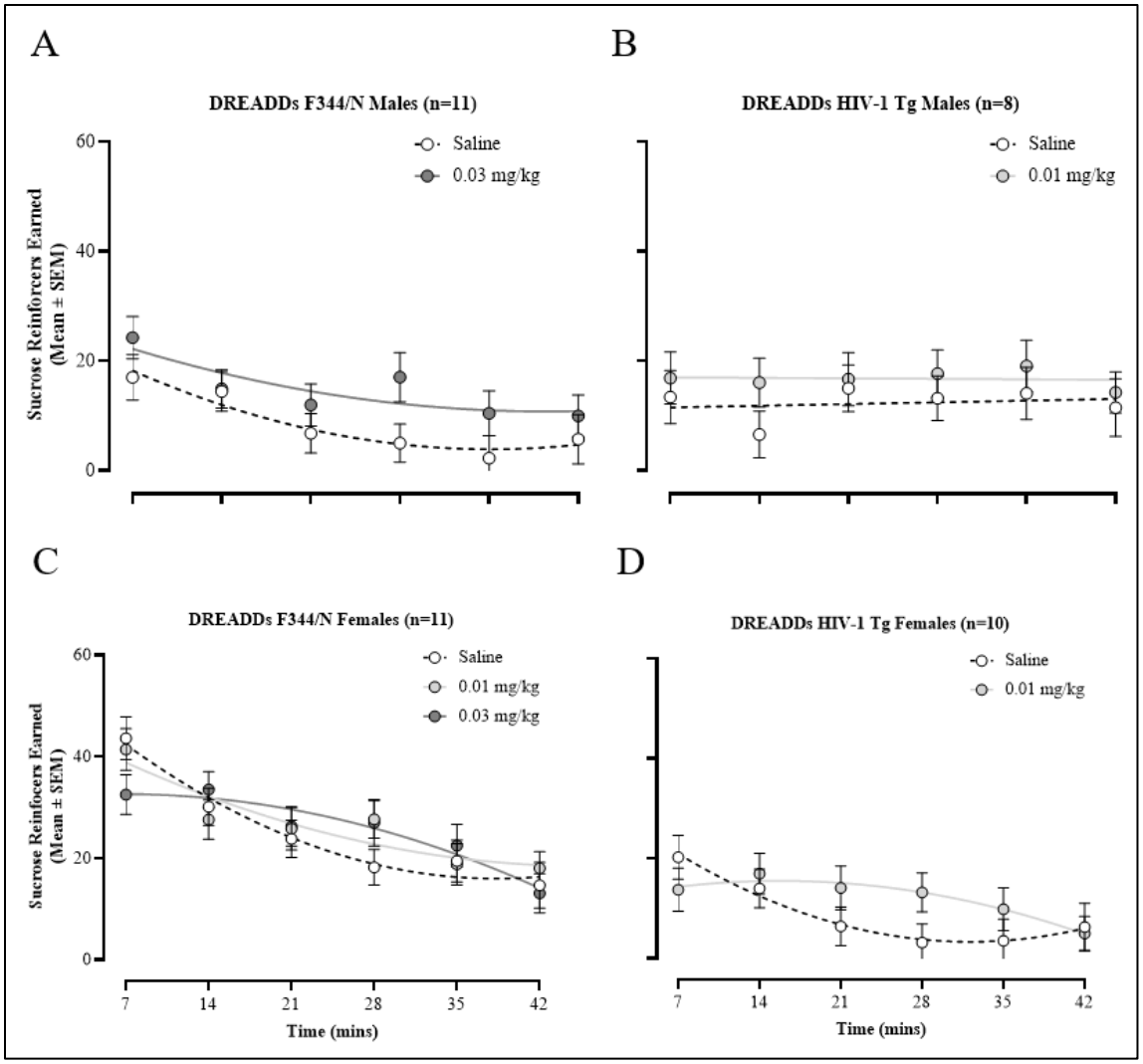


Figure 3.7. Sucrose Responding in the Presence of hM3D(Gq) Stimulation. Sucrose intake across time was altered by C21 administration, compared to saline administration, in each of the four groups. Compared to sucrose intake following saline administration, DREADDs F344/N males displayed a significant increase in sucrose intake between minutes 21-28 following the 0.03 mg/kg dose of C21 (A). DREADDs HIV-1 Tg males also displayed a significant increase in sucrose intake between minutes 7-14 following the 0.01 mg/kg dose of C21 (B). While sucrose intake by DREADDs F344/N females was also increased between minutes 21-28 of the session following the 0.01 mg/kg dose of C21, sucrose intake was significantly decreased between minutes 0-7 following the 0.03 mg/kg dose (C). Similarly, DREADDs HIV-1 Tg females displayed a significant decrease in sucrose intake between minutes 0-7 following the 0.01 mg/kg dose of C21 (D).

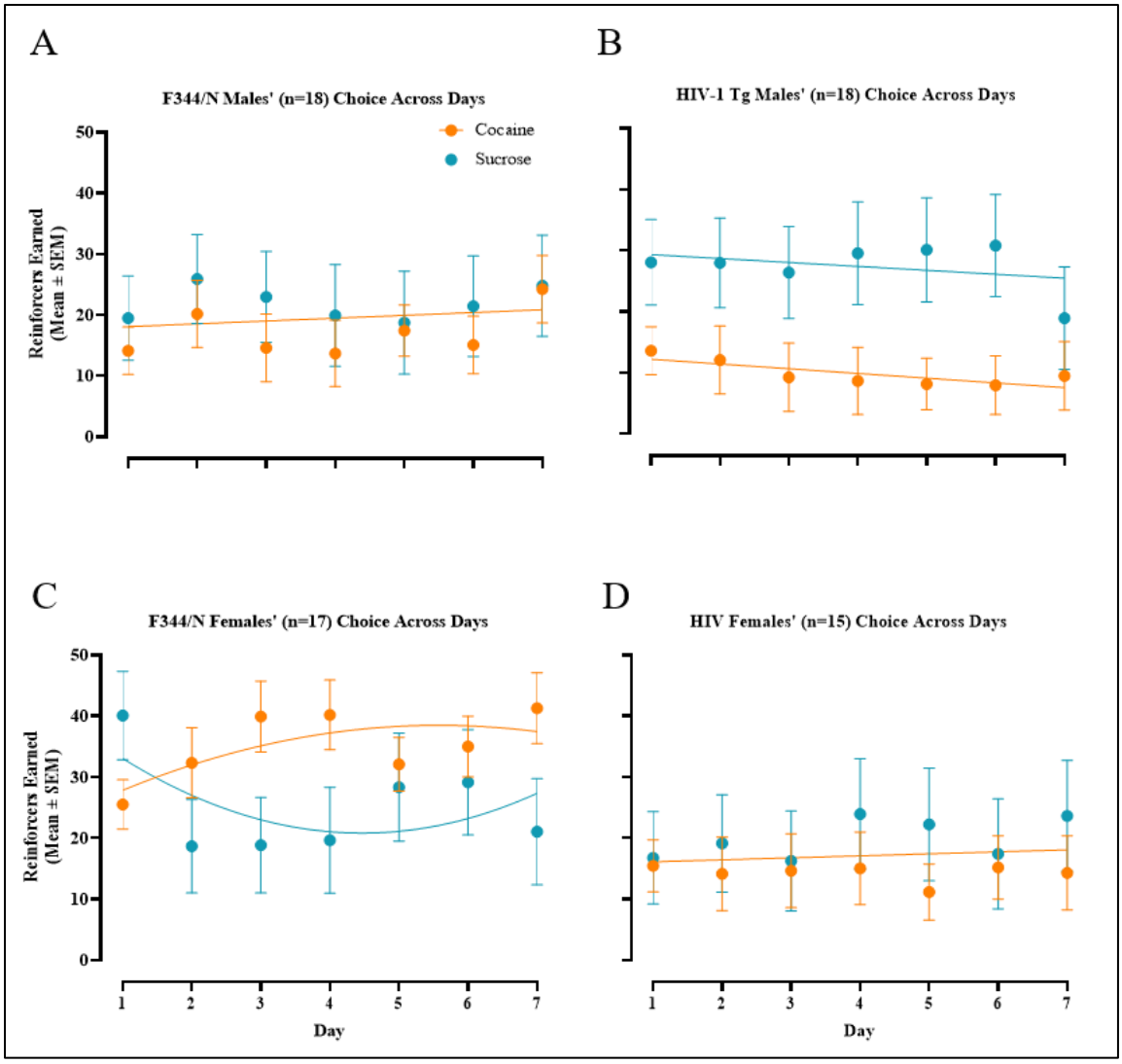


Figure 3.8. Choice Behavior by Group. The choice between 0.2 mg/kg infusions of cocaine or 5% sucrose solution was recorded across 7 days of testing under an FR(1) schedule of reinforcement, as described by Bertrand et al., (2018). A significant genotype × sex × day × reinforcer interaction supported that each of the four groups exhibited different patterns of choice behavior. F344/N males exhibited a linear pattern of intake across days and did not earn significantly different levels of cocaine and sucrose when both reinforcers were available simultaneously (A). In contrast, HIV-1 Tg males also exhibited a linear pattern of intake across days but also exhibited a significantly greater intake of sucrose than cocaine in days 5 and 6 of choice testing (B). F344/N females were the only group to exhibit a quadratic pattern of cocaine and sucrose intake across days, with cocaine intake being significantly greater than sucrose intake on testing day 3 (C). HIV-1 Tg females exhibited a pattern of choice behavior which was similar to that observed in F344/N males, with linear patterns of sucrose and cocaine across days being statistically similar (D). Thus, F344/N females did exhibit greater sensitivity to drug

choice than F344/N males as hypothesized. HIV-1 Tg females also exhibited disrupted choice behavior compared to F344/N females, supporting the findings of Bertand et al., (2018). However, HIV-1 Tg males did not exhibit what would be considered apathetic behavior compared to F344/N males, highlighting the importance of considering biological sex when reporting influences of the presence of HIV-1.

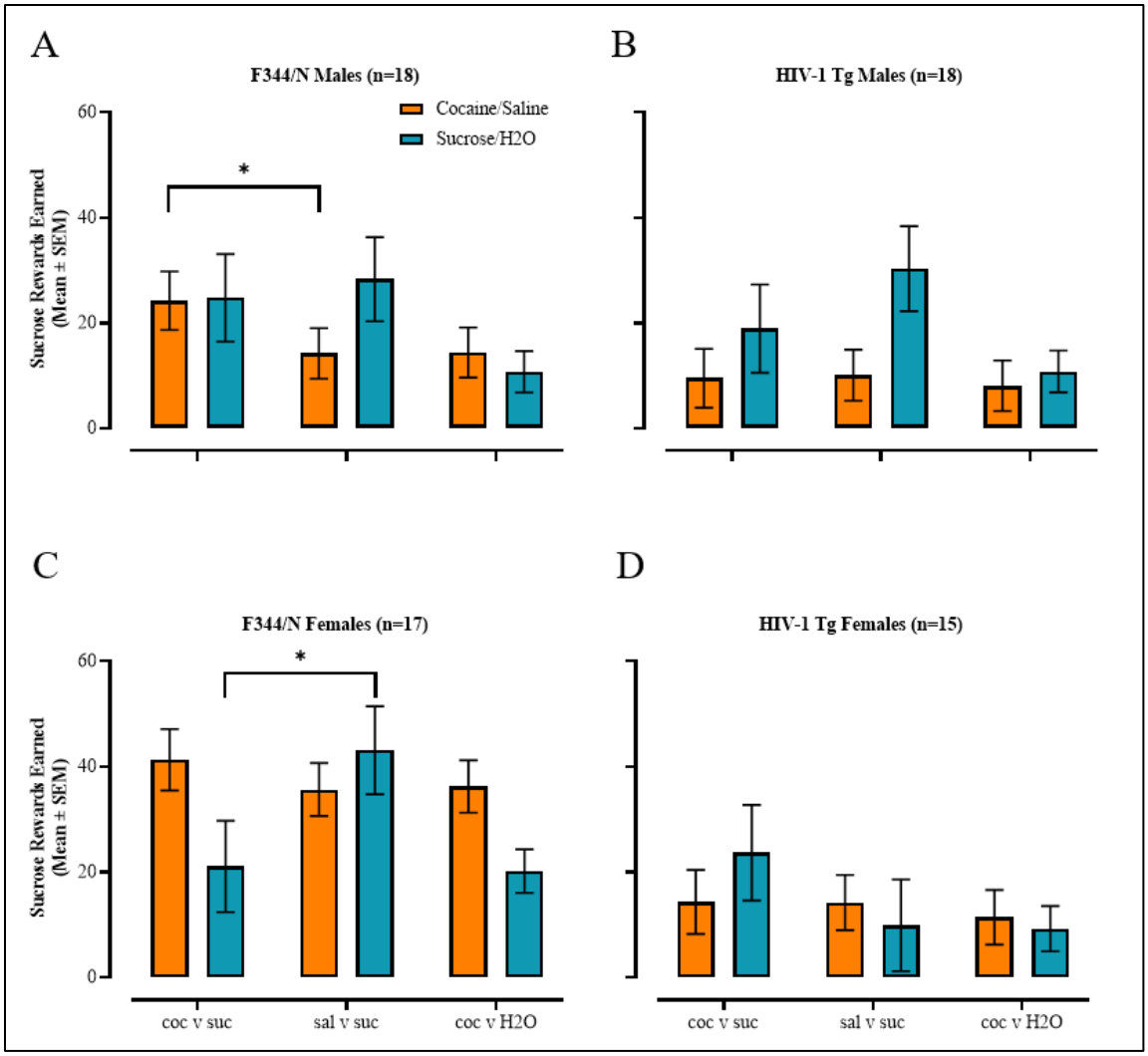


Figure 3.9. Group Responses to Choice Conditions. Cocaine was switched with saline and then sucrose was switched with water to determine if each group appropriately altered their choice behavior rather than exhibiting responding purely for the reinforcement from cocaine- or sucrose-associated stimuli. A significant genotype × sex × choice condition × reinforcer interaction suggested that each group responded to the choice conditions differently. F344/N males significantly reduced their intake of "cocaine" when cocaine was replaced with saline (A). While "sucrose" intake also appeared reduced when sucrose was replaced with water, this reduction was not significantly different compared to that under the cocaine vs. sucrose choice condition. HIV-1 Tg males exhibited low intake of cocaine under the cocaine vs. sucrose choice condition which did not appear to decrease further when cocaine was replaced with saline (B). Similar to what was observed in F344/N males, while "sucrose" intake appeared reduced when sucrose was replaced with water, this reduction was not significantly different. F344/N females did not significantly reduce their "cocaine" intake when cocaine was replaced with saline, but they did

significantly increase their intake of sucrose (C). When cocaine was returned and sucrose was replaced with water, sucrose intake was reduced back to levels observed under the cocaine vs. sucrose condition. HIV-1 Tg females appeared to decrease their intake of sucrose/"sucrose" under both the saline vs. sucrose and the cocaine vs. water conditions compared to the cocaine vs. sucrose condition, but this decrease was not statistically significant. *: $p \leq 0.05$

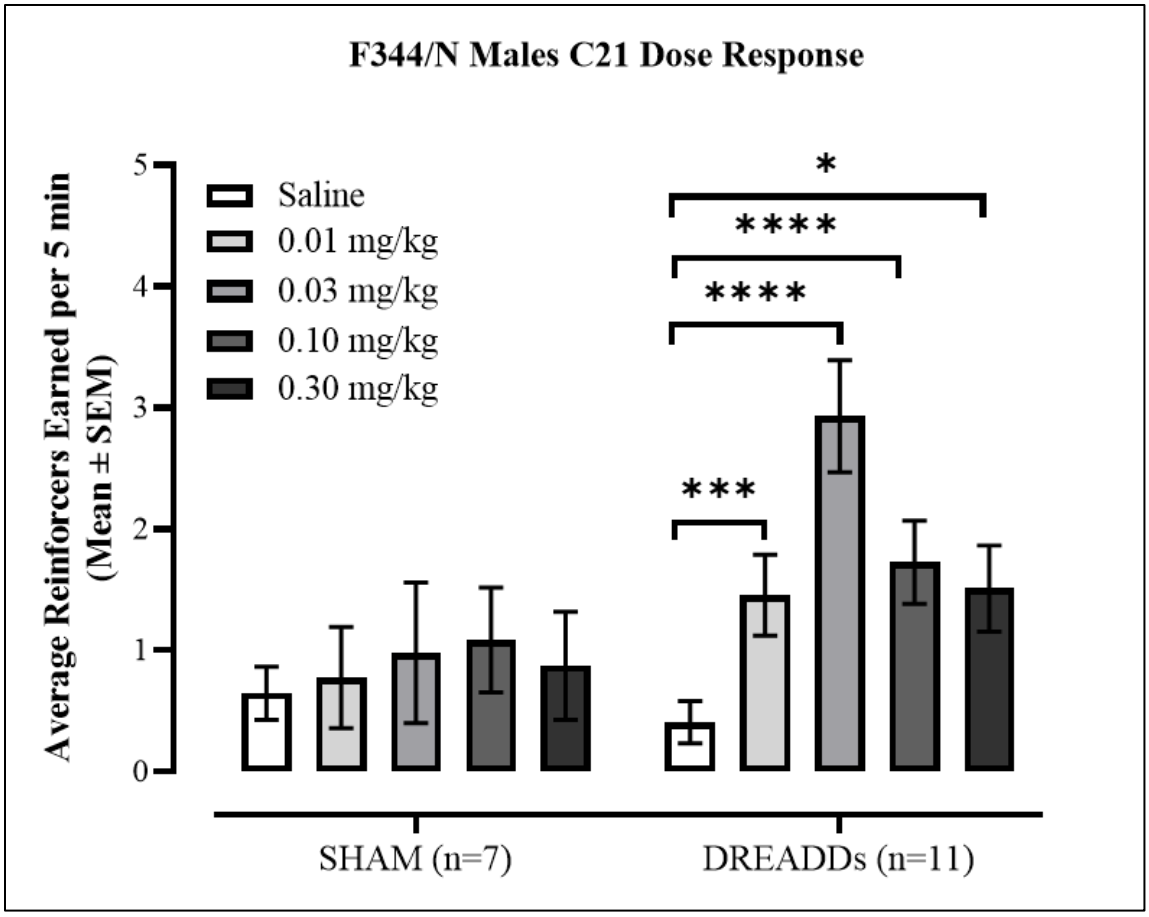


Figure 3.10. F344/N Males' Choice Across C21 Doses. Administration of the DREADDs hM3DG(q) receptor ligand, C21, increased average reinforcer intake/5 minutes in DREADDs, but not sham, F344/N males. Specifically, a VTA infusion × C21 dose × time × reinforcer mixed-models ANOVA suggested that, when compared to choice behavior following administration of saline, DREADDs F344/N males increased their average reinforcer intake following each of the administration of each of the doses of C21 tested, with the largest change in intake observed following administration of the 0.03 mg/kg dose of C21. Further, examination of the results did, however, support that the influence of C21 on DREADDs F344/N males' reinforcer intake was dependent on the type of reinforcer (cocaine vs. sucrose). *: $p \leq 0.05$; ***: $p \leq 0.005$; ****: $p \leq 0.001$.

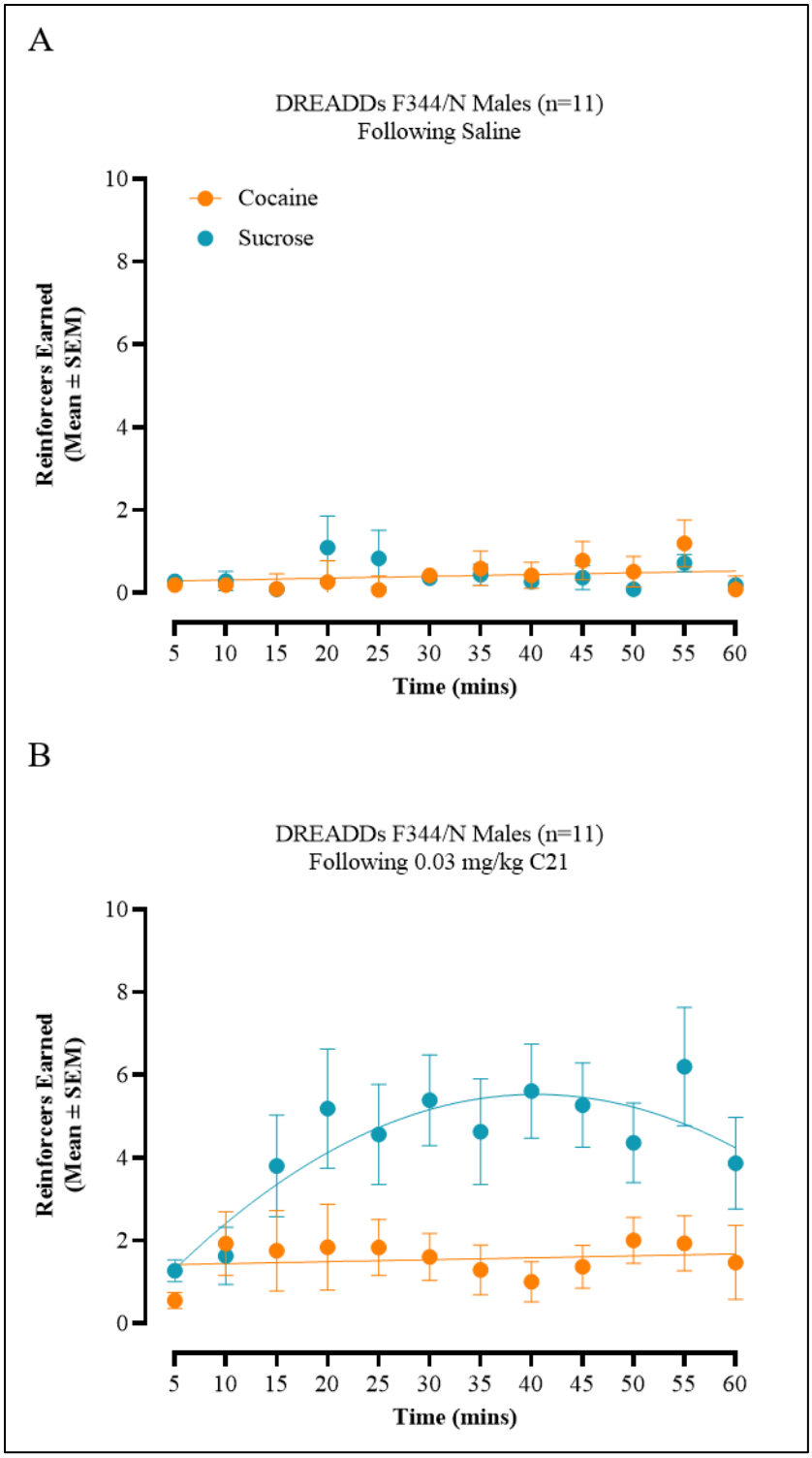


Figure 3.11. F344/N Males' Choice in the Presence of hM3D(Gq) Stimulation. Administration of the 0.03 mg/kg dose of C21 significantly increased the choice of sucrose over cocaine in

DREADDs F344/N males. Following administration of saline (A), DREADDs F344/N males did not exhibit significantly different levels of cocaine and sucrose intake at any of the 5-minute periods represented here. In contrast, following administration of the 0.03 mg/kg dose of C21 (B), intake of sucrose shifted from following a linear to a quadratic pattern across time, and intake of sucrose was significantly greater than that of cocaine for approximately 25 minutes of the session (i.e., minutes 0-5, 25-30, 35-345, and 50-55). This was the greatest change in DREADDs F344/N males' choice behavior observed following C21 administration, although all of the other tested C21 doses (0.01, 0.10, 0.30 mg/kg) did elicit greater intake of sucrose than cocaine in the first five minutes of testing sessions. Further, when the factor of time was removed and data were censored to remove any animals that did not earn at least 2 of each reinforcer, the analysis supported that the number of sucrose reinforcers earned was significantly greater than the number of cocaine reinforcers earned following the 0.03 mg/kg and 0.30 mg/kg doses of C21.

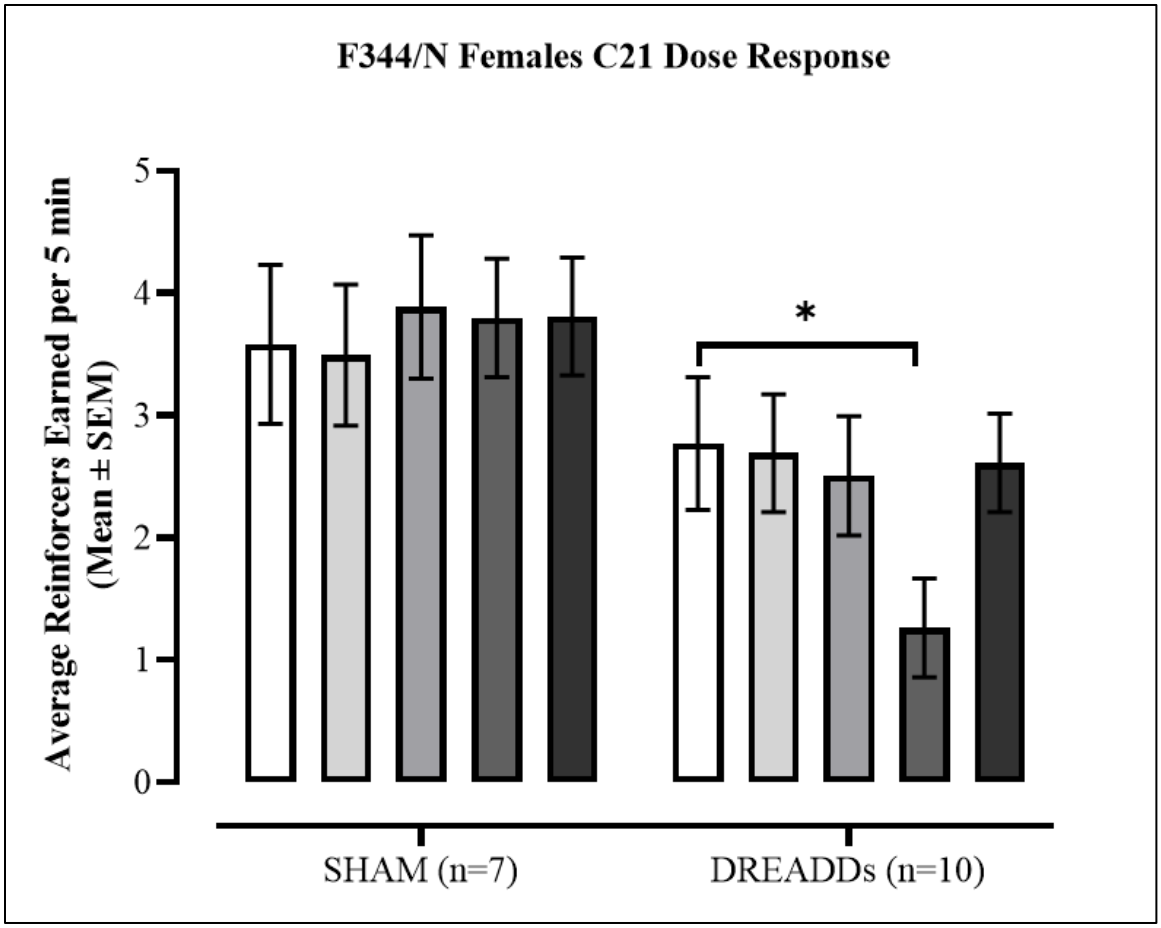


Figure 3.12. F344/N Females' Choice in the Presence of hM3D(Gq) Stimulation. Administration of the DREADDs hM3DG(q) receptor ligand, C21, decreased average reinforcer intake/5 minutes in DREADDs, but not sham, F344/N females. Specifically, a VTA infusion × C21 dose × time × reinforcer mixed-models ANOVA suggested that, when compared to choice behavior following administration of saline, DREADDs F344/N females increased their average reinforcer intake following each of the administration of each of the doses of C21 tested, with the largest change in intake observed following administration of the 0.03 mg/kg dose of C21. Further, when the factor of time was removed and data were censored to remove any animals that did not earn at least 2 of each reinforcer, the analysis supported that the number of reinforcers earned was significantly reduced following the 0.10 mg/kg dose of C21 compared to that observed following saline. *: $p \leq 0.05$.

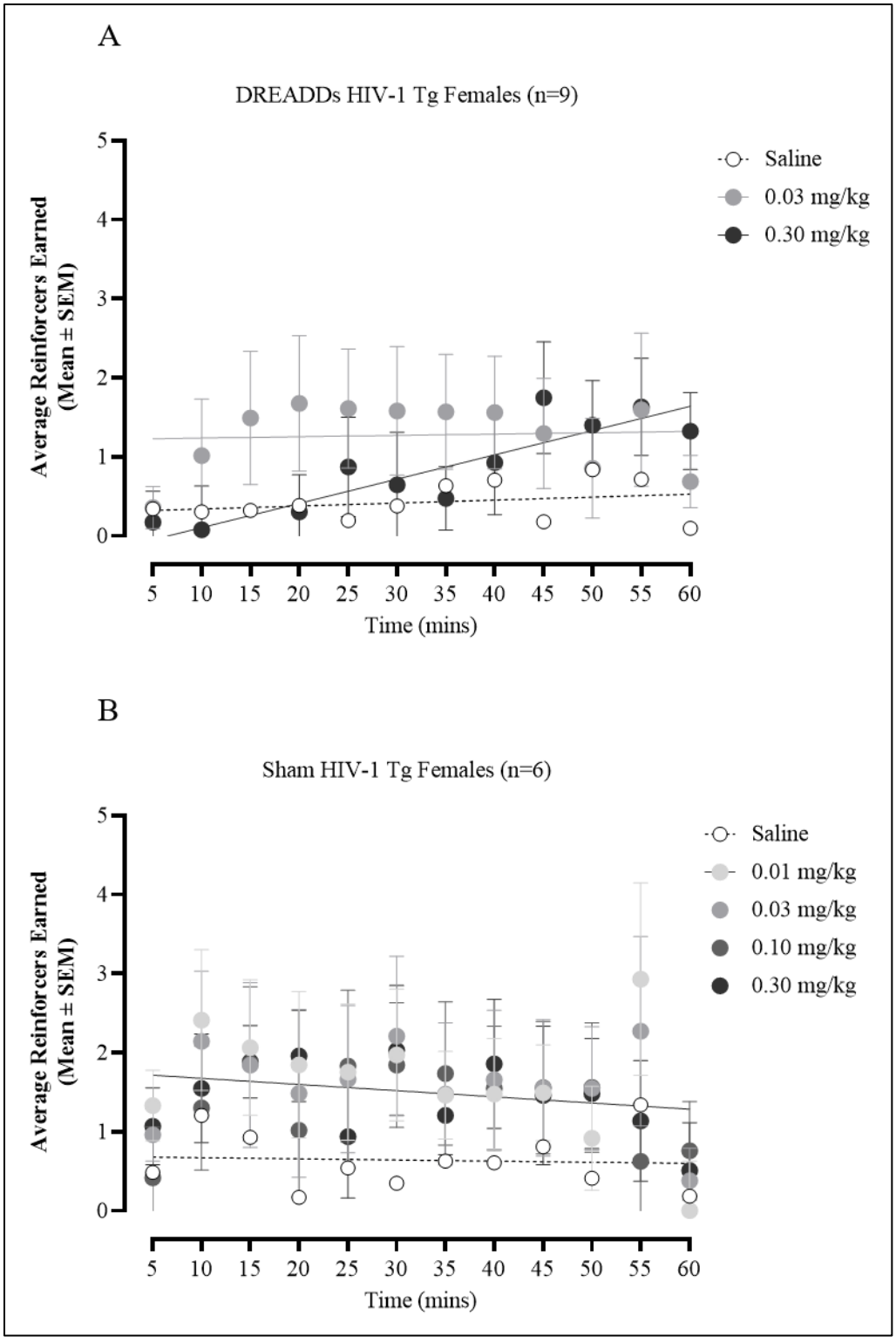


Figure 3.13. HIV-1 Tg Females' Choice in the Presence of hM3D(Gq) Stimulation. The influence of hM3DG(q) stimulation in HIV-1 Tg females was

not clear, as both DREADDs and sham animals displayed changes to choice behavior following administration of C21. Administration of the 0.03 and 0.30 mg/kg doses of C21 increased DREADDs HIV-1 Tg females (A) average reinforcer intake for approximately 10 minutes compared to intake following saline administration. In contrast, sham HIV-1 Tg females (B) displayed a significant increase in reinforcer intake for at least 5 minutes of the session following all tested doses of C21 (0.01, 0.03, 01.0, 0.30 mg/kg) compared to intake following saline administration. Notably, when the factor of time was removed and data were censored to remove any animals that did not earn at least 2 of each reinforcer, only 35% of intake data were available and the analysis was unable to converge.

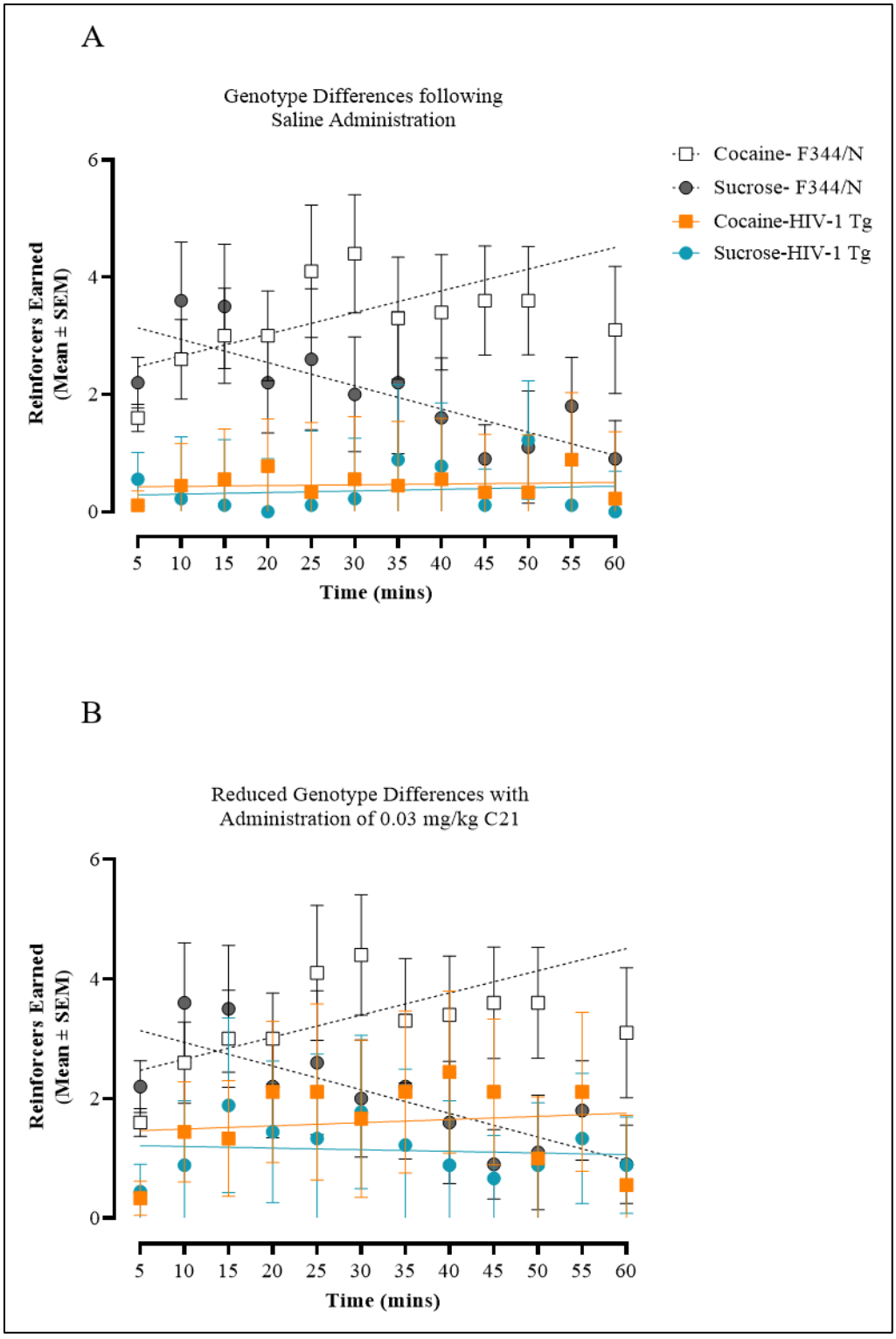


Figure 3.14. hM3D(Gq) Stimulation may Reduce Apathy in HIV-1 Tg Females. Stimulation of hM3DG(q) receptors in DREADDs HIV-1 Tg females significantly reduced genotype differences in choice behavior compared to

DREADDs F344/N females. When both DREADDs F344/N and DREADDs HIV-1 Tg females are administered saline (A), F344/N animals earned significantly more cocaine reinforcers during approximately 35 minutes of the session as well as significantly more sucrose reinforcers during approximately 15 minutes of the session. A genotype × C21 dose × time × reinforcer mixed-models ANOVA revealed that administration of all except for the 0.01 mg/kg dose of C21 (0.03, 0.10, 0.30 mg/kg) significantly reduced genotype differences observed following saline, however, this was dependent on reinforcer type (cocaine vs. sucrose). Administration of the 0.03 mg/kg dose of C21 to DREADDs HIV-1 Tg females (B) appeared most effective at reducing genotype differences in cocaine intake. Compared to DREADDs F344/N females following administration of saline, DREADDs HIV-1 Tg females following administration of 0.03 mg/kg C21 only displayed reduced sucrose intake for approximately 10 minutes. The 0.03 mg/kg dose of C21 also reduced genotype differences in sucrose intake so that DREADDs F344/N females following saline administration only earned significantly more sucrose reinforcers than DREADDs HIV-1 Tg females during the first 5 minutes of the session.

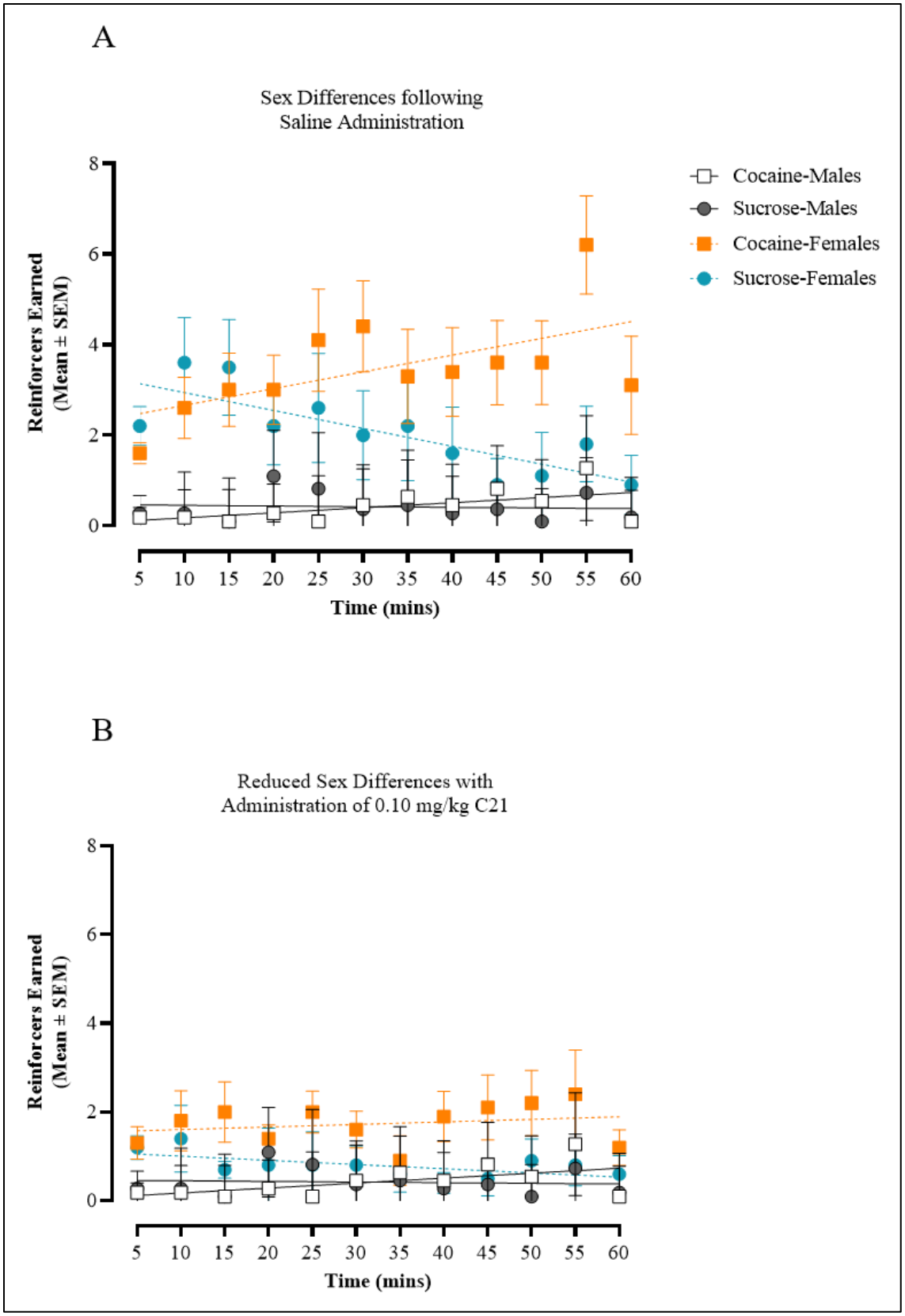

A
Sex Differences following
Saline Administration
Reinforcers Earned
(Mean ± SEM)
0
2
4
6
8
5
10
15
20
25
30
35
40
45
50
55
60
Time (mins)
Cocaine-Males
Sucrose-Males
Cocaine-Females
Sucrose-Females
B
Reduced Sex Differences with
Administration of 0.10 mg/kg C21
Reinforcers Earned
(Mean ± SEM)
0
2
4
6
8
5
10
15
20
25
30
35
40
45
50
55
60
Time (mins)

Figure 3.15. hM3D(Gq) Stimulation may Reduce Drug Dependence in F344/N Females. Stimulation of hM3DG(q) receptors in DREADDs F344/N females significantly reduced sex differences in choice behavior compared to DREADDs F344/N males. When both DREADDs F344/N males and females are administered saline (A), F344/N males earned significantly fewer cocaine reinforcers during approximately 50 minutes of the session as well as significantly fewer sucrose reinforcers during the first 15 minutes of the session. A genotype × C21 dose × time × reinforcer mixed-models ANOVA revealed that administration of all except for the 0.03 mg/kg dose of C21 to DREADDs F344/N females reduced sex differences in cocaine intake. Administration of the 0.10 mg/kg dose of C21 to DREADDs F344/N females (B) appeared most effective at reducing genotype differences in cocaine intake. Compared to DREADDs F344/N males following administration of saline, DREADDs F344/N females following administration of 0.10 mg/kg C21 only displayed increased cocaine intake for approximately 15 minutes of the session. The 0.10 mg/kg dose of C21 also reduced sex differences in sucrose intake so that DREADDs F344/N females following saline administration only earned significantly more sucrose reinforcers than DREADDs F34/N males during 10 minutes of the session.

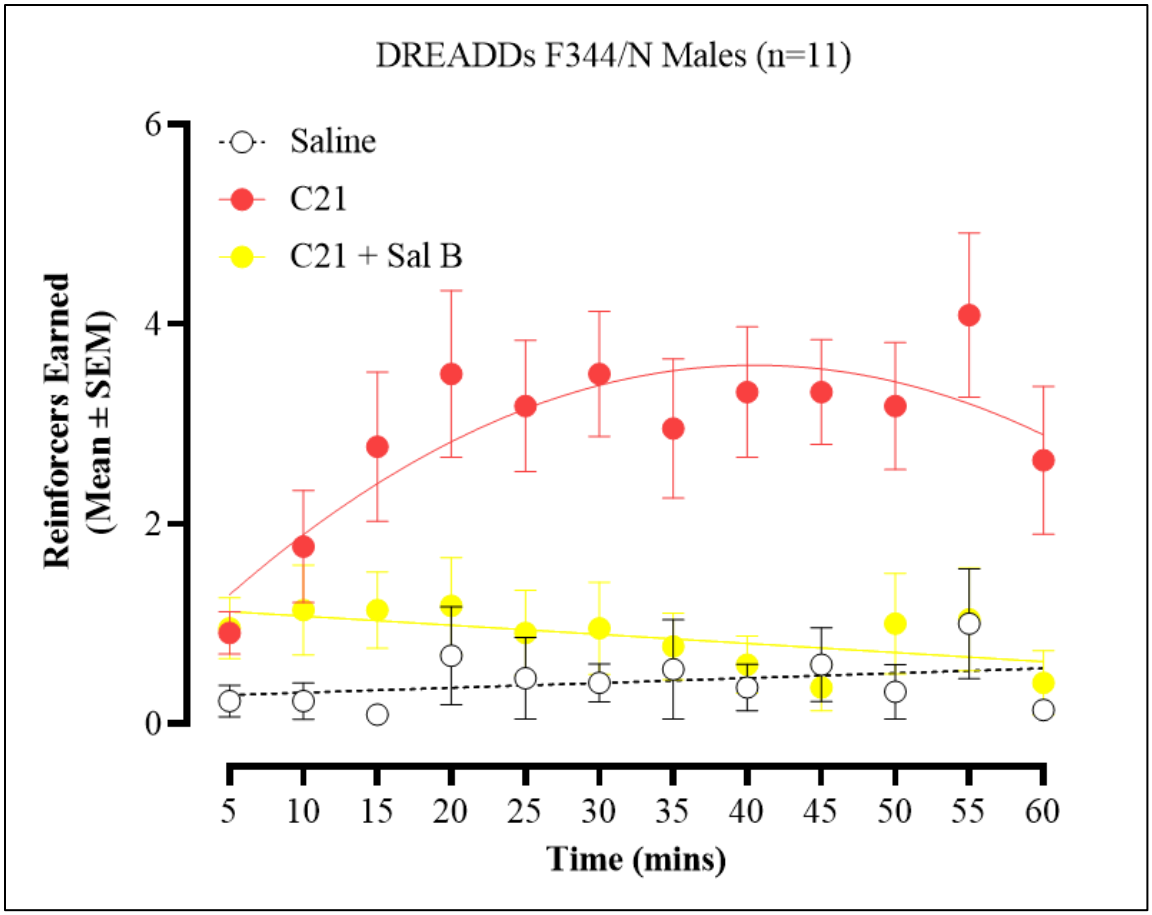


Figure 3.16. Sal B Reversal of C21 Influence on F344/N Males' Choice. To determine if the additional administration of the KORD (inhibitory receptor) ligand, Sal B, would block the observed effects of C21, C21 was administered before returning animals to their home cage for approximately 15 minutes. After 15 minutes animals were administered 0.15 mg/kg Sal B and were immediately placed directly into the choice testing chamber. In DREADDs F344/N males, administration of 0.03 mg/kg C21 and 0.15 mg/kg Sal B together significantly reduced reinforcer intake when compared to that following C21 alone and restored the linear pattern of intake that was observed following saline administration.

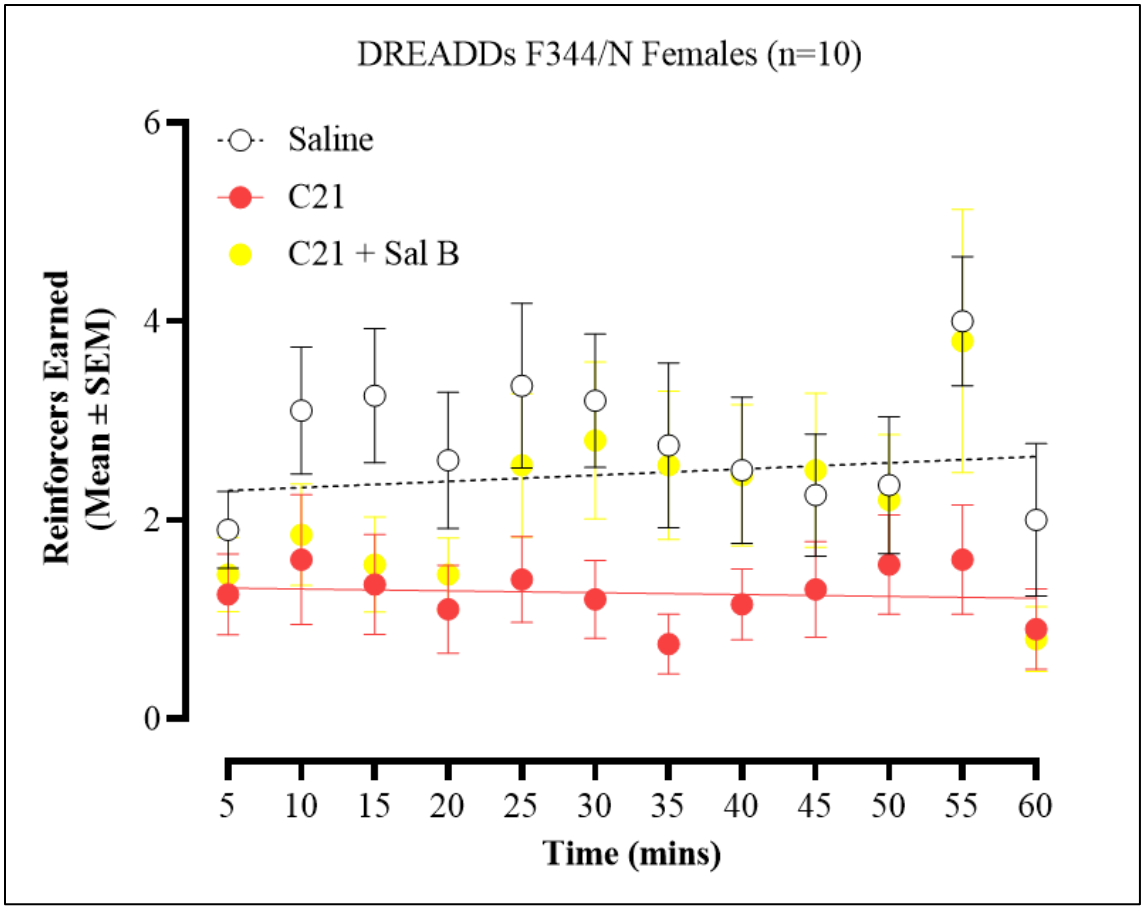
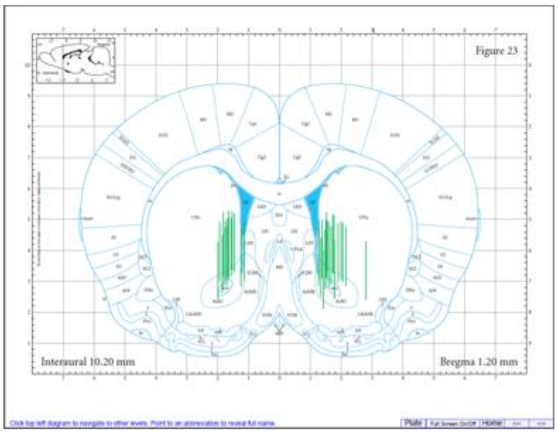
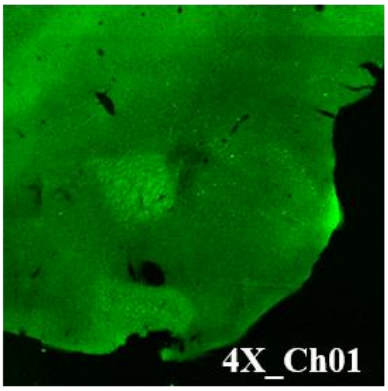


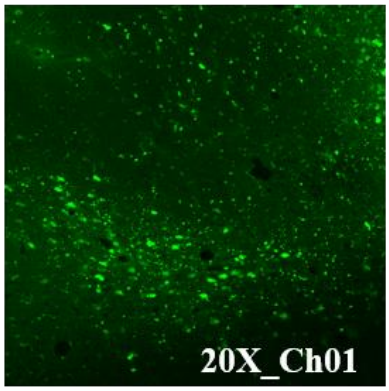
Figure 3.17. Sal B Reversal of C21 Influence on F344/N Females' Choice. To determine if the additional administration of the KORD (inhibitory receptor) ligand, Sal B, would block the observed effects of C21, C21 was administered before returning animals to their home cage for approximately 15 minutes. After 15 minutes animals were administered 0.15 mg/kg Sal B and were immediately placed directly into the choice testing chamber. In DREADDs F344/N females, administration of 0.10 mg/kg C21 and 0.15 mg/kg Sal B together significantly increased reinforcer intake compared to that following 0.10 mg/kg C21 alone so that intake in the latter half of the 1-hour session more closely resembled intake following saline administration.

A

Nucleus Accumbens

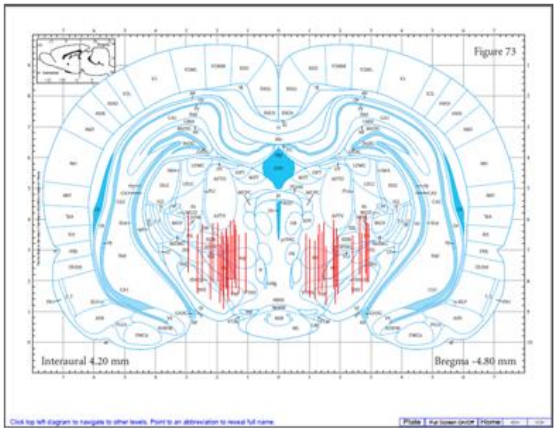


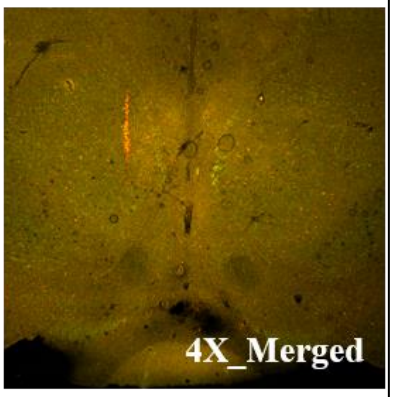




B

Ventral Tegmental Area





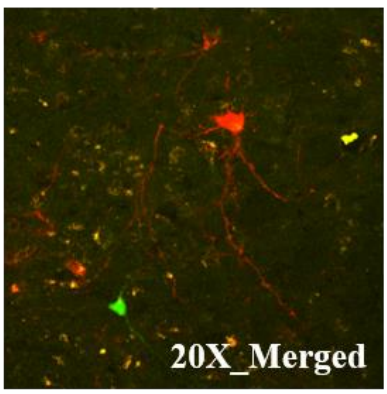


Figure 3.18. Verification of Cannula Placement and DREADDS Expression. Placement of DREADDs cannula tracts and expression of DREADDs in the NAc (A) and VTA (B) were verified following behavioral testing. Cannula tracts were observed within the targeted regions of tissue from 28 randomly-selected animals. Expression of green fluorescent protein indicates the presence of Cre in the NAc of DREADDs animals (A). Expression of mCherry (red) and mCitrine (yellow) indicates the presence of DREADDs hM3DG(q) and KORD receptors in the VTA of DREADDs animals (B).

Chapter 4. Discussion

The current experiment provides a translational and adaptable method to inform the development of and assess the effectiveness of proposed treatments to reduce drug use or other harmful choice behaviors. Specifically, the current experiment attempted to mitigate symptoms of drug dependence or apathy by manipulating the activity of cells projecting from the VTA to the NAc using chemogenetic techniques and reducing drug choice or enhancing reinforcer-seeking behavior, respectively. Successful modulation of sucrose intake and cocaine vs. sucrose choice supports that the mesolimbic circuit is involved in mediating choice between one or more reinforcers and informing the development of intriguing hypotheses regarding the neurobiology which determines choice behavior. Biological sex and the presence of the HIV-1 transgene significantly influenced the reinforcing efficacy of sucrose and choice between cocaine vs. sucrose in the current experiment. Females exhibited faster reinforcement learning, greater reinforcing efficacy for a high (30%) sucrose solution, and a stronger response vigor for low doses of sucrose under single-schedule procedures and increased response vigor for cocaine under the choice procedures. The likelihood of choosing cocaine over sucrose in the choice procedure was also significantly greater in females, supporting that females may exhibit a greater vulnerability to the effects of repeated cocaine use compared to males. HIV-1 Tg females exhibited reduced reinforcing efficacy of sucrose under a single-schedule procedure and of sucrose and cocaine under choice procedures compared to F344/N females, supporting that

biological sex interacts with genotype to influence the display of apathetic behavior in HIV-1 Tg animals. Additionally, HIV-1 Tg animals generally exhibited reduced response vigor for and escalation of cocaine under the single-schedule procedures when compared to F344/N animals, supporting the results of Bertrand et al., (2018).

Stimulation of the mesolimbic circuit increased the choice of sucrose over cocaine in F344/N males and decreased the choice of cocaine over sucrose in F344/N females, suggesting that the mesolimbic circuit may be a promising target for the treatment of psychopathologies characterized by disrupted reinforcement processing. In contrast, while mesolimbic stimulation appeared to alter sucrose intake by all groups under the single-schedule procedure, the influence of mesolimbic stimulation on choice behavior in HIV-1 Tg rats was less clear and may need to be re-assessed under different experimental conditions. The level of reinforcer intake in HIV-1 Tg animals may have been too low to reliably detect changes to choice behavior elicited by mesolimbic stimulation, supporting the findings of McLaurin et al., (under review). The described methods may be used under some conditions to assess the effectiveness of said proposed treatments in populations with theorized vulnerabilities to harmful behavior patterns and/or under conditions that may promote or reduce the likelihood of said behavior patterns.

4.1. Implications

Foremost, the current experimental design should be noted regardless of whether the hypotheses of the current experiments were confirmed as patients exhibiting many psychopathologies (e.g., internet addiction, obesity, anorexia) could benefit from relieving harmful patterns of choice behavior. In the current experiment, stimulation of the mesolimbic circuit enhanced the choice of a non-drug reinforcer (sucrose) over that of

cocaine in F344/N male and female rats through mechanisms dependent on biological sex. While results in HIV-1 Tg animals were less clear under the current experimental conditions, changes to choice behavior elicited by a treatment of interest may be observed if the reinforcers available provoke a higher level of pre-treatment responding. Similar results were found when McLaurin et al., (under review) tested the efficacy of S-equol as a treatment for apathy in HIV-1 Tg animals. While the efficacy of various treatment strategies can be assessed using the choice procedure, other neural circuits of interest can also be explored to possibly promote the seeking of one reinforcer type over another. Such manipulation of neural circuit activity may provide a novel method by which to intervene in harmful choice-making behaviors that affect many psychopathologies (e.g., addiction, obesity, anorexia, apathy) that greatly impact public health. As such, it should be determined if manipulation of the mesolimbic or other circuits can influence engagement in other goal-directed behaviors of interest. Likewise, choice procedures can be used to detect circumstances that may promote risky behavior and/or treatment resistance and may be adapted to uncover the neurophysiological mechanisms driving behavioral pathologies. For example, in the current experiment, F344/N females were more likely than F344/N males to exhibit behaviors that characterize drug-dependence whereas HIV-1 Tg females were more likely than F344/N females to exhibit behaviors that characterize apathy. It may thus be beneficial for researchers interested in exploring the etiology of drug-dependence or apathy to identify factors outside of sex and/or genotype which influence the display of behaviors associated with disrupted reinforcement processing. Additionally, researchers developing treatments for such behavioral pathologies should ensure that said treatments are effective in populations that are found to be particularly vulnerable to dysregulation.

Aside from the utility of the choice procedure itself, the current results demonstrated successful modulation of choice behavior with chemogenetic manipulation to reduce the prominence of drug choice in a sex-dependent manner in F344/N rats. The successful alteration of choice behavior in male and female rats supports our hypothesis and the current literature in that the activity of the mesolimbic circuit is likely involved in determining choices between one or more reinforcers, specifically drug vs. non-drug choice behavior. Prior to stimulation, F344/N males exhibited statistically similar intake of cocaine and sucrose during choice procedures and exhibited reduced intake of cocaine and sucrose compared to F344/N females in single-schedule and choice procedures. F344/N females, however, exhibited greater choice of cocaine than sucrose before stimulation. Stimulation of the mesolimbic circuit increased sucrose choice in F344/N males, so that sucrose was chosen significantly more than cocaine, and decreased cocaine choice in F344/N females, such that intake of cocaine and sucrose were statistically similar for a longer portion of the session.

While the specific mechanisms by which mesolimbic activity influences choice in the current experiment are still undetermined, a previous experiment from our laboratory suggested that stimulation of this circuit increased the salience of novelty in the environment (e.g., removal of background noise) in ovariectomized F344/N females (Li et al., 2019). In the current experiment, it is possible that increased salience or value of sucrose contributed to observed increases in intake in all but HIV-1 Tg females under the single-schedule procedure and F344/N male rats under the choice procedure. Such results suggest that, when both cocaine and sucrose were available to F344/N males, the salience of sucrose, which appeared to be greater than cocaine prior to treatment, was increased to

a greater degree than the salience of cocaine. Behaviors maintained by a reinforcer with greater value may be influenced by the activity of a greater number of cells than behaviors in pursuit of a reinforcer of lesser value (Cromwell et al., 2018). It is possible that, in the choice setting, stimulation of a majority of mesolimbic cells in F344/N males promotes sucrose intake compared to cocaine intake. This is supported by the current results that mesolimbic stimulation in both F344/N males and females had a greater influence on intake of the reinforcer which was chosen more frequently prior to stimulation.

Nevertheless, it is unlikely that altering the firing activity of any given mesolimbic cell will always promote either sucrose or cocaine seeking. Certain cells of the ventral striatum have been observed to be selectively active when pursuing a reinforcer in a choice setting but not a single-schedule setting (Cromwell et al., 2018). The current results support that stimulation of the same group of mesolimbic cells can produce different, and even opposing, influences on behavior depending on the availability of reinforcers. In females, mesolimbic stimulation significantly altered sucrose intake under the single-schedule procedure but analyses on censored data suggested that mesolimbic stimulation altered cocaine intake more so than sucrose intake when both reinforcers were available simultaneously. Additionally, stimulation of the mesolimbic circuit increased sucrose intake in HIV-1 Tg males when sucrose was the only reinforcer available but not when sucrose and cocaine were both available simultaneously. Treatment effects observed in the current experiment which were dependent on the available reinforcers may also be observed for other forms of addiction treatments, particularly pharmacological methods targeting mesolimbic activity. Further, while not tested in the current experiment, to elucidate the mechanisms of choice behavior, it is of interest to determine the influence of

mesolimbic stimulation on reinforcer intake when cocaine, rather than sucrose is the only reinforcer available. If drug intake in addicted animals is more resistant to treatment than that in non-addicted animals, it is likely that cocaine intake, particularly in F344/N female rats, will be reduced to a lesser extent, or even increased, by mesolimbic stimulation if cocaine is the only reinforcer available.

However, it should also be noted that sucrose and cocaine intake under the choice procedure in F344/N females was decreased rather than increased. Given that F344/N females displayed greater intake of cocaine than sucrose prior to mesolimbic stimulation, the suggestion that the reinforcer with greater value will be represented by a greater number of mesolimbic cells and will thus display greater changes when mesolimbic activity is manipulated is supported. However, the suggestion that an increase in salience is responsible for the observed changes to choice behavior is less consistent with the observed results in F344/N females. Another possibility is that the treatment in F344/N females overstimulated the mesolimbic circuit to a point where intake begins to decrease with increased stimulation, beyond the point which maximal responding would be observed. Changes to sucrose intake elicited by C21 administration in the single-schedule procedure could indicate that similar overstimulation occurs in HIV-1 Tg females, but not males, under these conditions. Such observations indicate that it may be beneficial to test other targets for the treatment of apathy, at least in HIV-1 Tg females. Specifically, between-subjects differences in the connectivity between or organization within various regions likely influence the ability of mesolimbic circuit activity to mediate reinforcer intake and/or choice behavior. Likewise, differences in the connectivity between different regions related to drug reinforcement processing may be responsible for the observed sex-dependent

changes to choice behavior elicited by mesolimbic stimulation. Specifically, it is also possible that prior exposure to cocaine may have elicited the emergence of opposing neurobiological processes in F344/N females sooner than in F344/N males so that mesolimbic stimulation in F344/N females triggered a response from opposing circuits that was more prominent than that which promotes goal-directed behavior and that which occurred in males, resulting in a decrease rather than an increase in reinforcer intake.

F344/N males and females exhibited the hypothesized sex differences in sucrose intake under the single-schedule procedures, with females earning significantly more reinforcers than males; and, under choice procedures, with F344/N females, but not males, earning more cocaine reinforcers than sucrose prior to mesolimbic stimulation. The existing literature suggests that higher response levels in females may be due to a faster progression to habitual behavior, maintained on stimulus-response learning rather than the value of the consequence, in females compared to males (Schoenberg et al., 2019). Interestingly, female rats appeared to exhibit an increased reinforcing efficacy for high concentrations of sucrose solution (30% w/v) and an increased response vigor for sucrose concentrations up to 30% (w/v). While conducted in adolescent, rather than adult rats, Reichel et al., (2016) suggested that females exposed to sucrose daily exhibited higher breakpoints for a 15% sucrose solution. Regarding cocaine intake, it should be noted that differences in drug choice when both cocaine and sucrose are available were observed although males and females displayed similar rates of cocaine escalation. It is possible that sex differences in cocaine intake did not appear until after at least 12 days of daily cocaine intake and, therefore, repeating single-schedule cocaine testing after more extensive drug experiences may have revealed sex differences described in the literature. Additionally, the

current experiment observed similar levels of responding for both reinforcers, with all groups choosing approximately 23-68% cocaine (See Table 3.1.), although females were determined to have a greater chance of being a "high drug responder" than males. This is different from what was observed by Perry et al. (2013), reporting that food-preferring males and females chose food (45mg) close to 100% of the time, and cocaine-preferring rats chose IV cocaine (0.4 mg/kg) approximately 96% or the time. It should be noted that the animals described by Perry et al. (2013) were placed on choice procedures prior to single-schedule training and all animals increased responding for food pellets over choice sessions while only cocaine-preferring rats displayed an increase in responding for cocaine. The lack of cocaine experience prior to choice testing may be a source of differences between the results of Perry et al. (2013) and the current experiment, as animals, in the current experiment, all demonstrated escalation of cocaine-intake prior to choice testing.

Females may be more vulnerable to the changes to neural circuitry which occur with repeated drug use and differences in the state of the mesolimbic and surrounding circuits may be the reason that choice behavior was affected in the opposite direction by mesolimbic stimulation (lower reinforcer intake) than that observed in males. It is of note that sex differences in choice prior to and following treatment were present despite all rats displaying escalation of cocaine intake prior to choice testing. The hypothesis that female rats in the current experiment exhibited behaviors, and possibly neural states, which more closely characterize an addicted phenotype was supported by females exhibiting greater drug than non-drug intake prior to administration of C21, compared to males which did not exhibit such a preference for cocaine. Sex-dependent patterns of intake within the 1-hour testing sessions following mesolimbic stimulation also support that the activity of the

mesolimbic circuit is dependent on biological sex and/or the interaction between biological sex and drug use. It should be noted that while females were more likely to choose cocaine over sucrose prior to treatment, the choice behavior of females was not less sensitive to manipulation than that of males. This is of interest as many theories of addiction suggest that chronic exposure to drugs leads to "automatic" or "involuntary" drug-taking behavior. It is possible that stimulation of the mesolimbic circuit successfully reduced drug choice because females in the current experiment had not yet reached the threshold past which drug-taking becomes "automatic." However, stimulation of the mesolimbic circuit may also reduce drug-taking by overcoming the processes which drive "automatic" drug-taking. It should be determined by future researchers if the current treatment remains effective despite more evidence of addiction and/or drug exposure.

Our hypothesis that biological sex would interact with HIV-1 exposure was also confirmed and supports the current literature. Sex differences were often observed between F344/N males and females in single-schedule and choice procedures whereas HIV-1 Tg males and females exhibited relatively similar behaviors across single-schedule, but not choice, tasks. Additionally, the behavior of F344/N males and HIV-1 Tg animals was often not statistically different. These results support the current literature indicating that biological sex is a significant predictor of the display of apathy related to HIV-1 exposure. Specifically, reports from our lab have suggested that biological sex may moderate the influence of HIV-1 genotype on performance of signal detection tasks (McLaurin et al., 2017) and on accumbal MSN dendritic spine morphology (McLaurin et al., 2018a). In the current experiment, while HIV-1 Tg males exhibited slower reinforcement learning than F344/N males, the choice behavior of HIV-1 Tg males would not likely be considered

apathetic compared to F344/N males. Specifically, HIV-1 Tg males exhibited a stable choice for sucrose over cocaine under all choice conditions. It is of interest that mesolimbic stimulation in F344/N males elicited a similar statistically significant choice for sucrose over cocaine, although the pattern of intake across time was quadratic rather than linear. In contrast, HIV-1 Tg females displayed behaviors that could be characterized as consequences of HIV-1-related apathy (e.g., reduced goal-directed behavior) such as slower rates of reinforcement learning and reduced response vigor for sucrose and cocaine, consistent with the results of Bertrand et al., (2018) in F344/N and HIV-1 Tg ovariectomized rats. While McLaurin et al., (under review) also reported slower reinforcement learning in HIV-1 Tg ovariectomized rats, these animals were also reported to exhibit a higher response vigor for cocaine of the same dose used in the current experiment. The choice behavior reported by Bertrand et al., (2018) in ovariectomized rats also closely resembled that observed in F344/N and HIV-1 Tg females in the current experiment. F344/N females decreased sucrose intake and increased cocaine intake over the first 7 days of choice testing while HIV-1 Tg females displayed a lack of choice, not earning more cocaine than sucrose or vice versa on any of the 7 testing days. While the animals tested by McLaurin et al., (under review) did undergo choice testing according to the current procedures, censoring of choice data by removing those that did not meet choice criteria reduced sample sizes by approximately 50% and thus prevented reliable interpretations of data. Administration of C21 and presumed stimulation of the mesolimbic circuit in the current experiment's HIV-1 Tg females did reduce genotype differences in choice behavior compared to F344/N females. However, it is not clear if the reductions in genotype differences were indeed the result of mesolimbic stimulation as effects of C21

administration were also observed in sham HIV-1 Tg females. More so, closer examination of the data revealed that, similar to what was reported by McLaurin et al., (under review), 40% of HIV-1 Tg females did not meet the choice criteria of earning at least 2 of each reinforcer type. Although this finding does support a reduction in goal-driven behavior in HIV-1 Tg females, it prevents reliable interpretation of how mesolimbic stimulation may have altered such behavior. Thus, if the choice procedure is to be used to assess the efficacy of a treatment to alter choice behavior in HIV-1 Tg animals, it may be beneficial to increase choice response levels at baseline (by increasing reinforcer magnitude or changing reinforcer frequency) prior to introducing a treatment (Beckmann et al., 2019). A relative inability to alter choice behavior in HIV-1 Tg animals even following collection of data sufficient to make reliable interpretations would support our hypothesis that mesolimbic function and, particularly, its involvement in determining goal-directed behavior is altered in the presence of HIV-1 viral proteins. In contrast, the observation that S-equol can restore synaptodendritic integrity of MSNs suggests that S-equol may serve as a more promising, although less immediate, treatment strategy for mitigating symptoms of apathy in HIV-1+ populations (Bertrand et al., 2015; Moran et al., 2019; McLaurin et al., 2020a;McLaurin et al., 2020b).

4.2. Directions for Future Researchers

This, to our knowledge, is the first experiment to administer C21 and/or Sal B through the IV route of administration. Following IP administration, C21 has been reported to exert effects on inhibitory G(i) protein-coupled human M4 muscarinic DREADDs receptor activity between 60-180 minutes following administration (Thompson et al., 2018; Goutaudier et al., 2020) but that a high dose (3-5 mg/kg) of C21 remains relatively stable

in the brain following the first half-hour after administration (Jendryka et al., 2019; Thompson et al., 2018). Sal B has been reported to elicit its most prominent effects on feeding behavior approximately 60-90 minutes following subcutaneous administration (Vardy et al., 2015). It was hypothesized that effects of IV-administered C21 and Sal B would be observed sooner than IP-administered C21 and Sal B as IV administration bypasses the processes of first-pass metabolism by the liver. While the complete timecourse of C21 effects may not have been observed in the 42-minute single-schedule sessions or the hour-long choice sessions; graphing timecourse data did not reveal patterns of behavior that appeared to be changing rapidly surrounding the end of these sessions. Nevertheless, the influence of IV-administered C21 on sucrose intake in the single-schedule procedure was examined to attempt to determine the timecourse of effects on behavior, as choice behavior is more complex and less likely to provide clear results. The analysis and Figure 3.7. support that IV-administered C21 appeared to elicit a peak change in sucrose intake between minutes 21-28 following administration.

A primary concern of researchers interested in using chemogenetic techniques is the possibility of off-target effects of DREADDs ligand administration. For example, CNO is a DREADDs hM3D(Gq) receptor ligand which has been shown to elicit off-target effects on behavior in sham animals when it is metabolized to clozapine, an endogenously active substance (Jendryka et al., 2019). As was done in the current experiment, the effects of CNO in DREADDs animals should be considered in conjunction with those in sham animals to ensure that the described effects are not off-target effects of ligand administration, rather than the assumed manipulation of neural activity (Li et al., 2019). Similarly, a recent publication suggested that IP administration of C21 can elicit off-target

effects on neural activity in sham animals (Goutaudier et al., 2020). However, Goutaudier et al., (2020) also reported that the off-target effects observed following administration of 1 mg/kg C21 were mitigated following administration of a lower, 0.5 mg/kg dose of C21, both of which are higher than any of the doses tested in the current experiment. Some off-target effects of C21 administration were observed in sham F344/N females when tested under the single-schedule procedure and in sham HIV-1 Tg females when tested under the choice procedure and these should be taken into consideration when interpreting the corresponding effects in DREADDs F344/N and HIV-1 Tg females, respectively. Nevertheless, compared to optogenetic methods, chemogenetic methods are far less invasive and may eventually be available for use in humans through insufflation methods (Urban & Roth, 2015). Scientists should thus aim to continue optimizing chemogenetic techniques such as those used in the current experiment.

While the lack of C21-induced effects in sham control groups in the current experiment support that the observed influences of C21 administration on choice behavior were not due to off-target effects of C21, it may also be of future researchers to compare the results described here to those observed when mesolimbic circuit activity is altered by other means. For example, if the results observed in the current experiment are due to off-target effects of C21, it is unlikely that that the same results would be observed following administration of CNO. Similarly, although stimulation of the mesolimbic circuit in the current experiment was not specific to DA-positive neurons, we would expect that intracranial infusion of a DA receptor agonist into NAc would elicit similar results. Support for this hypothesis would support not only that the changes to choice behavior described in the current experiment are indeed due to hM3DG(q) stimulation and not off target effects

of C21, but also that said changes are due to stimulation of mesolimbic DA activity. Supporting the current results with those observed following intracranial infusion of a DA receptor agonist into the NAc would also support that the current results are not due to off-target effects of infecting neurons in a rat with human proteins (such as those reported by Johnston et al., 2020). However, both chemogenetic techniques and intracranial infusions require cannula placement within brain tissue which can cause damage to tissues between the region of interest and the dorsal surface of the skull. It is thus also of interest to compare the currently described results of chemogenetic stimulation of mesolimbic activity to results of mesolimbic stimulation initiated by means that do not require stereotaxic surgeries. Although methods that do not require cranial invasion, such as transcranial magnetic stimulation, do not provide the same regional specificity as chemogenetic or intracranial infusion, it is of interest to reproduce the current experiment using such methods to ensure that the results of the current experiment are not dependent on off-target effects of tissue damage which may have occurred in the current experiment. Additionally, the stress of single-housing animals after IV catheter implantation may be another confound within the current experiment (Engeln et al., 2020). Reproducing the current experiment in rats that are not single-housed would be difficult as external IV catheters can easily be damaged during interactions between animals. Repeating the current experiments in mice (as described by Engeln et al., 2020) that are pair housed throughout experimentation would help identify if the effects described in the current experiment are indeed dependent on the stress elicited by single-housing conditions.

While the current experiment did not include physiological methods to measure the activity of the mesolimbic circuit prior to and during DREADDs stimulation, it is unlikely

that the observed results are due to off-target effects, as they were not consistently observed in sham animals across tasks. More so the observed influence of C21 administration in DREADDs F344/N animals was blocked by the additional administration of Sal B approximately 15 minutes later. However, one shortcoming of the current experiment is that the specific neuron-type(s) (e.g. DA, Glu, and/or GABA-releasing) necessary for the observed changes to reinforcement intake were not identified. We can examine the literature and our results indicating that mCherry and mCitrine expression were primarily observed in the posterior parabrachial nucleus of the VTA to speculate on the specific cell-types which may be involved. The cells of the parabrachial nucleus of the VTA primarily project to the NAc shell (Lammel et al., 2014) and contains a high number of DA neurons relative to other neurotransmitter types (Goncalves et al., 2012). Likewise, the posterior VTA is reported to contain more DAergic cells than other cell types while the anterior VTA is reported to contain more GABAergic cells (Nair-Roberts et al., 2008). VTA GABAergic neurons that project to the NAc are reported to make inhibitory synapses on cholinergic interneurons of the NAc, but not MSNs or parvalbumin interneurons of the NAc (Brown et al., 2012). Importantly, Brown et al., (2012) also reported that stimulation of VTA GABA cells using optogenetic techniques elicited a pause in the activity of cholinergic interneurons of the NAc which may serve as a brief period when MSNs can be more easily influenced by DA inputs, facilitating (negative) reinforcement learning. Glu cells of the VTA are reported to form excitatory synapses with GABAergic parvalbumin interneurons of the medial NAc shell to indirectly inhibit MSN activity, and thus act against DA stimulation of DA activity (Dobi et al., 2010). Of note, another report suggested that antagonizing Glu cells of the posterior VTA reduces ethanol-seeking (Czachowski et al.,

2012), although these effects may have been the result of Glu actions outside of or in addition to the NAc. Nevertheless, Glu cells are primarily located in the anterior midline nuclei of the VTA supporting that cells in which expression was observed are most likely to be DA cells (Morales & Root, 2014). A report by Qi et al., (2016) suggests that cells of the VTA that co-express Glu and GABA do not project to the NAc, and thus would not have been able to receive Cre to elicit DREADDs expression.

Additionally, it is hypothesized based on the current results that stimulation of DA neurons of the VTA were primarily involved in altering choice behavior. However, it is not clear if group differences in the ability to alter choice behavior are due to group differences in the magnitude of the DA response assumed to be elicited by the current methods. It is also possible that group differences in the ability to alter choice behavior are due to differences in the composition of circuits surrounding and/or acting on the mesolimbic circuit that was targeted. Future researchers should consider determining if similar effects on choice behavior are indeed observed following stimulation of feedforward or feedback circuits that modulate VTA and or NAc activity (Bernard, 2020).

The behavioral consequences of stimulating hM3D(Gq) receptors were blocked with stimulation of KORD receptors in the same circuit. While not tested in the current experiment, it is of interest to determine if inhibiting the mesolimbic circuit with the administration of Sal B alone would elicit effects on choice behavior which directly oppose those effects observed following administration of C21 alone. Additionally, stimulation of the mesolimbic circuit is thought to promote goal-directed behavior and it is thus not surprising that the effects of stimulating the mesolimbic circuit may be more or less apparent depending on the value of the reinforcers available. It is currently unclear if the

consequences of inhibiting the mesolimbic circuit would also be dependent on the value of reinforcement available. Likewise, to better understand the functional consequences of the mesolimbic circuit, it is of interest to test the hypothesis that the effects of Sal B alone on reinforcer intake will not be as dependent on the availability of reinforcers as were the effects of C21.

Another aspect of the current experiment which should be noted is that while the choice between 0.2 mg/kg IV cocaine and 5% sucrose solution was probed, the current experiment did not test whether animals had a *preference* for cocaine or sucrose. Choices between reinforcers depend on various factors (e.g. "price", magnitude/dose, price of other available reinforcers, satiety) which were not manipulated in the current choice procedures. As such, repeating choice testing under different conditions (i.e., by manipulating one of the aforementioned factors) may reveal different patterns of choice behavior, sometimes even reversing choice (Beckmann et al., 2019). Notably, because behaviors are usually reallocated in a way that maximizes utility or minimizes deviations form a set point (Bickel et al., 1995), the absolute value of a reinforcer can be estimated using behavioral economics techniques to study how the preference among options is altered by various conditions. Thus, it should be emphasized that the current experiment utilized a choice procedure to assess between- and within-subjects differences in behavior and underlying reinforcement processes. While still of interest, the current experiment did not examine the relative value of cocaine and sucrose by determining parameters such as price elasticity, consumption with no price, or true break point. Nevertheless, sucrose magnitude was manipulated under the single-schedule FR(1) and PR procedures, and thus some insight is provided into how reinforcer magnitude may influence responding for sucrose in each group. Specifically,

dose-response curves represented in Figure 3.3. suggest that biological sex may be a significant predictor of the value of sucrose. However, it is not clear if the choice for sucrose in males and females will exhibit similar responses to changes in reinforcer magnitude nor if altering the price of a sucrose reinforcer will reveal different patterns of intake. Collectively, it is important for researchers positing that one reinforcer is preferred or valued over another to demonstrate that the reported preferences are not dependent on the chosen experimental parameters. Future researchers should aim to determine how biological sex and/or the presence of HIV-1 influences the value of or preference between cocaine and/or sucrose as such findings would have important implications regarding why certain populations may be vulnerable to dysregulation of reinforcement processing.

Overall, the current experiment utilized the choice procedure to identify hypothesized disruptions in reinforcement processing and to evaluate the role of the mesolimbic circuit in determining such behaviors. Generally, the current experiment demonstrated the utility of the choice procedure to identify factors that may render some individuals more vulnerable to dysregulation of reinforcement processing and supported that females may be more vulnerable to exhibiting disrupted reinforcement processing, such as drug dependence or apathy, compared to males. Additionally, the choice procedure can be utilized to assess the efficacy of treatments that aim to reduce less desirable choice behavior. Importantly, the current experiment also demonstrated that the activity of the mesolimbic circuit may be manipulated to alleviate some aspects of disrupted reinforcement processing and may be helpful to assess the efficacy of other proposed treatments, such as S-equol administration for apathy. Specifically, drug choice was reduced not only in F344/N females which exhibited greater choice for drug over sucrose

prior to stimulation, but also in males F344/N males which did not. Altering choice behavior to reduce harmful choice-making and/or increase desired choice-making could significantly inform treatments for psychobehavioral pathologies characterized by dysregulated reinforcement processing. While the exact mechanism by which drug choice was reduced is not yet clear, the current results support that the behavioral outcomes of the mesolimbic circuit's activity can be influenced by both the biological (e.g., biological sex, genotype) and environmental (single-schedule, choice) conditions under which said activity occurs. The current experiment has thus provided various hypotheses for future researchers interested in choice, addiction, apathy, or any behavioral pathology related to disrupted reinforcement processing and more so provides an adaptable and translational method through which such hypotheses can be tested.

References

Ahmed, S.H., Lenoir, M., & Guillem, K. (2013). Neurobiology of addiction versus drug driven by lack of choice. *Current Opinions in Neurobiology*, 23, 581-587. https://doi.org/10.1016/j.conb.2013.01.028

Amemori, S., Amemori, K., Yoshida, T., Papageorgiou, G.K., Xu, R., Shimazu, H., Desimone, R., & Graybiel, A.M. (2020). Microstimulation of primate neocortex targeting striosomes induces negative-decision-making. *European Journal of Neuroscience, 51*(3), 731-741. https://doi.org/10.1111/ejn.14555

Arbisi, P.A., Billington, C.J., & Levine, A.S. (1999). The effect of naltrexone on taste detection and recognition threshold. *Appetite, 32*, 241-249. https://doi.org/10.1006/appe.1998.0217

Banks, M.L., & Negus, S.S. (2017). Insights from Preclinical Choice Models on Treating Drug Addiction. *Trends in Pharmacological Sciences*, 38(2), 181-194. https://doi.org/10.1016/j.tips.2016.11.002

Becker, J.B. (2016). Sex differences in addiction. *Dialogues in Clinical Neuroscience*, 18(4), 395-402. https://doi.org/10.31887/DCNS.2016.18.4/jbecker

Beckmann, J.S., Chow, J.J., Hutsell, B.A. (2019). Cocaine-associated decision-making: Towards isolating preference. *Neuropharmacology*, 153, 142-152. https://doi.org/10.1016/j.neuropharm.2019.03.025

Berger, J.R., Kumar, M., Kumar, A., Fernandez, J.B., & Levin, B. (1994). Cerebrospinal Fluid Dopamine in HIV-1 Infection. *AIDS*, 8(1), 67-71. https://doi.org/10.1097/00002030-199401000-00010

Bernard, C. (2020). Optogenetics: Keep interpretations light. *eNeuro*, 7(2). https://doi.org/10.1523/ENEURO.0091-20.2020

Berridge, K.C. (1996). Food Reward: Brain Substrates of Wanting and Liking. *Neuroscience and Biobehavioral Reviews*, 20(1), 1-25. https://doi.org/10.1016/0149-7634(95)00033-B

Berridge, K.C., & Robinson, T.E. (1998). What Is the Role of Dopamine in Reward: Hedonic Impact, Reward Learning, or Incentive Salience? *Brain Research*, 28(3), 309-369. https://doi.org/10.1016/s0165-0173(98)00019-8

Berridge, K.C., Venier, I.L., & Robinson, T.E. (1989). Taste reactivity analysis of 6-hydroxydopamine-induced aphagia: implications for arousal and anhedonia hypotheses of dopamine function. *Behavioral Neuroscience, 103*, 36-45. https://doi.org/10.1037/0735-7044.103.1.36

Bertrand, S.J., Hu, C., Aksenova, M.V., Mactutus, C.F., Booze, R.M. (2015). HIV-1 Tat and cocaine mediated synaptopathy in cortical and midbrain neurons is prevented by the isoflavone Equol. *Frontiers in Microbiology*, 6, 894. https://doi.org/10.3389/fmicb.2015.00894

Bertrand, S., Mactutus, C., Harrod, S., Moran, L., & Booze, R. (2018). HIV-1 proteins dysregulate motivational processes and dopamine circuitry. *Scientific Reports*, 8, 7869. https://doi.org/10.1038/s41598-018-25109-0

Bickel, W.K., DeGranpre, R.J., & Higgins, S.T. (1995). The behavioral economics of concurrent drug reinforcers: a review and reanalysis of drug self-administration research. *Psychopharmacology, 118*(3), 250-259. https://doi.org/10.1007/BF02245952

Brown, M.T.C., Tan, K.R., O'Conner, E.C., Nikonenko, I., Muller, D., & Luscher, C. (2012). Ventral tegmental area GABA projections pause accumbal cholinergic interneurons to enhance associative learning. *Nature*, 492(7429), 452-456. https://doi.org/10.1038/nature11657

Calipari, E., Bagot, R., Purushothaman, I., Davidson, T., Yorgason, J., Pena, C., Walker, D., Pirpinias, S., Guise, K., Ramakrishnan, C., Deisseroth, K., & Nestler, E. (2016). In vivo imaging identifies temporal signature of D1 and D2 medium spiny neurons in cocaine reward. *Proceedings of the National Academy of Science of the United States of America* 113(10), 2726-2731. https://doi.org/10.1073/pnas.1521238113

Calipari, E.S., Juarez, B., Morel, C., Walker, D.M., Cahill, M.E., Ribeiro, E., Roman-Ortiz, C., Ramakrishnan, C., Deisseroth, K., Han, M.H., & Nestler, E.J. (2017). Dopaminergic dynamics underlying sex-specific cocaine reward. *Nature Communications*, 8, 13877. https://doi.org/10.1038/ncomms13877

Castellon, S.A., Hinkin, C.H., & Myers, H.F. (2000). Neuropsychiatric Disturbance is Associated with Executive Dysfunction in HIV-1 infection. *Journal of the International Neuropsychological Society*, 6(3), 336-347. https://doi.org/10.1017/S1355617700633088

Castellon, S.A., Hinkin, C.H., Wood, S., & Yarema, K.T. (1998). Apathy, Depression, and Cognitive Performance in HIV-1 Infection. *The Journal of Neuropsychiatry and Clinical Neurosciences*, 10(3), 320-329. https://doi.org/10.1176/jnp.10.3.320

Chang, L., Wang, G., Volkow, N., Ernst, T., Telang, F., Logan, J., & Fowler, J. (2008). Decreased brain dopamine transporters are related to cognitive deficits in HIV patients with or without cocaine abuse. *NeuroImage*, 42(2), 869-878. https://doi.org/10.1016/j.neuroimage.2008.05.011

Chaudhri, N., Caggiula, A.R., Donny, E.C., Booth, S., Gharib, M.A., Craven, L.A., Allen, S.S., Sved, A.F., & Perkins, K.A. (2005). Sex differences in the contribution of nicotine and nonpharmacological stimuli to nicotine self-administration in rats. *Psychopharmacology*, 180, 258-266. https://doi.org/10.1007/s00213-005-2152-3

Cole, M.A., Castellon, S.A., Perkins, A.C., Ureno, O.S., Robinet, M.B., Reinhard, M.J., Barclay, T.R., & Hinkin, C.H. (2007). Relationship Between Psychiatric Status and Frontal-Subcortical Systems in HIV-infected Individuals. *Journal of the International Neuropsychological Society*, 13(3), 549-554. https://doi.org/10.1017/S135561770707066X

Colwill, R. M., & Rescorla, R. A. (1985a). Postconditioning devaluation of a reinforcer affects instrumental responding. Journal of Experimental Psychology: *Animal Behavior Processes*, 11(1), 120–132. https://doi.org/10.1037/0097-7403.11.1.120

Colwill, R.M., Rescorla, R.A. (1985b). Instrumental responding remains sensitive to reinforcer devaluation after extensive training. *Journal of Experimental*

Psychology- animal behavior processes, 11(4), 520-536. https://doi.org/10.1037/0097-7403.11.4.520

Crockett, M.J., Clark, L., & Robbins, T.W. (2009). Reconciling the role of serotonin in behavioral inhibition and aversion: acute tryptophan depletion abolishes punishment-induced inhibition in humans. *Journal of Neuroscience, 29*(38), 11993-11999. https://doi.org/10.1523/JNEUROSCI.2513-09.2009

Cromwell, H.C., Tremblay, L., & Schultz, W. (2018). Neural encoding of choice during a delayed response task in primate striatum and orbitofrontal cortex. *Experimental Brain Research, 236*(6), 1679-1688. https://doi.org/10.1007/s00221-018-5253-z

Cysique, L.A., & Brew, B.J. (2019). Comorbid depression and apathy in HIV-associated neurocognitive disorders in the era of chronic HIV infection. In V.I. Reus & D. Lindqvist (Eds.), *Handbook of Clinical Neurology* (pp. 71-82). Psychopharmacology of Neurologic Disease.

Czachowski, C.L., Delory, M.J., & Pope, J.D. (2012). Behavioral and neurotransmitter specific roles for the ventral tegmental area in reinforcer-seeking and intake. *Alcoholism: Clinical and Experiment Research*, 36(10), 1659-1668. https://doi.org/10.1111/j.1530-0277.2012.01774.x

Day, J.J., Jones, J.L., Wightman, M., & Carelli, R.M. (2010). Phasic Nucleus Accumbens Dopamine Release Encodes Effort-and Delay-Related Costs. *Biological Psychiatry, 68*, 306-309. https://doi.org/10.1016/j.biopsych.2010.03.026

Denton, A., Samaranayake, S., Kirchner, K., Roscoe, R., Berger, S., Harrod, S., Mactutus, C.F., Hashemi, P., & Booze, R.M. (2019). Selective monoaminergic

and histaminergic circuit dysregulation following long-term HIV-1 protein exposure. *Journal of Neurovirology*, 25(4), 540-550. https://doi.org/10.1007/s13365-019-00754-x

Desplats, P., Dumaop, W., Smith, D., Adame, A., Everall, I., Letendre, S., Ellis, R., Cherner, M., Grant, I., & Masliah, E. (2013). Molecular and Pathologic Insights from Latent HIV-1 Infection in Human Brain. *Neurology*, 80(15), 1415-1423. https://doi.org/10.1212/WNL.0b013e31828c2e9e

Dickinson, A., Balleine, B. (1994). Motivational control of goal-directed action. *Animal Learning & Behavior*, 22, 1–18. https://doi.org/10.3758/BF03199951

Di Chiara, G. (1998). A motivational learning hypothesis of the role of mesolimbic dopamine in compulsive drug use. *Journal of Psychopharmacology*, 12(1), 54-67. https://doi.org/10.1177/026988119801200108

Di Rocco, A., Bottiglieri, T., Dorfman, D., Werner, P., Morrison, C., & Simpson, D. (2000). Decreased Homovanilic Acid in Cerebrospinal Fluid Correlates with Impaired Neuropsychologic Function in HIV-1-infected Patients. *Clinical Neuropharmacology*, 23(4), 190-194. https://doi.org/10.1097/00002826-200007000-00004

Dobi, A., Margolis, E.B., Wang, H.L., Harvey, B.K., & Morales, M. (2010). Glutamatergic and nonglutamatergic neurons of the ventral tegmental area establish local synaptic contacts with dopaminergic and nondopaminergic neurons. *Journal of Neuroscience*, 30, 218-229. https://doi.org/10.1523/JNEUROSCI.3884-09.2010

Dunlop, O., Bjorklund, R.A., Abdelnoor, M., & Myrvang, B. (1992). Five Different Tests of Reaction Time Evaluated in HIV Seropositive Men. *Acta Neurologica Scandinavica*, 86(3), 260-266. https://doi.org/10.1111/j.1600-0404.1992.tb05082.x

Du Plessis, S., Vink, M., Joska, J., Koutsilieri, E., Stein, D., & Emsley, R. (2014). HIV infection and the fronto- striatal system: a systematic review and meta- analysis of fMRI studies. *AIDS*, 28(6), 803-811. https://doi.org/10.1097/QAD.0000000000000151

Elbirt, D., Mahlab-Guri, K., Bezalel-Rosenberg, S., Gills, H., Attali, M., & Asher, I. (2015). HIV-associated neurocognitive disorders (HAND). *The Israel Medical Association Journal*, 17, 54-59.

Engeln, M., Fox, M.E., Lobo, M.K. (2020). Housing conditions during self-administration determine motivation for cocaine in mic following chronic social defeat stress. *Psychopharmacology*, 238, 41-45. https://doi.org/10.1007/s00213-020-05657-y

Everitt, B.J., & Robbins, T.W. (2005). Neural systems of reinforcement for drug addiction: from actions to habits to compulsion. *Nature Neuroscience*, 8(11), 1481- 1489. https://doi.org/10.1038/nn1579

Ferster, C.B. (1957). Concurrent Schedules of Reinforcement in the Chimpanzee. *Science*, 125(3257), 1090-1091. https://doi.org/10.1126/science.125.3257.1090

Ferster, C.B., & Skinner, B.F. (1957). *Schedules of Reinforcement*. Appleton-Century-Croft. http://dx.doi.org/10.1037/10627-000

Floresco, S. (2015). The nucleus accumbens: an interface between cognition emotion, and action. *Annual Review of Psychology, 66,* 25-52. https://doi.org/10.1146/annurev-psych-010213-115159

Friedman, A., Homma, D., Gibb, L.G., Amemori, K.I., Rubin, S.J., Hood, A.S., Riad, M.H., & Graybiel, A.M. (2015). A corticostriatal path targeting striosomes controls decision-making under conflict. *Cell*, 161(6), 1320-1333. https://doi.org/10.1016/j.cell.2015.04.049

Garcia, J., Kimeldorf, D.J., & Koelling, R.A. (1955). Conditioned aversion to saccharin resulting from exposure to gamma radiation. *Science, 122*, 157-158. https://doi.org/10.1126/science.122.3160.157

Goncalves, L., Sego, C., & Metzger, M. (2012). Differential projections from the lateral habenula to the rostromedial tegmental nucleus and ventral tegmental area in the rat. *Journal of Comparative Neurology*, 520(6), 1278-1300. https://doi.org/10.1002/cne.22787

Gorman, A., Foley, J., Ettenhofer, M., Hinkin, C., & van Gorp, W. (2009). Functional Consequences of HIV-Associated Neuropsychological Impairment. *Neuropsychology Review*, 19(2), 186-203. https://doi.org/10.1007/s11065-009-9095-0

Goulding, D., Kraft, A., Mouton, P.R., McPherson, C.A., Avdoshina, V., Mocchetti, I., & Harry, G.J. (2019). Age-related decrease in tyrosine hydroxylase immunoreactivity in the substantia nigra and region-specific changes in microglia

morphology in HIV-1 Tg rats. *Neurotoxicity Research*, 36(3), 563-582. https://doi.org/10.1007/s12640-019-00077-z

Goutaudier, R., Coizet, V., Carcenac, C., & Carnicalla, S. (2020). Compound 21, a two-edged sword with both DREADD-selective and off-target outcomes in rats. *PLoS One*, 15(9), e0238156. https://doi.org/10.1371/journal.pone.0238156

Guitart-Masip, M., Economides, M., Huys, Q.J.M., Frank, M.J., & Chowdhury, R. (2014). Differential, but not opponent effects of L-DOPA and citalopram on action learning with reward and punishment. *Psychopharmacology, 231*(5), 955-966. https://doi.org/10.1007/s00213-013-3313-4

Heaton, R., Clifford, D., Franklin, D.J., Woods, S., Ake, C., Vaida, F., Ellis, R.J., Letendre, S.L., Marcotte, T.D., Atkinson, J.H., Rivera-Mindt, M., Vigil, O.R., Taylor, M.J., Collier, A.C., Marra, C.M., Gelman, B.B., McArthur, J.C., Morgello, S., Simpson, D.M., McCutchan, J.A., Abramson, I., Gamst, A., Fennema-Notestine, C., Jernigan, T.L., Wong, J., Grant, I., & CHARTER Group. (2010). HIV-associated neurocognitive disorders persist in the era of potent antiretroviral therapy: CHARTER Study. *Neurology*, 75(23), 2087-2096. https://doi.org/10.1212/WNL.0b013e318200d727

Heyman, G.M. (2009). *Addiction: A disorder of* choice. Harvard University Press.

Huynh, Y.W., Thompson, B.M., Larsen, C.E., Buch, S., Guo, M., Bevins, R.A., & Murray, J.E. (2020). Male HIV-1 transgenic rats show reduced cocaine-maintained lever pressing compared to F344 wildtype rats despite similar baseline

locomotion. *Journal of the Experimental Analysis of Behavior*, 113, 468-484. https://doi.org/10.1002/jeab.586

Illenberger, J.M., Harrod, S.B., Mactutus, C.F., McLaurin, K.A., Kallianpur, A., & Booze, R.M. (2020). HIV infection and Neurocognitive Disorders in the Context of Chronic Drug Abuse: Evidence for Divergent Findings Dependent upon Prior Drug History. *Journal of NeuroImmune Pharmacology*, 47. https://doi.org/10.1007/s11481-020-09942-7

Ipser, J., Brown, G., Bischoff-Grethe, A., Connolly, C., Ellis, R., Heaton, R., Grant, I., & TMARC Group. (2015). HIV Infection Is Associated with Attenuated Frontostriatal Intrinsic Connectivity: A Preliminary Study. *Journal of the International Neuropsychological Society*, 21(3), 203-213. https://doi.org/10.1017/S1355617715000156

Jackson, L.R., Robinson, T.E., & Becker, J.B. (2006). Sex differences and hormonal influences in acquisition of cocaine in self-administration in rats. *Neuropsychopharmacology*, 31, 129-138. https://doi.org/10.1038/sj.npp.1300778

Javadi-Paydar, M., Roscoe, R.J., Denton, A., Mactutus, C., & Booze, R. (2017). HIV-1 and cocaine disrupt dopamine reuptake and medium spiny neurons in female rat striatum. *PloS One*, 12(11). https://doi.org/10.1371/journal.pone.0188404

Jendryka, M., Palchaudhuri, M., Ursu, D., van der Veen, B., Liss, B., Katzel, D. Nissen, W., & Pekcec, A. (2019). Pharmacokinetic and pharmacodynamic actions of clozapine-N-oxide, clozapine, and compound 21 in DREADD-based

chemogenetics in mice. *Scientific Reports*, 9, 4522. https://doi.org/10.1038/s41598-019-41088-2

Jentsch, J.D., & Taylor, J.R. (1999). Impulsivity resulting from frontostriatal dysfunction in drug abuse: implication for the control of behavior by reward-related stimuli. *Psychopharmacology*, 146(4), 373-390. http://dx.doi.org/10.1007/PL00005483

Johnston, S.T., Parylak, S.L., Kim, S., Mac, N., Lim, C.K., Gallina, I.S., Bloyd, C.W., Newberry, A., Saavedra, C.D., Novak, O., Goncalves, J.T., Gage, F.H., Shtrahman, M. (2020). AAV ablates neurogenesis in the adult murine hippocampus. *bioRxiv.* http://dx.doi.org/10.1101/2020.01.18.911362

June, H.L., Yang, A., Bryant, J.L., Jones, O., & Royal III, W. (2010). Vitamin A Deficiency and Behavioral and Motor Deficits in the HIV-1 Transgenic Rat. *Journal of Neurovirology*, 15(5-6), 380-389. http://dx.doi.org/10.3109/13550280903350200

Kamat, R., Brown, G., Bolden, K., Fennema-Notestein, C., Archibald, S., Marcotte, T., Letendre, S.L., Ellis, R.J., Woods, S.P., Grant, I., Heaton, R.K., & TMARC Group. (2014). Apathy is associated with white matter abnormalities in anterior, medial brain regions in persons with HIV infection. *Journal of Clinical and Experimental Neuropsychology*, 36(8), 854-866. http://dx.doi.org/10.1080/13803395.2014.950636

Kamat, R., Woods, S.P., Cameron, M.V., ludicello, J.E., & HIV Neurobehavioral Research Program (HNRP) Group. (2016). Apathy is Associated with Lower Mental and Physical Quality of Life in Persons Infected with HIV. *Psychology,*

Health, and Medicine, 21(7), 890-901. http://dx.doi.org/10.1080/13548506.2015.1131998

Kamat, R., Woods, S.P., Marcotte, T.D., Ellis, R.J., & Grant, I. (2012). Implications of apathy for everyday functioning outcomes in persons living with HIV infection. *Archives of Clinical Neuropsychology*, 27(5), 520-531. http://dx.doi.org/10.1093/arclin/acs055

Kanoski, S.E. (2012). Cognitive and neuronal systems underlying obesity. *Physiology and Behavior, 106*, 337- 344. http://dx.doi.org/10.1016/j.physbeh.2012.01.007

Kaspar, B.K., Vissel, B., Bengoechea, T., Crone, S., Randolph-Moore, L., Muller, R., Brandon, E.P., Schaffer, D., Verma, I.M., Lee, K.F., Heinemann, S.F., & Gage, F.H. (2002). Adeno-associated virus effectively mediates conditional gene modification in the brain. Proceedings of the National Academy of Sciences USA, 99(4), 2320-2325. http://dx.doi.org/10.1073/pnas.042678699

Kelley, A.E., Bakshi, V.P., Haber, S.N., Steininger, T.L., Will, M.J., & Zhang, M. (2002). Opioid modulation of taste hedonics within the ventral striatum. *Physiology and Behavior, 76*, 365-377. http://dx.doi.org/10.1016/S0031-9384(02)00751-5

Kerstetter, K.A., Ballis, M.A., Duffin-Lutgen, S., Carr, A.E., Behrens, A.M., & Kippin, T.E. (2012). Sex Differences in Selecting Between food and cocaine reinforcement are mediated by estrogen. *Neuropsychopharmacology*, 37, 2605-2614. http://dx.doi.org/10.1038/npp.2012.99

Khodr, C.E., Chen, L., Al-Harthi, L., & Hu, X. (2018). Aging alters voltage-gated calcium channels in prefrontal cortex pyramidal neurons in the HIV brain. *Journal of Neurovirology*, 24(1), 133-118. http://dx.doi.org/10.1007/s13365-017-0588-y

Koob, G., & Le Moal, M. (2001). Drug Addiction, Dysregulation of Reward, and Allostasis. *Neuropsychopharmacology*, 24(2), 97-129. http://dx.doi.org/10.1016/S0893-133X(00)00195-0

Koob, G., & Le Moal, M. (2008). Addiction and the brain antireward system. *Annual Review of Psychology*, 59, 29-53. http://dx.doi.org/10.1146/annurev.psych.59.103006.093548

Kosten, T.A., Gawin, F.H., Kosten, T.R., & Rounsaville, B.J. (1993). Gender differences in cocaine use and treatment response. *Journal of Substance Abuse Treatment*, 10(1): 63-66. http://dx.doi.org/10.1016/0740-5472(93)90100-G

Kumar, A., Ownby, R., Waldrop-Valverde, D., Fernandez, B., & Kumar, M. (2011). Human immunodeficiency virus infection in the CNS and decreased dopamine availability: relationship with neuropsychological performance. *Journal of Neurovirology*, 17(1), 26-40. http://dx.doi.org/10.1007/s13365-010-0003-4

Kuper, M., Rabe, K., Esser, S., Gizewski, E.R., Husstedt, I.W., Maschke, M., & Obermann, M. (2011). Structural Gray and White Matter Changes in Patients with HIV. *Journal of Neurology*, 258(6), 1066-1075. http://dx.doi.org/10.1007/s00415-010-5883-y

Lammel, S., Lim, B.K., & Malenka, R.C. (2014). Reward and aversion in heterogeneous midbrain dopamine system. *Neuropharmacology*, 76, 351-359. https://doi.org/10.1016/j.neuropharm.2013.03.019

Levesque, D., Gagnon, S., & Di Paolo, T. (1989). Striatal D1 dopamine receptor density fluctuates during the rat estrous cycle. *Neuroscience Letters, 98,* 345-350. https://doi.org/10.1016/0304-3940(89)90426-6

Li, M.D., Cao, J., Wang, S., Wang, J., Sarkar, S., Vigorito, M. Ma, J.Z., & Chang, S.L. (2013). Transcriptome Sequencing of Gene Expression in the Brain of the HIV-1 Transgenic Rat. *PloS ONE,* 8(3), e59582. https://doi.org/10.1371/journal.pone.0059582

Li, H., Illenberger, J.M., Cranston, M.N., Mactutus, C.F., McLaurin, K.A., Harrod, S.B., & Booze, R.M. (2019). Posterior ventral tegmental area-nucleus accumbens shell circuitry modulates response to novelty. *PloS ONE,* 14(3), e0213088. https://doi.org/10.1371/journal.pone.0213088

Levy, R., & Dubois, B. (2006). Apathy and the Functional Anatomy of the Prefrontal Cortex-Basal Ganglia Circuits. *Cerebral Cortex*, 16(7), 916-928. https://doi.org/10.1093/cercor/bhj043

Lopez, O.L., Smith, G., Meltzer, C.C., & Becker, J.T. (1999). Dopamine Systems in Human Immunodeficiency Virus-Associated Dementia. *Neuropsychiatry, Neuropsychology, and Behavioral Neurology,* 12(3), 184-192.

Lynch, W.J., & Carroll, M.E. (1999). Sex Differences in the Acquisition of Intravenously Self-Administered Cocaine and Heroin in Rats. *Psychopharmacology*, 144(1), 77-82. https://doi.org/10.1007/s002130050979

Mahler, S.V., Brodnik, Z.D., Cox, B.M., Buchta, W.C., Bentzley, B.S., Quintanilla, J. Cope, Z.A., Lin, E.C., Riedy, M.D., Scofield, M.D., Messinger, J., Ruiz, C.M., Riegel, A.C., Espana, R.A., & Aston-Jones, G. (2019). Chemogenetic Manipulations of Ventral Tegmental Area Dopamine Neurons Reveal Multifaceted Roles in Cocaine Abuse. *Journal of Neuroscience*, 39(3), 503-518. https://doi.org/10.1523/JNEUROSCI.0537-18.2018

Matt, S.M., Gaskill, P.J. (2019). Dopaminergic impact of cART and anti-depressants on HIV neuropathogenesis in older adults. *Brain Research*, 1723, 146398. https://doi.org/10.1016/j.brainres.2019.146398

Marchant, N.J., Whitaker, L.R. Bossert, J.M., Harvey, B.K., Hope, B.T., Kaganovsky, K., Adhikary, S., Prisinzano, T.E., Vardy, E., Roth, B.L., & Shaham, Y. (2016). Behavioral and Physiological Effects of a Novel Kappa-Opioid Receptor-Based DREADDs in Rats. *Neuropsychopharmacology*, 41(2), 402-409. https://doi.org/10.1038/npp.2015.149

Marie, N., Canestrelli, C., & Noble, F. (2012). Transfer of neuroplasticity from nucleus accumbens core to shell is required for cocaine reward. *PLoS One*, 7(1), e30241. https://doi.org/10.1371/journal.pone.0030241

Markou, A., & Koob, G.F. (1992). Construct-validity of a self-stimulation threshold paradigm-effects of reward and performance manipulations. *Physiology & Behavior, 51*(1), 111-119. https://doi.org/10.1016/0031-9384(92)90211-J

McIntosh, S., Sexton, T., Pattison, L., Childers, S., & Hemby, S. (2015). Neuropathological sequelae of Human Immunodeficiency Virus and Apathy: A Review of Neuropsychological and Neuroimaging Studies. *Neuroscience and Biobehavioral Reviews*, 55, 147-164. https://doi.org/10.1016/j.neubiorev.2015.04.008

McLaurin, K.A., Bertrand, S.J., Illenberger, J.I., Harrod, S.B., Mactutus, C.F., & Booze, R.M. (under review). HIV-1-induced apathy: Mitigation by the gut metabolite, S-Equol. *Scientific Reports*

McLaurin, K.A., Booze, R.M., Mactutus, C.F., & Fairchild, A.J. (2017). Sex Matters: Robust Sex Differences in Signal Detection in the HIV-1 Transgenic Rat. *Frontiers in Behavioral Neuroscience*, 11, 212. https://doi.org/10.3389/fnbeh.2017.00212

McLaurin, K., Cook, A., Li, H., League, A., Mactutus, C., & Booze, R. (2018a). Synaptic Connectivity in Medium Spiny Neurons of the Nucleus Accumbens: A Sex-Dependent Mechanism Underlying Apathy in the HIV-1 Transgenic Rat. *Frontiers in Behavioral Neuroscience*, 12, 285. https://doi.org/10.3389/fnbeh.2018.00285

McLaurin, K.A., Li, H., Booze, R.M., Fairchild, A.J., & Mactutus, C.F. (2018b). Unraveling individual differences in the HIV-1 transgenic rat: therapeutic efficacy

of methylphenidate. *Scientific Reports*, 8(1), 136. https://doi.org/10.1038/s41598-017-18300-2

McLaurin, K.A., Li, H., Booze, R.M., & Mactutus, C.F. (2018c). Disruption of Timing: NeuroHIV Progression in the Post-cART Era. *Scientific Reports*, 9, 287. https://doi.org/10.1038/s41598-018-36822-1

McLaurin, K.A., Li, H.L., Cook, A.K., Booze, R.M., Mactutus, C.F. (2020a). S-EQUOL: a neuroprotective therapeutic for chronic neurocognitive impairments in pediatric HIV. *Journal of Neurovirology*, 26(5), 704-718. https://doi.org/10.1007/s13365-020-00886-5

McLaurin, K.A., Moran, L.M., Booze, R.M., Mactutus, C.F. (2020b). Selective estrogen receptor beta agonists: a therapeutic approach for HIV-1 associated neurocognitive disorders. *Journal of Neuroimmune Pharmacology*, 15(2), 264-279. https://doi.org/10.1007/s11481-019-09900-y

Midde, N.M., Gomez, A.M., Harrod, S.B., & Zhu, J. (2011). Genetically expressed HIV-1 viral proteins attenuate nicotine induced-behavioral sensitization and alter mesocorticolimbic ERK and CREB signals in rats. *Pharmacology Biochemistry Behavior*, 98, 587-597. https://doi.org/10.1016/j.pbb.2011.03.013

Mohebi, A., Pettibone, J.R., Hamis, A.A., Wong, J.T., Vinson, L.T., Patriarchi, T., Tian, L., Kennedy, R.T., & Berke, J.D. (2019). Dissociable dopamine dynamics for learning and motivation. *Nature, 570*, 65-70. https://doi.org/10.1038/s41586-019-1353-6

Morales, M., & Root, D.H. (2014). Glutamate neurons within the midbrain dopamine regions. *Neuroscience,* 282, 60-68. https://doi.org/10.1016/j.neuroscience.2014.05.032

Moran, L.M., Aksenov, M.Y., Booze, R.M., Webb, K.M., & Mactutus, C.F. (2012). Adolescent HIV-1 Transgenic Rats: Evidence for Dopaminergic Alterations in Behavior and Neurochemistry Revealed by Methamphetamine Challenge. *Current HIV Research*, 10, 415-424.

Moran, L.M., Booze, R.M., Webb, K.M., & Mactutus, C.F. (2013). Neurobehavioral Alterations in HIV-1 Transgenic Rats: Evidence for Dopaminergic Dysfunction. *Experimental Neurology,* 239, 139-147. https://doi.org/10.1016/j.expneurol.2012.10.008

Moran, L.M., McLaurin, K.A., Booze, R.M., Mactutus, C.F. (2019). Neurorestoration of sustained attention in a model of HIV-1 associated neurocognitive disorders. *Frontiers in Behavioral Neuroscience*, 13, 169. https://doi.org/10.3389/fnbeh.2019.00169

Morgan, D., Liu, Y., & Roberts, D.C.S. (2006). Rapid and Persistent Sensitization to the Reinforcing Effects of Cocaine. *Neuropsychopharmacology*, 31, 121-128. https://doi.org/10.1038/sj.npp.1300773

Muller-Oehring, E.M., Hong, J., Hughes, R.L., Kwon, D., Bronte-Stewart, H.M., Poston, K.L., & Schulte, T. (2020). Alterations of Brain Signal Oscillations in Older Individuals with HIV Infection and Parkinson's Disease. *Journal of Neuroimmune Pharmacology*. https://doi.org/10.1007/s11481-020-09914-x

Nair-Roberts, R.G., Chatelain-Badie, S.D., Benson, E., White-Cooper, H., Bolam, J.P., & Ungless, M.A. (2008). Stereological estimates of dopaminergic, GABAergic, and glutamatergic neurons in the ventral tegmental area, substantia nigra and retrorubral field in the rat. *Neuroscience*, 152, 1024-1031. https://doi.org/10.1016/j.neuroscience.2008.01.046

Nickoloff-Bybel, E.A., Mackie, P., Runner, K., Matt, S.M., Khoshbouei, H., Gaskill, P.J. (2019). Dopamine increases HIV entry into macrophages by increasing calcium release via an alternative signaling pathway. *Brain, Behavior, and Immunity*, 82, 239-252. https://doi.org/10.1016/j.bbi.2019.08.191

Nicola, S.M., Surmeier, D.T., & Malenka, R.C. (2000). Dopaminergic modulation of neuronal excitability in the striatum and nucleus accumbens. *Annual Review of Neuroscience, 23*, 185-215. https://doi.org/10.1146/annurev.neuro.23.1.185

Nemeth, C.L., Glasper, E.R., Harrell, C.S., Malviya, S.A., Otis, J.S., & Neigh, G.N. (2014). Meloxicam Blocks Neuroinflammation, but Not Depressive-Like Behaviors, in HIV-1 Transgenic Female Rats. *PloS One*, 9(10), e108399. https://doi.org/10.1371/journal.pone.0108399

Norgren, R., & Pfaffmann, C. (1975). Pontine taste area in rat. *Brain Research, 91*(1), 99-117. https://doi.org/10.1016/0006-8993(75)90469-2

Oleson, E., Talluri, S., Childers, S., Smith, J., Roberts, D., Bonin, K., & Budygin, E. (2009). Dopamine uptake changes associated with cocaine self-administration. *Neuropsychopharmacology, 24*(5), 1174-1184. https://doi.org/10.1038/npp.2008.186

Pang, X., Panee, J., Liu, X., Berry, M.J., Change, S.L., & Chang, L. (2013). Regional variations of antioxidant capacity and oxidative stress responses in HIV-1 transgenic rats with and without methamphetamine administration. *Journal of NeuroImmune Pharmacology*, 8(3), 691-704. https://doi.org/10.1007/s11481-013-9454-8

Parvaz, M.A., Konova, A.B., Proudfit, G.H., Dunning, J.P., Malaker, P., Moeller, S.J., Maloney, T., Alia-Klein, N., & Goldstein, R.Z. (2015). Impaired neural response to negative prediction errors in cocaine addiction. *Journal of Neuroscience,* 35(5): 1872-1879. https://doi.org/10.1523/JNEUROSCI.2777-14.2015

Paul, R.H., Brickman, A.M., Hinkin, C., Bradford, N., Hinkin, C., Malloy, P.F., Jefferson, A.L., Cohen, R.A., Tate, D.F., & Flanigan, T.P. (2005). Apathy is associated with volume of the nucleus accumbens in patients infected with HIV. *Journal of Neuropsychiatry and Clinical Neuroscience,* 17(2), 167-171. https://doi.org/10.1176/appi.neuropsych.17.2.167

Perry, A.N., Westenbroek, C., & Becker, J.B. (2013). The Development of a Preference for Cocaine over Food Identifies Individual Rats with Addiction-Like Behaviors. *PloS ONE,* 8(11), e79465. https://doi.org/10.1371/journal.pone.0079465

Pessiglione, M., Seymour, B., Flandin, G., Dolan, R.J., & Frith, C.D. (2006). Dopamine-dependent prediction errors underpin reward-seeking behavior in humans. *Nature, 442*(7106), 1042-1045. https://doi.org/10.1038/nature05051

Purohit, V., Rapaka, R., & Shurtleff, D. (2011). Drugs of Abuse, Dopamine, and HIV-associated Neurocognitive disorders/HIV-associated Dementia. *Molecular Neurobiology*, 44(1), 102-110. https://doi.org/10.1007/s12035-011-8195-z

Qi J, Zhang S, Wang HL, Barker DJ, Miranda-Barrientos J, & Morales M. (2016). VTA glutamatergic inputs to nucleus accumbens drive aversion by acting on GABAergic interneurons. *Nature Neuroscience*, 19, 725-733. https://doi.org/10.1038/nn.4281

Reichel, C.M., Linkugel, J.D., & Bevins, R.A. (2008). Bupropion differential impacts acquisition of methamphetamine self-administration and sucrose-maintained behavior. *Pharmacology Biochemistry Behavior*, 89(3), 463-472. https://doi.org/10.1016/j.pbb.2008.02.002

Reid, W.C., Ibrrahim, W.G., Kim, S.J., Denaro, F., Casas, R., Lee, D.E., Maric, D., & Hammoud, D.A. (2016). Characterization of neuropathology in the HIV-1 transgenic rat at different ages. *Journal of Neuroimmunology*, 292, 116-125. https://doi.org/10.1016/j.jneuroim.2016.01.022

Reid, W., Sadowska, M., Denaro, F., Rao, S., Foulke, J., Hayes, N., Jones, O., Doodnauth, D., Davis, H., Sill, A., O'Driscoll, P., Huso, D., Fouts, T., Lewis, G., Hill, M., Kamin-Lewis, R., Wei, C., Ray, P., Gallo, R.C., & Reitz, M. (2001). An HIV-1 transgenic rat that develops HIV-related pathology and immunologic dysfunction. *Proceedings of the National Academy of Sciences*, 98(16), 9271-9276. https://doi.org/10.1073/pnas.161290298

Reichelt, A.C., Abbott, K.N., Westbrook, R.F., & Morris, M.J. (2016). Differential motivational profiles following adolescent sucrose access in male and female rats. *Physiology & Behavior*, 157, 13-19. https://doi.org/10.1016/j.physbeh.2016.01.038

Rescorla, R.A., Solomon, R.L. (1967). Two-process learning theory: Relationships between Pavlovian conditioning and instrumental learning. *Psychological Review*, 74(3), 151-182. https://doi-org.pallas2.tcl.sc.edu/10.1037/h0024475

Risacher, S.L., & Saykin, A.J. (2013). Neuroimaging Biomarkers of Neurodegenerative Diseases and Dementia. *Seminars in Neurology*, 33(4), 386-416. https://doi.org/10.1055/s-0033-1359312

Ritz, M.C., Lamb, R.J., Goldberg, S.R., & Kuhar, M.J. (1987). Cocaine receptors on dopamine transporters are related to self-administration of cocaine. *Science*, 237(4819), 1219-1223. https://doi.org/10.1126/science.2820058

Robinson, T.E., & Berridge, K.C. (2003). Addiction. *Annual Review of Psychology*, 54, 25-53. https://doi.org/10.1146/annurev.psych.54.101601.145237

Robinson, T.E., & Berridge, K.C. (2008). The incentive sensitization theory of addiction: some current issues. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *363*(1507), 3137-3146. https://doi.org/10.1098/rstb.2008.0093

Robinson, T., Carr, C., & Kawa, A. (2018). The Propensity to Attribute Incentive Salience to Drug Cues and Poor Cognitive Control Combine to Render Sign-

Trackers Susceptible to Addiction. In Morrow J & Tomie A (Eds.) *Sign Tracking and Drug Addiction* (pp. 75-105).

Roscoe, R., Mactutus, C., & Booze, R. (2014). HIV-1 Transgenic Female Rat: Synaptodendritic Alterations of Medium Spiny Neurons in the Nucleus Accumbens. *Journal of NeuroImmune Pharmacology*, 9, 642-653. https://doi.org/10.1007/s11481-014-9555-z

Rowson, S.A., Harrell, C.S., Bekhbat, M., Gangavelli, A., Wu, M.J., Kelly, S.D., Reddy, R., & Neigh, G.N. (2016). Neuroinflammation and Behavior in HIV-1 Transgenic Rats Exposed to Chronic Adolescent Stress. *Frontiers in Psychiatry*, 7, 102. https://doi.org/10.3389/fpsyt.2016.00102

Runegaard, A.H., Sorensen, A.T., Fitzpatrick, C.M., Jorgensen, S.H., Petersen, A.V., Hansen, N.W. Weikop, P., Andreasen, J.T., Mikkelsen, J.D., Perrier, J., Woldbye, S., Rickhag, M., Wortwein, G., & Gether, U. (2018). Locomotor- and Reward-Enhancing Effects of Cocaine are Differentially Regulated by Chemogenetic Stimulation of Gi-Signaling Dopaminergic Neurons. *eNeuro* 5(3), eneuro.0345-17.2018. https://doi.org/10.1523/ENEURO.0345-17.2018

Salamone, J. D., Cousins, M. S., & Bucher, S. (1994). Anhedonia or anergia? Effects of haloperidol and nucleus accumbens dopamine depletion on instrumental response selection in a T-maze cost/benefit procedure. *Behavioral Brain Research, 65*, 221–229. https://doi.org/10.1016/0166-4328(94)90108-2

Sanders, R.M. (1968). Note on Magnitude of a Monetary Reinforcer in a Concurrent Schedule of Reinforcement. *Psychological Reports*, 22, 407-414. https://doi.org/10.2466/pr0.1968.22.2.407

Sardar, A.M., Czudek, C., & Reynolds, G.P. (1996). Dopamine Deficits in the Brain: The Neurochemical Basis of Parkinsonian Symptoms in AIDs. *Neuroreport*, 7(4), 910-912. https://doi.org/10.1097/00001756-199603220-00015

Schoenberg, H.L., Sola, E., X., Seyller, E., Kelberman, M., & Toufe xis, D.J. (2019). Female rats express habitual behavior earlier in operant training than males. *Neuroscience*, 133(1), 110-120. https://doi.org/10.1037/bne0000282

Schultz, W. (2016). Dopamine reward prediction error coding. *Dialogues in Clinical Neuroscience, 18*(1), 23-32.

Schultz, W., Dayan, P., & Montague, P. (1997). A neural substrate of prediction and reward. *Science*, 275(5306), 1593-1599. https://doi.org/10.1126/science.275.5306.1593

Shah, S., Maric, D., Denaro, F., Ibrahim, W., Mason, R., Kumar, A., Hammoud, D.A., & Reid, W. (2019). Nitrosative Stress Is Associated with Dopaminergic Dysfunction in the HIV-1 Transgenic Rat. *American Journal of Pathology*, 189(7), 1375-1385. https://doi.org/10.1016/j.ajpath.2019.03.004

Siegel, S. (2005). Drug tolerance, drug addiction, and drug anticipation. *Current Directions in Psychological Science, 14*(6), 296-300. https://doi.org/10.1111/j.0963-7214.2005.00384.x

Sinharay, S., Lee, D., Shah, S., Muthusamy, S., Papadakis, G.Z., Zhang, X., Maric, D., Reid, W.C., & Hammoud, D.A. (2017). Cross-sectional and longitudinal small animal PET shows pre and post-synaptic striatal dopaminergic deficits in an animal model of HIV. *Nuclear Medicine and Biology*, 55, 27-33. https://doi.org/10.1016/j.nucmedbio.2017.08.004

Skinner, B. (1938). *The Behavior of Organisms: An Experimental Analysis*. Appleton-Century.

Smith, M.A. (2020). Nonhuman animal models of substance use disorders: Translational value and utility to basic science. *Drug and Alcohol Dependence*, 206, 107733. https://doi.org/10.1016/j.drugalcdep.2019.107733

Solomon, R.L., & Corbit, J.D. (1974). An opponent-process theory of motivation: 1. Temporal dynamics of affect. *Psychological Reviews*, 81, 119-145.

Sultana, S., Li, H., Puche, A., Jones, O., Bryant, J.L., Royal, III W. (2010). Quantitation of parvalbumin+ neurons and human immunodeficiency virus type 1 (HIV-1) regulatory gene expression in the HIV-1 transgenic rat: effects of vitamin A deficiency and morphine. *Journal of Neurovirology*, 16, 33-40. https://doi.org/10.3109/13550280903555712

Teeraananchai, S., Kerr, S.J., Amin, J. Ruxrungtham, K., & Law, M.G. (2016). Life expectancy of HIV-positive people after starting combination antiretroviral therapy: a meta-analysis. *HIV Medicine*, 18(4), 256-266. https://doi.org/10.1111/hiv.12421

Thompson, K.J., Khajehali, E., Bradley, S.J., Navarrete, J.S., Huang, X.P., Slocum, S. Jin, J., Liu, J., Xiong, Y., Olsen, R., Diberto, J., Boyt, K., Pina, M., Pati, D., Molloy, C., Bundgaard, C., Sexton, P., Kash, T., Krashes, M., Christopoulos, A., Roth, B., & Tobin, A. (2018). DREADD Agonist 21 is an effective agonist for Muscarinic-Based DREADDs in Vitro and in Vivo. *ACS Pharmacology and Translational Sciences*, 1(1), 61-72. https://doi.org/10.1021/acsptsci.8b00012

Urban, D.J., & Roth, B.L. (2015). DREADDs (designer receptors exclusively activated by designer drugs): chemogenetic tools with therapeutic utility. *Annual Review of Pharmacology and Toxicology*, 55, 399-417. https://doi.org/10.1146/annurev-pharmtox-010814-124803

Valcour, V., Watters, M.R., Williams, A.E., Sacktor, N., McMurtray, A., & Shikuma, C. (2008). Aging Exacerbates Extrapyramidal Motor Signs in the Era of HAART. *Journal of Neurovirology*, 14(5), 362-367. https://doi.org/10.1080/13550280802216494

Vanderschuren, L.J., & Everitt, B.J., (2004). Drug seeking becomes compulsive after prolonged cocaine self-administration. *Science*, 305, 1017-1019. https://doi.org/10.1097/00008877-200409000-00106

Van Reekum, R, Stuss, D., & Ostrander, L. (2005). Apathy: why care? *Journal of Neuropsychiatry and Clinical Neuroscience*, 17(1), 7-19.

Vardy, E., Robinson, J.E., Li, C., Olsen, R.H.J., DiBerto, J.F., Giguere, P.M., Sassano, F.M., Huang, X., Zhu, H., Urban, D.J., White, K.L., Rittiner J.E., Crowley, N.A., Pleil, K.E., Mazzone, C.M., Mosier, P.D., Song, J., Kash, T.L., Malanga, C.J.,

Krashes, M.J., & Roth, B.L. (2015). A New DREADD Facilitates the Multiplexed Chemogenetic Interrogation of Behavior. *Neuron*, 86, 936-946. https://doi.org/0.1016/j.neuron.2015.03.065

Volkow, N.D., & Fowler, J.S. (2000). Addiction, a disease of compulsion and drive: Involvement of the orbitofrontal cortex. Cerebral Cortex, 10:318–325. http://dx.doi.org/10.1093/cercor/10.3.318

Walker, K.A., & Brown, G.G. (2018). HIV-associated Executive Dysfunction in the Era of Modern Antiretroviral Therapy: A Systematic Review and Meta-Analysis. *Journal of Clinical and Experimental Neuropsychology*, 40(4), 357-376. http://dx.doi.org/10.1080/13803395.2017.1349879

Wang, G., Chang, L., Volkow, N.D., Telang, F., Logan, J., Ernst, T., & Fowler, J.S. (2004). Decreased Brain Dopaminergic Transporters in HIV-associated Dementia Patients. *Brain*, 127(11), 2452-2458. http://dx.doi.org/10.1093/brain/awh269

Wayman, W.N., Chen, L., Hu, X., & Napier, T.C. (2016). HIV-1 Transgenic Rat Prefrontal Cortex Hyper-Excitability Is Enhanced by Cocaine Self-Administration. *Neuropsychopharmacology*, 41(8), 1965-1973. http://dx.doi.org/10.1038/npp.2015.366

Wise, R. & Koob, G. (2014). The Development and Maintenance of Drug Addiction. *Neuropsychopharmacology*, 39(2), 254-262. https://doi.org/10.1038/npp.2013.261

Wise, R., & McDevitt, R. (2018). Drive and Reinforcement Circuitry in the Brain: Origins, Neurotransmitters, and Projection Fields. *Neuropsychopharmacology, 43*(4), 680-689. https://doi.org/10.1038/npp.2017.228

Wolffgramm, J. & Heyne, A. (1995). From controlled drug intake to loss of control- the irreversible development of drug-addiction in the rat. *Behavioral Brain Research*, 70, 77-94. https://doi.org/10.1016/0166-4328(95)00131-C

Woods, S.P., Moore, D.J., Weber, E., & Grant I. (2009). Cognitive neuropsychology of HIV-associated neurocognitive disorders. *Neuropsychology Review*, 19, 152-168. https://doi.org/10.1007/s11065-009-9102-5

Yang, Z., Nesil, T., Connaghan, K.P., Li, M.D., & Chang, S.L. (2016). Modulation effect of HIV-1 viral proteins and nicotine on expression of the immune-related genes in brain of the HIV-1 transgenic rats. *Journal of NeuroImmune Pharmacology*, 11(3), 562-571. https://doi.org/10.1007/s11481-016-9679-4

Yao, W., Spealman, R.D., & Zhang, J. (2008). Dopaminergic signaling in dendritic spines. *Biochemical Pharmacology, 75*(11), 2055-2069. https://doi.org/10.1016/j.bcp.2008.01.018

Zhu, H., & Roth, B.L. (2015). DREADD: a chemogenetic GPCR signaling platform. *International Journal of Neuropsychopharmacology*, 18(1). https://doi.org/10.1093/ijnp/pyu007

Zhu, J., Yuan, Y., Midde, N.M., Gomez, A.M., Sun, W., Quizon, P.M., & Zhan, C. (2016). HIV-1 Transgenic Rats Display an Increase in [(3)H]dopamine uptake in the Prefrontal Cortex and Striatum. *Journal of Neurovirology*, 22(3), 282-292. https://doi.org/10.1007/s13365-015-0391-6

Appendix A. SAS Code

```
*This code determines the number of missing observations and total observations in data from each of the 4 groups;

proc means data=F344Males nmiss n; run;

proc means data=HIVMales nmiss n; run;

proc means data=F344Females nmiss n; run;

proc means data=HIVFemales nmiss n; run;

*This code determines the number of missing observations and total observations in the censored data from each of the 4 groups;

proc means data=F344MalesCensored nmiss n; run;

proc means data=HIVMalesCensored nmiss n; run;

proc means data=F344FemalesCensored nmiss n; run;

proc means data=HIVFemalesCensored nmiss n; run;

*This code is used to run mix-model analyses on the censored data from each of the 4 groups;

Proc mixed data=F344MalesCensored;

Class animal DOB surgery ligand reward;

model intake = surgery surgery*ligand ligand*reward surgery*ligand*reward/noint solution;

random animal ligand(animal) reward(ligand);

run;

Proc mixed data=HIVMalesCensored;

Class animal DOB surgery ligand reward;

model intake = surgery surgery*ligand ligand*reward surgery*ligand*reward/noint solution;

random animal ligand(animal) reward(ligand);
```

```
run;

Proc mixed data=F344FemalesCensored;

Class animal DOB surgery ligand reward;

model intake = surgery surgery*ligand ligand*reward surgery*ligand*reward/ noint
solution;

random animal ligand(animal) reward(ligand);

run;

Proc mixed data=HIVFemalesCensored;

Class animal DOB surgery ligand reward;

model intake = surgery surgery*ligand ligand*reward surgery*ligand*reward/noint
solution;

random animal ligand(animal) reward(ligand);

run;
```