Structural, Functional, And Behavioral Alterations To The Dopamine System In The Female HIV-1 Transgenic Rat

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STRUCTURAL, FUNCTIONAL, AND BEHAVIORAL ALTERATIONS TO THE
DOPAMINE SYSTEM IN THE FEMALE HIV-1 TRANSGENIC RAT

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DEDICATION

I would like to dedicate this document to my loving parents and the sacrifices they have made to make my dreams a reality. I would also like to dedicate this work to all the individuals who have suffered or will suffer from HIV-1 infection in the past, present, and future.
ACKNOWLEDGEMENTS

I would like to acknowledge the contributions of my lab mates, including Landhing Moran, Sarah J. Bertrand, Kristen McLaurin, Michael N. Cranston, Alex Steiner, Adam Denton, Hailong Li, Marina Aksenova, and all the temporary undergraduate supporters throughout the years. Without this team of people, the work herein would not have come to fruition. I would also like to acknowledge the incredible accomplishments of my mentor, Dr. Rosemarie M. Booze, and her husband, Dr. Charles F. Mactutus. The pleasure of learning from these top caliber scientists will follow me for the rest of my life.
ABSTRACT

HIV-associated cognitive disorders continue to affect approximately 25 million individuals worldwide, and its prevalence is expected to increase as the lifespan of HIV-1 infected individuals continues to improve. The HIV-1 transgenic rat expresses 7 of the 9 genes that encompass the virus, and is an appropriate model for studying chronic HIV-1 infection at a level that is controlled through combined antiretroviral therapy. Psychostimulant abuse is known to exacerbate HAND symptomology, but the psychostimulant methylphenidate is a first line of treatment for HIV associated cognitive inhibition. Dopamine transport abnormalities in the HIV-1 Tg rat have been well characterized through behavioral testing; however, this study is the first to analyze real-time dopamine transport differences via fast scan cyclic voltammetry. Dopamine release from the nucleus accumbens is greatly diminished in HIV-1 transgenic rats, especially in females. Acquisition rates of oral self-administration of methylphenidate in female HIV-1 Tg rats are increased compared to control rats, and this phenomenon is mirrored in locomotor activity following self-administration. Additionally, MPH has an equalizing effect on dendritic spine morphology of the nucleus accumbens in both groups of rats, and causes a slight relative increase in the length and head diameter of spines on pyramidal neurons of the prefrontal cortex. These findings suggest abuse lability for methylphenidate among individuals with HIV-1 infection.
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CHAPTER 1

GENERAL INTRODUCTION
Although advances in combined antiretroviral therapy have reduced global
mortality rates from HIV-1 infection, approximately 50% of infected individuals will
develop some degree of HIV-associated neurocognitive impairment (HAND) in
adulthood (Heaton et al., 2015; Joseph et al., 2013). The associated decrease in mortality
has led to HIV becoming better defined as a chronic illness, and as of 2014
approximately 47 million individuals are living with the disease, with 2 million of these
patients being between the ages of 10-19 years old (UNICEF). Moreover, prenatally
infected HIV patients are thought to have at least the same rate and susceptibility to
psychiatric disorders than the general population, while rates of prescribed
psychostimulant therapies are increased compared to peers (Gadow et al., 2010). HIV-1
infected individuals are also more susceptible to illicit psychostimulant abuse (Blackstone
et al., 2013; Dunne et al., 2014), which in turn increases the spread of infection and may
lead to a poorer neurocognitive outcome in HAND (Blackstone et al., 2013).

Dopaminergic disruption occurs in the absence of psychostimulant abuse (Chang
et al., 2008) and is associated with neuronal degeneration (Chana et al., 2006) and
reduction in DAT availability in human patients (Chang et al., 2008). Alterations to
dopamine terminals are compound-specific (Calipari et al., 2014) and HAND
symptomology is attenuated by cocaine and methamphetamine use (Anderson et al.,
2015; Buch et al., 2011; Dahal et al., 2015; di et al., 2000; Hoefer et al., 2015). Use of both
of these compounds reduces propensity for neocortical and NAc neurons to regulate
structural plasticity (Kolb et al., 2003), and interaction with HIV-1 attenuates behavioral
alterations of dopamine-dependent behavioral systems (Kolb et al., 2003; Liu et al.,
2009a; Liu et al., 2014; Maragos et al., 2002; Moran et al., 2012; Moran et al., 2013b).
However, HIV-1 infected individuals suffering from HIV-1 associated fatigue may be prescribed the C-II therapeutic methylphenidate (MPH), a dopamine transporter (DAT) inhibitor and dopamine agonist/CNS stimulant (Challman and Lipsky, 2000). MPH is typically used to treat inattentive/hyperactive subtypes of attention deficit hyperactivity disorder (ADHD) (Challman and Lipsky, 2000), but is also used in the HIV population to combat apathy (Kamat et al., 2015) and improve symptoms of cognitive slowing (Hinkin et al., 2001). Regardless, the possible increased susceptibility to psychostimulant abuse in adulthood because of MPH prescription or abuse during adolescence is considerably more consequential to the HIV-1+ adolescent population. Moreover, MPH exposure during adolescence in mice exhibits a sex-specific effect, with female mice exhibiting increased sensitization to illicit psychostimulants over male mice (Shanks et al., 2015).

Psychostimulant attenuation of disease severity has been established in animal models (Moran et al., 2012; Moran et al., 2013b; Webb et al., 2010; Blackstone et al., 2013; Liu et al., 2009a; Liu et al., 2014) and in human patients (Kesby et al., 2015; Meade et al., 2015; Panee et al., 2015), but most exploration includes the use of illicit psychostimulants (cocaine, methamphetamine) instead of the prescription medication methylphenidate. Methylphenidate remains the standard primary treatment in children suffering from ADHD (Challman and Lipsky, 2000); however, studies of adults have shown a decrease in ADHD symptomology during HIV-1 coinfection (Kamat et al., 2014; Kamat et al., 2015), further supporting HIV-1 Tat disruption of dopamine transport. Methylphenidate also binds to the dopamine transporter (DAT) and inhibits reuptake similarly to cocaine but has distinctly different pharmacokinetics, as indicated by a reduced clearance rate at the binding site (Challman and Lipsky, 2000; Clemow,
Cognitive performance in HIV-1 infected adults indicates possible uses for methylphenidate to combat cognitive slowing and forgetfulness (Hinkin et al., 2001; Vance et al., 2013), however self-administration of MPH in rodents causes an intake-pattern independent of priming of the dopamine system similar to that of amphetamines (Calipari and Jones, 2014). Conclusions drawn by Calipari and Jones (2014) suggest a sensitized response to amphetamines after intermittent MPH self-administration, possibly opening the user to future psychostimulant susceptibilities and addiction (Calipari and Jones, 2014). The fact that MPH is prescribed to this population is troubling considering known dopaminergic deficits caused by shed viral proteins. Additionally, MPH abuse remains stable among college students (Clemow, 2015) while attention has shifted to comorbid alcohol abuse specifically in the college-age population (Jain and Stark, 2016). Comorbid substance abuse investigation must be followed up by comorbid psychiatric medication use, especially in the adolescent HIV-1 population, as it may be more susceptible to illicit stimulant abuse later in adulthood.

Analysis of post-mortem brains of HIV-1 infected patients has revealed decreases in dopamine and homovanilic acid in many DA rich regions including the prefrontal cortex and basal ganglia (Kumar et al., 2009). Executive function in living patients is seriously diminished, (di Rocco et al., 2000) and interactions with cocaine are thought to exacerbate this reduction in dopaminergic throughput (Buch et al., 2011; Purohit et al., 2011; Kumar et al., 2011). PET scans of living adults show decreased DA transporter bioavailability in the putamen and ventral striatum, with reductions in quantity slightly correlated to nadir CD4+ cell count (Wang et al., 2004). Viral burden is highest in the
basal ganglia (Kure et al., 1990) and neurocognitive impairment shows a slight positive correlation to viral load (Wang et al., 2004). Analysis of post-mortem brain tissue also reveals significant reductions in total dopamine concentration among HIV-1+ individuals in the caudate, putamen, and substantia nigra (Kumar et al., 2009). Obermann et al., (2009) used ultrasound to image the brain and found hyperechogenicity in the substantia nigra of HIV-1+ patients is positively correlated to impairment determined by the psychopathology assessment scale; the hyperechogenicity also significantly negatively correlated with nadir CD4 counts (Obermann et al., 2009). More recent neurocognitive assessment of HIV-1 infected individuals on cART revealed decreases in temporal memory ability compared to uninfected patients (Woods et al., 2013). The pharmacokinetics of MPH include a relatively long binding time to the DAT compared to illicit psychostimulants (Challman and Lipsky, 2000), but the molecular interactions, and the resulting behavior, of this drug with HIV-1 Tat allosteric modulation of the DAT have not been explored in either a human or animal model of HIV-1 infection. As age and comorbid psychostimulant use are high predictors of HAND severity, it is critical for the adolescent population that interactions of Tat and MPH be explored to determine possible contraindications for this therapy.

The current HIV-1 transgenic rat in the F344 background expresses 7 of the 9 HIV genes placed on one copy of chromosome 9, therefore mimicking viral protein expression without an active state of viremia, and is indicative of HIV-1 infection in the era of combined antiretroviral therapy (Peng et al., 2010; Reid et al., 2001). HIV-1 transgenic rats exhibit extracellular hyperdopaminergia (Ferris et al., 2009c; Ferris et al., 2010), and indeed dopaminergic tone shift in HIV-1 transgenic rats has been functionally
correlated with alterations in perceptual gating determined through prepulse inhibition (PPI) of the auditory startle response (ASR) (Moran et al., 2013b; Roscoe, Jr. et al., 2014; Moran et al., 2013a). Methamphetamine challenge to the dopamine system revealed altered responses to percent PPI as a function of dose in adolescent HIV-1 transgenic rats relative to controls, highlighting the altered functionality of dopamine transport in the HIV-1 Tg rat (Moran et al., 2012). Brains of HIV-1 Tg rats contain increases in phosphorylated tyrosine hydroxylase expression and decreased DAT mRNA expression (Webb et al., 2010). Throughout the lifespan of the rat, inhibitions to temporal gating persist, (Moran et al., 2013) indicating that damage done to the brain is consistent with early developmental effects that require a rescue event to achieve normal functioning.

HIV-1 transgenic rats experience behavioral sensitivity to cocaine stimulation in conjunction with alterations to precortical synapses (McIntosh et al., 2015). Behavioral sensitization in HIV-1 transgenic rats also occurs with low/moderate methamphetamine exposure (Liu et al., 2009b). However, rats treated with MPH in adolescence decrease cocaine self-administration in adulthood, and this change is associated with increased D2 receptor availability in the striatum (Thanos et al., 2007). In the striatum, competitive binding at the same active site occurs on the dopamine transporter for both cocaine and MPH (Challman and Lipsky, 2000; Volkow et al., 1995), but the interaction with MPH in DAT inhibited animals resulting from HIV-1 infection has not yet been explored. Lee et al (2014) used PET tomography to show decreases in striatal volume in HIV-1 transgenic rats compared to control rats, but only as a function of age; younger animals exhibit less-severe alterations to brain volumetrics compared to adult rats (Lee et al., 2014).
Therefore, increasing the severity of neurological insult during adolescence may exacerbate future neurocognitive impairment in adulthood. As methylphenidate is most often prescribed in childhood for attention deficits (Challman and Lipsky, 2000), it is necessary to analyze alterations to this system from an abusive perspective in an attempt to characterize alterations to the dopaminergic potential of the basal ganglia (Kalivas, 2001; Kalivas, 2003; Kalivas, 2004) that results in addiction.

Synaptodendritic complexity of prefrontal connections to the NAc via MSNs is believed to be the main neural network involved in reward-based seeking and reinforcement, including drug addiction (Kalivas, 2001; Kalivas, 2003; Kalivas, 2004). The nucleus accumbens is believed to be responsible for regulating reward-based behavioral sensitization (Steketee et al., 1992; Kalivas and Duffy, 1990). Analysis of post-mortem brains of HAND sufferers shows significant cortical and subcortical degeneration is negatively correlated to neuropsychological impairment in the midfrontal region and alters hippocampus connectivity (Moore et al., 2006). Human brain tissue presents with dendritic simplification, especially reduction of branching orders, determined through MAP-2 staining (Masliah et al., 1997). Neuronal connectivity and branch complexity is also reduced in the HIV-1 transgenic female rat and accompanies alterations to dendritic spine morphology (Roscoe, Jr. et al., 2014), indicating reductions of neuronal complexity as the functional counterpart to neurocognitive impairment in HIV-1 infection.

Sublethal neurodegeneration in HIV-1 infection occurs with comorbid opioid use (Fitting et al., 2010) and cocaine addition (Bertrand et al., 2015) in preclinical models. Cortical degeneration is significantly reduced in midfrontal regions and hippocampus
(Moore et al., 2006), and HIV-1 transgenic rats experience cocaine-mediated hyperexcitability of PFC neurons (McIntosh et al., 2015; Wayman et al., 2015) and changes in striatal DAT binding (McIntosh et al., 2015). However, cocaine-induced alteration to medium spiny neuron pathology is pharmacologically distinct and alters potential for synaptic connection (Khibnik et al., 2016). Analysis of dopamine transport using FAST-scan cyclic voltammetry has revealed a significant alteration to DAT functioning in HIV-1 transgenic rat striatal slices (Javadi-Paydar et al., in press). Additionally, methamphetamine-induced synaptic pathology results in a pruning of dendritic arbors and decreases in presynaptic quality and quantity (Sanchez et al., 2016). While cocaine and methamphetamine use has been characterized in terms of dendritic simplification comorbid effects, MPH abuse and MPH-specific psychostimulant alterations have not been explored in a model of HIV-1 infection. The sublethal comorbid effects of Tat and low doses of psychostimulants, which results in dendritic simplification, is of concern to adolescent HIV-1 sufferers who are prescribed MPH as a therapeutic for ADHD and adult HIV-1 sufferers who are prescribed the drug for apathy.

Analysis of gender influence of HIV-1 infection on neurocognition has been sparse, considering women make up over 50% of global infection rates (UNAIDS 2015). Female-specific pathology in HIV-1 has only been recently explored in preclinical models (Roscoe Jr et al., 2014), and phytoestrogenic compounds show promising effectiveness in reducing cocaine-mediated synaptopathy associated with HIV-1 Tat with an overall minor improvement in motivational behavior (Bertrand et al., 2015). Following seroconversion of HIV-1, females exhibit increased levels of peripheral inflammation resulting from innate differences in the immunologic response (Collazos et
al., 2007; Addo and Altfeld, 2014). It is currently unknown if this innate immune response to HIV-1 infection is associated with decreased neurocognitive performance, as neurocognitive batteries administered to both males and females show HIV-1 sufferers exhibit comparable levels of impairment. These detriments may be sex and genotype specific, as HIV-1 infected women that exhibit a Val58Met single nucleotide polymorphism in co-methyl transferase (COMT) display worse neurocognitive performance compared to uninfected Val/Val individuals (Sundermann et al., 2015). As mentioned, the quantity of individuals prescribed medication in this group is higher than average (Gadow et al., 2010) and, given our previous work regarding the interference of HIV-1 Tat at the site of the dopamine transporter (Moran et al., 2012; Zhu et al., 2011; Zhu et al., 2015; Ferris et al., 2009d; Ferris et al., 2009a), the proposed idea investigates the vulnerability to the DA transport system in the brain when exposed to a commonly prescribed DAT inhibitor, methylphenidate. Additionally, determination of gender-specific effects or interactions may elucidate sex-specific pharmacology of methylphenidate in both the control F344 and HIV-1 transgenic male and female rats. Due to the recent inclusion of sex as a biological variable for NIH funded investigation, further sex-specific effects will be indications of high-caliber research.
CHAPTER 2

HIV-1 TRANSGENIC FEMALE RAT: SYNAPTODENDRITIC ALTERATIONS
OF MEDIUM SPINY NEURONS IN THE NUCLEUS ACCUMBENS

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INTRODUCTION

A large proportion of individuals infected with HIV experience HIV-associated neurocognitive disorders (HAND), even in long-standing aviremic patients (Woods et al., 2009; Winston et al., 2013; Alfahad and Nath, 2013). Patients with latent HIV-1 infection (i.e., HIV+ without HIV-1 viral RNA or p24 present), which display mild to moderate neurocognitive dysfunction, show decreased expression of the presynaptic protein, synaptophysin and the dendritic microtubule activation protein 2, throughout the frontal cortex (Desplats et al., 2013). Thus, in the absence of active infection or viral replication, long-lived, transcriptionally silent, HIV-1 proviruses in the brain may produce synaptodendritic injury in HAND, yet the mechanism(s) of HAND synaptopathy remain unclear. Interestingly, the production of HIV-1 proteins, such as Tat, (produced during the early phase of transcription from proviral DNA) is, for the most part, unaffected by antiretroviral drugs. Tat protein is found in the CSF of aviremic patients (Johnson et al., 2013), suggesting a role for HIV-1 proteins/Tat in producing synaptodendritic injury in HAND.

Indeed, synaptodendritic injury has been observed in response to HIV-Tat protein. Tat protein induced synaptodendritic damage is highly specific and dependent upon the presence of the cysteine region of the Tat protein (aa 21-32) (Bertrand et al., 2013). Moreover, Tat-induced synaptodendritic damage occurs prior to cell death, at very low Tat concentrations, and may be reversible (Bertrand et al., 2014). Synaptic alterations in pyramidal cells of the hippocampus (Fitting et al., 2013), as well as decreased spine density in medium spiny neurons (Fitting et al., 2010), have also been reported in mice conditionally expressing the HIV-1 Tat protein in astrocytes. Interestingly, gender
differences have been reported in mice conditionally expressing the HIV-1 Tat protein (Hahn et al., 2013). Likewise, gp120 expressing mice demonstrate synaptic dysfunction (Gorantla et al., 2012; Toggas et al., 1994; Kang et al., 2010). Collectively, these single protein (Tat or gp120) transgenic mice suggest that acute exposure to individual HIV-1 proteins affects synaptodendritic processes; however, synaptodendritic processes have not been studied in chronic multi-HIV protein exposures, such as in the HIV-1 Tg rat, which expresses 7 of the 9 HIV-1 proteins including Tat and gp120 (Reid et al., 2001), without viral replication, similar to the state seen in aviremic patients (Peng et al., 2010).

Shifts in dendritic spine density/alterations in spine morphologies often reflect impaired neuronal processing capacity and adverse neurocognitive outcomes (Mancuso et al., 2012; Ovtscharoff, Jr. et al., 2008; Shen et al., 2009). Medium Spiny Neurons (MSNs) are the major inhibitory projection neurons in the core region of the nucleus accumbens (NAcc) (Gangarossa et al., 2013). MSNs are characterized by particularly high densities of dendritic spines (Cheng et al., 1997) and play a key role in many motivational and reward-related behaviors (Enoksson et al., 2012; Shen et al., 2009). Dysfunction of the NAcc is associated with apathy (Levy and Dubois, 2006), depression (Nestler and Carlezon, Jr., 2006) and drug addiction (Lesscher and Vanderschuren, 2012) which are often co-morbid with HAND (Alfahad and Nath, 2013; Weber et al., 2013). Thus, alterations in MSN dendritic spines may play an important role in HAND.

To determine synaptodendritic alterations in HIV-1, we employed the DiOlistic labeling technique, which may have the following advantages over Golgi-Cox silver impregnation: 1) the lipophylic dye DiI, or 1,1’-dioctadecyl-3,3,3’,3’-tetramethylindocarbocyanine perchlorate, has higher resolution to distinguish fine spine
morphological characteristics (Mancuso et al., 2012; Shen et al., 2009); 2) allows three-dimensional dendritic analysis when using Z-stack confocal imaging (Mancuso et al., 2012; Shen et al., 2009), and 3) is compatible with immunohistochemistry (Mancuso et al., 2012; Seabold et al., 2010) facilitating identification of the population of labeled cells.

HIV+ women have increased neurocognitive impairments relative to HIV- women (Manly et al., 2011; Maki et al., 2009), yet few translational studies have focused exclusively on females. Therefore, we used DiOlistic labeling to explore alterations in dendritic arborization and spine morphology of MSNs in the NAcc of HIV-1 Tg female rats. The MSNs in the NAcc represent an important component of the motivational/reward system mediating substance abuse (Ma et al., 2013), which is often co-morbid with HAND in women (Meyer et al., 2013; Maki and Martin-Thormeyer, 2009). Thus, identification of altered MSN synaptodendritic structures in HIV-1 Tg female animals is a key step in clarifying possible neuropathological substrates of HAND.

**METHODS**

**Subjects**

Adult female Fischer F344 rats (HIV-1 Tg, n=12; control, n=12; approximately 9-10 weeks of age) were purchased from Harlan (Indianapolis, IN). Food (Pro-Lab Rat, Mouse Hamster Chow #3000, NIH diet #31) and water were available *ad libitum*. Rats were maintained according to the National Institute of Health guidelines in AAALAC-accredited facilities. The animal housing room was maintained at 21±2 °C, 50%±10% relative
humidity, and had a 12:12 hr light/dark cycle with lights on at 07:00 EST. Animals were handled daily for approximately two weeks prior to behavioral testing, and weighed daily. The research protocol was approved by the Institutional Animal Care and Use Committee (IACUC) of the University of South Carolina, Columbia, SC; animal assurance number A3049-01.

**Estrous Cycle Tracking**

Vaginal lavage was performed daily at 11:00 AM (10-11 weeks of age) for approximately 3 weeks to examine estrous cyclicity. As previously described (Booze et al., 1999), cellular cytology was examined in vaginal smears using a light microscope (10X) with the predominant cell type used to determine stage of the estrous cycle (Waynforth and Flecknell, 1992; Westwood, 2008). Proestrus was identified by flat, circular squamous cells. Predominately cornified cells indicated day of estrus and was confirmed the following day by presence of macrophages and nucleated cells, indicating metestrus. Diestrus state was designated by the presence of thick epithelial cells and absence of stratum granulosum. The criterion for regular cycling was that each rat demonstrated a 4-6 day estrus cycle. Irregular cycling and acyclicity were defined by either persistent estrus, absence of proestrus, failure to progress from proestrus to estrus, or prolonged diestrus.

**Prepulse Inhibition Task**

Animals were tested in the prepulse inhibition (PPI) task (12-13 weeks of age) similar to our prior publication (Moran et al., 2013a). In brief, the startle apparatus (SR-Lab Startle Reflex System, San Diego Instruments, San Diego, CA) was enclosed in a sound attenuating chamber (Industrial Acoustic Company, Bronx, NY). All auditory stimuli were delivered via a high frequency loudspeaker (Radio Shack model #40-1278B) mounted
inside the chamber 30 cm above the Plexiglas test cylinder. A white LED light was mounted inside the chamber in front of the test cylinder (22 lux; light meter model #840006, Sper Scientific Ltd, Scottsdale, AZ). The whole body startle response to the auditory startle stimulus deflected the test cylinder, which was measured via an accelerometer at the base of the Plexiglas test cylinder. All test sessions were conducted in the dark.

All animals were habituated to the startle response chamber for one day prior to PPI testing (Moran et al., 2012). The habituation session consisted of 36 trials beginning with a 5-minute acclimation period (70 dB(A) background white noise), followed by 36 trials of a 100dB(A) white noise stimulus of 20 msec duration and 10 sec intertrial interval (ITI). The habituation session was administered during the metestrus phase of the estrous cycle.

One day after habituation, rats were tested with 36 PPI trials, i.e. 0, 8, 40, 80, 120, and 4000 msec interstimulus intervals (ISI) between prepulse (single light flash at 22 lux) and the pulse stimulus (100dB(A) white noise). A variable 20 sec ITI was used (15-25 sec). Six trials for each ISI were conducted using a Latin-square design. Percent PPI was calculated as the difference between average peak amplitude at 0 and 4000 msec ISI and the ISI at which peak inhibition was observed (40 msec) divided by average peak amplitude at 0 and 4000 msec ISI, multiplied by 100. All PPI testing was performed in the diestrus phase of the estrous cycle.

Preparation of Tissue

Animals were sacrificed in the diestrus phase of the estrous cycle (13-15 weeks of age). Animals were deeply anesthetized using sevoflurane (Abbot Laboratories, North Chicago IL) and transcardially perfused with 100 ml of 100 mM PBS wash followed by 100-150 ml of 4% paraformaldehyde buffered in PBS (Sigma-Aldrich, St. Louis, MO).
Brains were dissected and post-fixed in 4% paraformaldehyde for 30-60 minutes. After post-fixation, 200 µm thick coronal slices were cut using a rat brain matrix (ASI Instruments, Warren, MI). Serial coronal slices were then washed in PBS 3 times, notched for orientation, and placed in tissue cell culture plates (24 well plate; Corning, Tewksbury MA) until further processing.

Preparation of DiOlistic Cartridges

DiOlistic labeling was performed according to published techniques (Seabold et al., 2010). Approximately 300mg of tungsten beads (Bio-Rad, Hercules, CA) were dissolved in 99.5% pure methylene chloride (Sigma-Aldrich, St. Louis, MO) and sonicated in a water bath for 30 minutes. Crystallized DiI (14.5 mg; Invitrogen, Carlsbad, CA) was dissolved in methylene chloride and light protected. Following sonication, 100 µl of the bead solution was placed on a glass slide and 150 µl of the DiI solution titrated on top, and slowly mixed using a pipette tip. After air drying, a razor blade was used to collect the dye/bead mixture onto wax-coated weigh paper and the dye/bead mixture transferred to a 15 ml conical tube (BD Falcon, San Jose, California) with 3 ml ddH2O and subsequently sonicated for 45-60 minutes.

Preparation of Tefzel Tubing

Tefzel tubing (IDEX Health Sciences, Oak Harbor, WA) was cut into three 1.7 M lengths. Polyvinylpyrrolidone (PVP, 100 mg Sigma-Aldrich, St. Louis, MO) was dissolved in 10 ml ddH2O, briefly vortexed, then passed through each length of the tubing. The 3 ml bead/dye solution was slowly drawn into the tubing and placed in the tubing prep station (Bio-Rad) for 5 minutes. After draining the water from the tube, the dry tubing was spun in the prep station for approximately 10 minutes with nitrogen gas flow of 1.0 LPM. The
nitrogen gas flow through the tubing was adjusted to 0.4-0.5 LPM and the tubing was further spun for 50-60 minutes to ensure the tubing was fully dry. Once dry, tubing was cut into 13 mm segments and stored under anhydrous conditions until use.

**DiOlistic Labeling using the Helios Gene Gun**

The Helios gene gun (Bio-Rad, Hercules, CA) was loaded with the previously prepared cartridges, He gas flow adjusted to 80 PSI, and particles delivered through 3 µm pore filter paper directly onto the slice with the barrel placed approximately 2.5 cm away from the sample. After washing 3X in PBS, sections were stored overnight at 4˚ C to allow dye diffusion. Tissue sections were mounted using Pro-Long Gold Antifade (Invitrogen, Carlsbad CA), coverslipped (#1 coverslip; ThermoFisher Scientific, Waltham, MA), and stored in the dark at 4˚ C.

**MSN Dendritic Analysis and Spine Quantification**

MSNs were analyzed from the NAcc, located approximately 2.28 mm to 0.60 mm anterior to Bregma (Paxinos and Watson, 2007). For dendritic branch order analysis, HIV-1 Tg (n=5) and Controls (n=5), three MSNs per animal were randomly selected for analysis. For spine analysis, neurons with continuous dendritic staining extending from the soma, minimal diffusion of the DiI into the extracellular space, and low background/dye clusters were selected. DiI labeling from 4 rats from each group did not meet the selection criteria, yielding HIV-1 Tg n=10, Controls n=10; one MSN per animal was randomly selected for spine analysis.

For dendritic spine analysis, Z-stack images were obtained with a Nikon TE-2000E confocal microscope utilizing Nikon’s EZ-C1 software (version 3.81b). Dendritic spine analysis was performed at 60X (n.a. = 1.4) with Z plane intervals of 0.15 µm (pinhole size
30 µm; backprojected pinhole radius 167 nm). A green helium-neon (HeNe) laser with an emission of 533 nm was used for DiI fluorophore excitation. The analysis of spine parameters was performed using Neurolucida version 10.52, utilizing the AutoNeuron and AutoSpine extension modules (MicroBrightField, Williston, VT). Total dendritic length scanned per cell ranged from 500 to 3000 µm.

**Dendritic Spine Parameters**

Dendritic spine parameters of length, volume, and head diameter were analyzed. Spine lengths were defined as between .01 µm to 4 µm; lengths greater than 4 µm were considered to be filopodia and excluded from the study (Blanpied and Ehlers, 2004; Ruszczycki et al., 2012). Spine volume parameters were defined as those measures between 0.02 µm³ and 0.2 µm³ (Merino-Serrais et al., 2013). Spine head diameters were defined as those measures between 0.3 µm and 1.2 µm (Bae et al., 2012).

**Data Analysis**

Data were analyzed using SPSS version 20.0 (IBM). Differences in weights were compared using a mixed two-way ANOVA with age as the within-subjects factor and rat strain as the between-subjects factor, with a Bonferroni post-hoc correction. Estrous cycle differences were determined using chi-square analysis (Booze et al., 1999). PPI was analyzed using ANOVA with a Greenhouse-Geisser correction to p values (Moran et al., 2013a). Branching order differences were determined using a two-way repeated measures ANOVA with rat strain as the between-subjects factor and branch order as the within-
subjects factor. Spine parameters (length, volume, and head diameter) were analyzed via Student’s unpaired t-test with Welch’s correction.

**RESULTS**

*HIV-1 Tg and control animals grow at similar rates and have similar estrous cycles*

Although HIV-1 Tg animals weighed significantly less than F344 control animals, F(1,22) = 15.4, p≤0.001 (Fig. 1A), there was no significant interaction between rat strain and age; regression analysis further confirmed similar rates of growth for both control and HIV-1 Tg animals (Control B = 0.74 ± 0.09, HIV-1 Tg B= 0.96 ± 0.10).

All 12 of the F344 control animals and 10/12 of the HIV-1 Tg animals experienced typical 4-5 day estrous cycles over a series of 4-5 cycles. One HIV-1 Tg animal displayed a persistent diestrus state on earlier tracking days, while another missed one ovulatory cycle. Their cycle length was calculated from the cycle data obtained. Means for F344 control and HIV-1 Tg animals were 4.8 and 4.9 days/cycle (t(22) = 0.74, p>0.10), respective medians were both 5.0, and a Chi-square analysis of cycle length found no significant difference between the two groups, $\chi^2 = 4.7$ (df=11), p>0.10 (Fig 1B).

**Magnitude of prepulse inhibition does not differ between groups using a 20 msec visual stimulus**

During visual prepulse inhibition testing, a significant main effect on mean peak amplitude was observed for ISI length, F(5,110) = 44.2, pGG≤0.001 (Fig. 1B). A significant quadratic trend was observed for each group [Control: F(1,11) = 26.7, p≤0.001; HIV-1 Tg: F(1,11) = 43.4, p≤0.001]. There was neither a significant effect of group nor a significant interaction between group and ISI length. Maximum inhibition for
the visual stimulus was achieved at the 40 msec ISI for both groups as shown previously (Moran et al., 2013a), with no significant difference between groups in percent PPI (Controls: 79.6% ± 3.3; HIV-1 Tg: 82.0% ± 2.8).

**MSN dendritic branching is reduced in HIV-1 Tg animals**

DiOlistic labeling was found to completely fill the soma and surrounding dendritic fields of NAcc MSNs with little to no background staining (Fig. 2A,B). MSNs from F344 control animals had multiple proximal branches extending from the soma, a complex dendritic branching pattern, and an extensive fine network (Fig. 2A). Decreased dendritic branching complexity in HIV-1 Tg MSNs was apparent (Fig. 2B). Dendritic sections revealed spines with an increased head-width to neck-width ratio among control slices, indicating advanced spine maturity (Fig. 2C). HIV-1 Tg spines appear thinner and with a near equal head-diameter to neck-diameter ratio, producing a stubbier appearance (Fig. 2D). Quantities of dendrites differed significantly between groups as a function of both transgene and branch order, F(1,42) = 56.7, p≤0.0001 and F(2,42) = 10.4, p≤0.001, respectively (Fig. 2E).

**Dendritic spine length and volume, but not head diameter, was altered in HIV-1 Tg animals**

Measurement of spine length revealed a significant shift in the distribution from longer to shorter spines for the HIV-1 Tg rats compared to controls (Fig 3 A,B; student’s unpaired t-test with Welch’s correction, t(20763) = 20.5, p≤0.0001). Measurement of spine volume also showed a significant shift in distribution as a function of the HIV-1 transgene, with the HIV-1 Tg rats displaying a reduction in spine volume relative to controls (Fig 4 A,B, student’s unpaired t-test with Welch’s correction, t(6078) = 5.6, p≤0.0001).
Measurement of spine head diameter did not reveal any statistically significant reduction in the HIV-1 Tg animals compared to controls (Fig 5 A,B).

**DISCUSSION**

HIV-1 associated neurocognitive disorders (HAND) are thought to be the result of dendritic injury (Ellis et al., 2007; Moore et al., 2006) and synaptic dysfunction (Desplats et al., 2013; Gelman and Nguyen, 2010). In the present study, DiOlistic labeling in HIV-1 Tg female rats was used to identify synaptodendritic injury in the NAcc MSNs via reduction in dendritic branching. Furthermore, analysis of dendritic spines revealed a shift toward shorter, stubbier spines with an overall decreased spine volume in HIV-1 Tg animals, relative to controls. Collectively, these findings suggest that MSN synaptodendritic injury occurs in response to chronic low-level HIV-1 protein expression in the central nervous system.

The study of female animals is particularly important as, on a global scale, women represent at least 50% of people living with AIDS (UNAIDS, 2013), yet HIV-1+ women are under-represented in neuropsychological studies (Maki and Martin-Thormeyer, 2009). This under-representation may be due to the complexity of studying economically disadvantaged HIV-1+ women with many complicating comorbidities, including substance abuse. However, recent studies suggest that HIV+ women are neurocognitively impaired relative to HIV- women with similar comorbid conditions (Maki et al., 2009; Manly et al., 2011). Collectively, these studies emphasize the important role that translational work may provide in identifying HIV-1 induced neurocognitive impairments in females and identifying the underlying neuropathological alterations.
MSNs are important in the motivational/reward system and, more generally, the NAcc plays an important role in substance abuse, which is often comorbid with HAND (Bell et al., 2006). Indeed, HIV-1 Tg animals are particularly vulnerable to a variety of drugs of abuse including methamphetamine (Pang et al., 2013; Moran et al., 2012), nicotine (Vigorito et al., 2013) and ethanol (Sakar and Chang, 2013), suggesting a commonality of neuropathological impairment across many different drug classes. It is possible that MSNs might be a common neuropathological link in altered responses of the HIV-1 Tg animals to drugs of abuse, as alterations in MSN spines have been found following cocaine/amphetamine exposure (Shen et al., 2014; Dumitriu et al., 2012) nicotine exposure (Gipson et al., 2013) and alcohol (Gass and Olive, 2012). Although women with HIV+ status may be particularly vulnerable to the effects of substance abuse relative to matched HIV- women (Meyer et al., 2013), few translational studies have focused exclusively on females.

In general, the HIV-1 Tg young adult female rats were found to be healthy (i.e., normal estrous cycles, similar growth rate as controls), which is consistent with our prior studies (Moran et al., 2012; Moran et al., 2013a; Moran et al., 2013b; Webb et al., 2010). However, it is of note that the health status of our HIV-1 Tg animals differs from the original description of the HIV-1 Tg rat. Reid and colleagues (Reid et al., 2001) described several severe pathological phenotypes associated with the transgene expression (then located on chromosome 2 and 9), including 3 grades of cataracts, accompanied by severe neurological disorders (hind limb paralysis) and wasting at a relatively young age (5-9 months). The HIV-1 Tg animals used in the current studies are a derivation of these originally described phenotypes, on an inbred F344 background, with the transgene (now
limited to chromosome 9), and a 100% penetrant phenotype of light to moderate cataracts. We have found no significant alterations in detection of brief visual stimuli, hearing or growth rates in the present study and have found no general wasting in this contemporary HIV-1 Tg phenotype through 344 days of age (Moran et al., 2013a). Similarly, studies using these animals report alterations in behavioral tasks of spatial learning (Lashoub et al., 2009; Vigarito et al., 2007), without alterations in hearing or locomotor ability. Thus, as suggested by Peng et al., (2010) the moderate phenotype more closely resembles HAND, is suitable for long-term/aging longitudinal studies, and should be considered distinct from the original descriptions of the most severe phenotypes of the initially derived HIV-1 Tg rat.

Although the phenotype of the HIV-1 Tg rat used in the present studies includes the presence of light to moderate corneal cataracts, we found PPI to brief visual stimuli unchanged, compared to control, with the maximum inhibition at 40 msec ISI. Our previous visual PPI studies, comparing light levels of 22 lux (dim light) and 100 lux (bright light), also indicated similar levels of maximum inhibition between control and HIV-1 Tg ovariectomized animals at the 40 msec ISI, indicating detection of the brief (20 msec) dim (22 lux) visual prepulse by the HIV-1 Tg animals (Moran et al., 2013a). Collectively, these results suggest the presence of moderate corneal cataracts does not hinder the ability of HIV-1 Tg rats to detect light cues. It remains that, although visual acuity may be impaired by the presence of light-moderate cataracts in the HIV-1 Tg animals, the ability to respond to visual cues (even brief dim cues) in small behavioral testing chambers is intact in the contemporary HIV-1 Tg phenotype.

The HIV-1 clade B transgenic rat expresses HIV-1 proteins via regulation of the viral long-terminal repeat, with the exception of Gag and Pol proteins (Peng et al., 2010;
Protein expression in this rat is regulated by the viral long terminal repeat (LTR) and uses cyclin-T as a cofactor to regulate Tat; murine cyclin-T is not compatible with this mechanism (Reid et al., 2001). Endogenous rat cyclin-T acts as a modulator of Tat, thereby establishing proviral DNA without an accompanying state of active peripheral viremia (Reid et al., 2001). This aviremic state may represent individuals infected with HIV-1, but present seronegative viral loads, as seen in individuals on combined antiretroviral therapy (cART). Moreover, in the HIV-1 Tg rats, HIV-1 expression of vif, nef, tat and gp160 proteins, occurs in mononuclear phagocytes/astrocytes, but not in neurons (Royal et al., 2012) – similar to that seen in human HIV-1 brains – accompanied by chronic, low-level, immune activation in the brains of these animals (Royal et al., 2012). Immune activation produces a neuroinflammatory microenvironment (Rao et al., 2011), which may be detrimental to neuronal connectivity and dendritic spines. However, to our knowledge, no studies of synaptodendritic pathology have been reported using the HIV-1 Tg rat.

Previous studies of HIV-1 synaptodendritic and spine alterations have used a variety of methods for spine detection, including MAP-2 staining (Maragos et al., 2003) and Golgi-Kopsch silver impregnation (Fitting et al., 2010). However, Golgi staining is notoriously capricious, and not all Golgi staining techniques are capable of providing the resolution for differentiating fine structures. In particular, the Golgi-Cox method may not have the ability to distinguish thin filopodia, and MAP-2 immunostaining does not identify specific morphological parameters in spines. Also, because white light is reflected from silver stain, silver staining methods method may be impractical for 3-D morphometric analysis of confocal z-stacks (Staffend and Meisel, 2011). In one of the few direct comparisons
between Golgi staining and DiOlistic labeling, the DiOlistic method was found to produce higher spine counts via 3-D analysis relative to Golgi staining (Shen et al., 2009).

Indocarbocyanine dye labeling via ballistic methods can be done in a fraction of the time it takes to complete the Golgi method (Staffend and Meisel, 2011), and is compatible with retrograde tracing (Neely et al., 2009) and immunohistochemistry (Seabold et al., 2010; Staffend and Meisel, 2011). DiOlistic labeling can be used to analyze multiple neuronal structures, and is available in multiple fluorescent spectra to be compatible with the majority of immunohistochemical techniques (Gan et al., 2000). DiOlistics may have advantages over microinjection and electroporation as well, since the technique can be modified to allow for minimal tissue damage, many cells can be labeled simultaneously, and no special or toxic media are required (O'Brien and Lummis, 2007). However, the DiOlistic method requires careful optimization of the technique to prevent clustering of DiO crystals, excessive tissue damage, and other pressure artifacts (see Seabold et al., 2010, for discussion of DiO artifacts).

The present study calculated the length, volume and head width of each spine on the dendritic stalk, providing a population distribution for various spine parameters. Although spine morphological nomenclature suggests a continuum of morphological structure, a priori classification of spine morphology is prone to false negatives (Ruszczycki et al., 2012). However, it is commonly thought that dendritic spine morphology is indicative of functionality and capacity for structural change (Lai and Ip, 2013). Classic nomenclature divides spine morphology into three categories: thin, stubby, or mushroom. Thin spines are characterized by a relatively short neck length and small head volume, with relatively small quantities of synaptophysin (Brusco et al., 2010; González-Burgos, 2012). Stubby
spines are quantified by an equal head-to-body volume (Brusco et al., 2010; González-Burgos, 2012). Mushroom spines, indicating stability, contain a high head volume to neck volume ratio, in addition to high synaptophysin and PSD95 protein quantities located at the spine terminal (Blanpied and Ehlers, 2004; Golden and Russo, 2012; Ruszczycki et al., 2012). The size and shape of dendritic spines correlates with their capacity for structural plasticity, leading to the idea that small spines are preferentially involved in learning and attention, whereas the larger, more stable spines, mediate long-term processes, such as memory (Kasai et al., 2010).

The directly measured spine parameters of length and volume in the MSNs of HIV-1 Tg rats suggest a population shift from longer spines with defined head areas to shorter, less projected, spines. The shorter, smaller dendritic spines on the MSNs of HIV-1 transgenic animals indicates alterations in synaptic properties. Shorter, smaller spines have weaker synaptic connectivity, are more prone to potentiation (Matsuzaki et al., 2004) and are transient/dynamic (Kasai et al., 2010; Holtmaat et al., 2005), relative to the larger, more stable spines. Given that the HIV-1 animals have a significant shift in the dendritic spine population parameters (i.e., shorter spines), it might be anticipated that cognitive deficits would be present in these animals. Our prior behavioral studies support this idea as we have observed deficits in temporal information processing (Moran et al., 2013a), as well as in attention/inhibition processes (Moran et al., 2014), as does the work of others in which spatial memory deficits were identified (Vigorito et al., 2007). However, direct correlations between dendritic spine parameters and learning/memory deficits remains to be done with the HIV-1 Tg animals.
Collectively, this study found synaptodendritic simplification and spine population alterations following long-term exposure to HIV-1 viral proteins, without an active infection. The MSNs of HIV-1 Tg female rats had limited branching patterns, in addition to a population shift to shorter and smaller dendritic spines. In the future, DiOlistic labeling can be used in conjunction with immunostaining to determine the specific MSN subtype(s) and the corresponding cellular mechanisms most affected by chronic HIV-1 protein expression. Identification of altered synaptodendritic structures in HIV-1 Tg female rat is a key step in identifying neuropathological substrates of HAND and moreover, determining how such synaptic pathology evolves.
Figure 2.1 Body weight and prepulse inhibition for control and HIV-1 Tg animals. A: Body weights of control and HIV-1 Tg animals across weeks (mean ± 95% confidence interval). The HIV-1 Tg animals weighed significantly less than controls. However, regression analysis indicated a comparable growth rate of controls and HIV-Tg-1 rats (Control B = 0.74 ± 0.09, HIV-1 Tg B = 0.96 ± 0.10). B: Mean peak auditory startle response (ASR) amplitude is shown as a function of interstimulus interval (ISI). PPI to the brief 20 msec visual prepulse was not significantly different between groups. Furthermore, both control and HIV-1 Tg rats exhibited similar rates of inhibition at 40 msec ISI (Control: 79.6% ± 3.3; HIV-1 Tg: 82.0% ± 2.8).
**Figure 2.2** DiOlistic labeling of medium spiny neurons (MSN) in the nucleus accumbens core. A: MSNx from control animals displayed a complex branching pattern with increased number of dendrites as a function of branch order (60X). B: HIV-1 Tg MSNs exhibited stunted branching complexity among primary, secondary, and tertiary branch orders (60X). C: Control MSN spines were longer and exhibited a high head-width to neck-width ratio. D: MSN HIV-1 Tg MSN spines exhibited a stubbier appearance and were shorter. E: Quantity of MSN dendritic branching differed significantly between HIV-1 Tg and controls F(2,42)=56.7, p≤0.0001. * indicates p<0.0001.
Figure 2.3 Spine length histograms illustrating relative (A) and cumulative (B) frequencies. The overall reduction in spine length reflects the significant shift in the population of spines observed; HIV-1 Tg rats had predominantly shorter spines relative to controls (Student’s unpaired t-test with Welch’s correction, t(20763) = 20.5, p≤0.0001).
Figure 2.4 Spine volume histograms illustrating relative (A) and cumulative (B) frequencies. The overall reduction in spine volume reflects the significant shift in the population of spines observed; the population of observed spines of the HIV-1 Tg rats had predominantly less volume relative to controls (Student’s unpaired t test with Welch’s correction, $t(6078) = 5.6, \ p \leq 0.0001$).
Figure 2.5 Spine head diameter histograms illustrating relative (A) and cumulative (B) frequencies. The overall slight reduction in spine head diameter as a function of expression of the HIV-1 transgene was not statistically significant.
CHAPTER 3

ACQUISITION OF SELF-ADMINISTRATION OF METHYLPHENIDATE IS ALTERED IN THE HIV-1 TRANSGENIC RAT: BEHAVIORAL AND PHYSICAL CONSEQUENCES OF DOPAMINE TRANSPORTER INHIBITION

2 Roscoe Jr. RF, Samaranayake S, Li H, Hashemi P, Harrod SB, Mactutus CF, Booze RM. To be submitted for peer-reviewed publication.
INTRODUCTION

Visualization and quantification of altered morphological parameters in the dendritic spines of medium spiny neurons from resultant HIV-1 pathology, from an unbiased viewpoint, is necessary to determine both cellular consequences of Tat induced DAT disruption and behavioral consequences of the altered neural network.

Synaptodendritic injury has been characterized in the HIV-1 transgenic rat using adult tissue (Roscoe, Jr. et al., 2014) and cell cultures (Bertrand et al., 2013; Bertrand et al., 2014; Bertrand et al., 2015); MSNs in the NAc present with a decreased complexity in dendritic branching, along with a higher percentage of short, stubby spines (Roscoe, Jr. et al., 2014). Reduced connectivity and increased synaptodendritic injury in nucleus accumbens projections are also seen during cocaine exposure to a wild-type background (Neumann et al., 2016). MPH addition has been shown to increase percentages of long thin spines, while cocaine addition results in an increase in shorter spines (Kim et al., 2009). Typically, spine density and dendritic complexity are increased with both chronic and acute cocaine and methylphenidate addition (Kim et al., 2009), yet subtoxic comorbidities with illegal psychostimulants results in reduction in dendritic arborization and neuronal complexity (Aksenova et al., 2009; Bertrand et al., 2015).

Additionally, pyramidal neurons experience alterations to synapse quantity and spine density as a result of cocaine addition (Rasakham et al., 2014). In 2008, Kolb et. al investigated the plasticity of MSNs in the NAc and pyramidal neurons in the parietal cortex following environmental enrichment after amphetamine or cocaine injection;
physical measures of plasticity (at least, dendritic spine density) were reduced in animals who had previous psychostimulant experience in the enriched environmental condition (Kolb et al., 2003). No direct investigation of MPH’s effect on dendritic spine morphology or plasticity has been performed in the HIV-1 transgenic rat thus far. Annis et al. (1994) suggests that electrophysiological activity on cortical neurons attenuate dendritic spine density on pyramidal neurons; however investigation into striatocortical circuitry alterations resulting from Tat-induced DAT interference will better categorize behavior-based alterations to motivation and choice behavior in the HIV-1 transgenic rat. Interpretation of similar or altered effects on these two neuronal systems will better categorize and describe Tat-induced behavioral disruptions in the HIV-1 Tg rat model using a bottom-up approach.

Cyclic voltammetry exploits the electroactive Red/Ox reactions of monoamines hydrolyzed during active transport into the cell, allowing for recording of phasic dopamine changes and highlighting the functional activity of the dopamine transporters in real time (Stamford et al., 1984; O’Neill and Fillenz, 1985). Because HIV-1 transgenic rats’ dopaminergic dysfunction has been well behaviorally established (Moran et al., 2012b; Moran et al., 2013b; Wayman et al., 2015a; Webb et al., 2010) and the HIV-1 Tat protein has been identified as a key neurotoxin in cell culture models (Aksenov et al., 2008; Aksenov et al., 2012; Aksenova et al., 2009), it is necessary to probe the functioning of the dopamine transporter in NAc neurons to determine functional alterations to dopamine release in the presence of MPH.

NAc activity correlates to dichotomous behavioral responses in acute and chronic methylphenidate administration, into either sensitization or tolerant groups of rats.
Chronic administration of methylphenidate did not seem to alter cellular responding of the NAc of sensitized vs tolerant animals, but a slightly greater inhibition of the NAc occurred at a dosage of 10mg/kg (Claussen et al., 2014). Expounding on this behavioral premise, it is necessary to determine functional activity of dopamine transport and clearance in the NAc of HIV-1 transgenic rats. Evidence of allosteric inhibition of the DAT by shed Tat protein (Zhu et al., 2011a) is further supported by in vivo microdialysis, showing altered dopamine flux in the striatum (Ferris et al., 2009b). FSCV in the HIV-1 transgenic animal will further support the aforementioned phenomenon, and our experimental design will specifically identify any gender-specific effects of dopamine transport in the NAc with and without expression of the HIV-1 transgene.

Electrostimulatory FSCV has not yet been performed in the HIV-1 Tg rat, but previous efforts using coronal slices report an altered clearance rate of dopamine compared to control slices (Javadi-Paydar et al., manuscript under review). Future study will determine effects of MPH administration in this population, building upon previous efforts detailing the functional kinetics of dopamine transport in the rat (Ferris et al., 2013) and the altered kinetics induced by MPH administration signified by hyperdopaminergia (Calipari et al., 2014; Calipari et al., 2015). Moreover, MPH is not inhibited in its ability to increase Km for DA reuptake following cocaine self-administration (Ferris et al., 2012) which indicates a specific and unique pharmacoactivity relative to that of cocaine. While not a model of HIV-1, spontaneously hypertensive rats exhibit an increase in cocaine sensitivity after long MPH withdrawal (dos Santos Perieria et al., 2015) suggesting a pharmacological relationship
between the two psychostimulants and may indicate a cross-tolerance or cross-sensitization phenomenon between the substances.

MPH has a distinct clinical pharmacology and functions as a dopamine and norepinephrine transporter inhibitor (Challman and Lipsky, 2000). MPH is clinically used to treat neurocognitive impairment associated with ADHD in adolescents and HIV-1 associated apathy in adults (Challman and Lipsky, 2000; Treisman et al., 2001), yet the discrete biochemical effects in an HIV-1 environment in these populations have not been explored. As MPH binds to the extracellular DAT domain similarly to cocaine, but exhibits an altered intrastriatal elimination rate, (Volkow et al., 1995), it is necessary to determine possible dopamine disruptions and addictive labilities of MPH in a preclinical model of HIV. Under normal conditions, therapeutic dosages of MPH occupy approximately 50% of active dopamine transporters (Volkow et al., 1998), but originally no clinical efficacy has been determined using MPH in HIV-1 associated neurocognitive impairment (van Dyck et al., 1997). Hinkin et al (2001) has indicated the use of MPH as beneficial to patients that display a great amount of cognitive impairment, however this effect has not been seen in HIV-1+ patients that exhibit minimal cognitive slowing (Hinkin et al., 2001). For these reasons, in addition to the number of HIV-1 perinatally infected youth that may be taking MPH therapeutically for ADHD, it is necessary to examine the abuse potential of MPH in detail.

Previous rat studies using methylphenidate have explored behavioral locomotor sensitization and tolerance resulting from drug exposure, with rats usually falling into one of the two categories following a dose-response (Frolov et al., 2015). Both sensitization and tolerance are associated with alterations in addictive lability and may indicate greater
responding after a period of acute withdrawal. Expounding on this binary phenomenon, methylphenidate’s effect on locomotor activity has been varied and it is unknown whether the route of administration is a causative factor (see (Askenasy et al., 2007) for thorough review). As the proposed experiment is performed using oral-route self-administration, and normal rats exhibit a mix of sensitization and tolerance, correctly hypothesizing the final results of this portion of the experiment may prove difficult. However, based on previous determination of sensitization in the HIV-1 transgenic rats as a result of cocaine addition (Moran et al., 2013b), abusive doses (~8mg/kg/day) of MPH may cause similar behavioral sensitization.

The proposed study would determine if abusive doses of methylphenidate (~2-8X typical daily dose) cause dopaminergic transport alterations that have been seen in cocaine self-administration. Because of Tat’s disruption of the DAT, increased MPH self-administration should also cause greater synaptodendritic injury in HIV-1 TG rats compared to F344 controls. These alterations will be reflected in reduced proliferative dendritic spine morphology on medium spiny neurons of the NAc. The behavioral result of these alterations will manifest in an enhanced addiction paradigm via a relative rate of increase in self-administration for MPH in a masked solution both chronically and after a short period of withdrawal.

**METHODS**

*Subjects*

Female F344 rats (n=20) and female HIV-1 Tg rats (n=19) (Harlan Laboratories, Indianapolis, Indiana) were commercially ovarectomized and pair housed by genotype.
Food and water were provided *ad libitum* throughout the experiment. Animals were kept in an AALAC-accredited facility at 21 +/- 2 C, 50% +/- 10% relative humidity, in a 12-hr light-dark cycle with lights on at 07:00h. All experiments were performed according to the National Institute of Health guidelines for AAALAC accredited facilities. All behavioral testing was performed during the animal’s light cycle. The research protocol was approved by the Institutional Animal Care and Use Committee (IACUC) of the University of South Carolina, Columbia, SC, under animal assurance number A3049-01.

**Drugs**

MPH hydrochloride was purchased from Sigma-Aldrich Corporation (St. Louis, MO) containing racemic isomers (50% l-threo methylphenidate, 50% d-threo methylphenidate). Drug was prepared daily by dissolving individual weight-controlled doses of MPH with sucrose in ddH$_2$O. Caps of 1L Nalgene bottles were used as a reservoir in the operant box to minimize drug waste. Food-grade commercially available refined white sugar was used during the experiment as a response reward (5, 10, or 26% concentration) or masking agent for MPH.

**Operant Testing Apparatus**

Operant chambers (ENV-008; Med-ED Associates, St. Albans, VT) were controlled by Med-PC IV interface software (MED Associates, St. Albans, VT) and were enclosed in a sound-attenuated cabinet. The front panel of the chamber (relative to the animal) contained a recessed dipper (ENV-202M) which became available in a 5cm X 5cm window containing an infrared sensor (ENV-245-CB) to track head pokes. Two retractable metal levers (ENV-112BM) were offset on each side of the opening and located 7.3cm above the chamber floor. A 0.3ml dipper cup attached to the dipper arm
was raised into the receptacle allowing self-administration of reward after 1 lever press (FR1 schedule). A third lever was affixed to the back wall and while presses were counted, the lever itself was inactive. All three levers were presented immediately at the beginning of testing and rats learned to respond for continuous reinforcement on an FR1 schedule during 42-minute sessions. An active lever response (either left or right of the access chamber) provides a 4-second access window to the dipper. Subjects were previously exposed to a habituation responding paradigm in an effort to counter conditioned place preference of lever responding.

**Locomotor Activity Apparatus**

Locomotor activity was tested daily approximately 15 minutes after conclusion of the rats’ respective self-administration operant work. The test chamber was a square Plexiglas box, 40cm by 40cm, containing photocell pairs in the X and Y dimensions. A circular Plexiglas insert was used to prevent corner bias. Tests were conducted for 60 minutes under low-light conditions (20 lux) to simulate a nocturnal environment. The photocell grid (Hamilton-Kinder Inc., Ponway, CA) (32 X 32 cm, individual cells spaced 2.5cm apart) was manufacturer-tuned to control for increase in perspex width associated with the circular insert. Fine and basic movements were recorded by Motor Monitor software v140 (Hamilton-Kinder Inc, Ponway, CA). A basic movement is defined as clearing of the anchor beam while a new beam is broken, indicating a step, while fine movements are defined as a front beam break without clearance of the anchor beam (see Motor Monitor Operations Manual version 3.11 (Hamilton-Kinder Inc, Ponway, CA).
Experimental Design

We performed 3 experiments: 1, sucrose preference; 2, drug discrimination; and 3, behavioral locomotor activity sensitization. Animals were habituated to the operant and locomotor activity chambers for 3 days prior to testing involving MPH. For sensitization experiments, rats were tested for 14 consecutive days and retested 1 week later.

Drug Detection

The first experiment performed was a drug detection task. Rats performed an FR1 schedule to receive a 5% sucrose reward. MPH was added on alternating days for 12 days, before sucrose concentration was increased to 10%. Any animals that failed to complete the requisite response (60 rewards/42-minute session) were placed on a 26% sucrose schedule in an effort to increase responding.

Self-administration/Locomotor activity

For the behavioral sensitization experiment, MPH was dissolved in 26% sucrose at a dose corresponding to each individual rat’s body weight. We used the maximum sucrose concentration employed to ensure maximal responding from each subject. The chambers were programmed to end the session upon completion of 120 rewards, corresponding to a maximum dosage of 4 mg/kg. Approximately 15 minutes after completion of the operant paradigm, animals performed a 60-minute locomotor activity session. The experiment was performed over 14 consecutive days, followed by a 14 day break with no testing, prior to a one-day retest.

Subjects
Control F344 (n=9 males, n=9 females) were age-matched to HIV-1 transgenic animals (n=8 males, n=8 females), obtained from Harlan laboratories (Indianapolis, IN). Animals were pair-housed with same-sex and genotype partners, as best available, throughout the study; animals were housed 3 per cage as necessary during sacrifices. Food (Pro-Lab Rat, Mouse, and Hamster chow no 3000, NIH diet #31) and water were available ad libitum. Animals were maintained in AAALAC-accredited facilities with a 12-hour light:dark cycle corresponding to lights on at 0700 hrs, EDT. All animal procedures were approved by the Institutional Animal Care and Use Committee (IACUC) at the University of South Carolina (assurance number A3049-01)

_Estrous Cycle Tracking_

Stages of estrous cycle were tracked daily via cytology obtained from vaginal lavage performed daily at approximately 11:00 AM. Cytology was examined under a light microscope, with predominant cell type used to indicate cycle state. Proestrus was characterized by flat, circular squamous cells with the absence of macrophage infiltration. Estrus was defined as cornified, keratinized cells and further confirmed the day after by determining presence or absence of macrophage infiltration and nucleated cells. Diestrus was defined by the presence of thickened epithelial cells with absence of abundant cells from the stratum granulosum.

_Carbon-fiber microelectrodes_

CFM’s were manufactured according to (Samaranayake et al., 2016). In brief, 7 um-diameter carbon fibers (Goodfellow Inc, Coraopolis, PA) were aspirated into glass capillary tubes (OD 0.6mm, ID 0.4mm, A-M Systems INC, Sequim, WA). A vertical pipette puller was used to seal the carbon fiber to the capillary tube (Narishige Group,
Tokyo, Japan). The carbon fiber was then trimmed to the necessary 150 um under a low-power light microscope. All microelectrodes are Nafion-coated as per (Hashemi et al., 2009).

**Animal Surgery**

Prior to surgery, animals were anesthetized using 2-3% sevoflurane inhalant (manufacturer). Full anesthesia was determined via a tail-pull and foot pinch. Once the animal was fully anesthetized, it was placed in the stereotaxic apparatus and the head was leveled with respect to Bregma (David Kopf Instruments, Tujunga, CA). A heating pad (37degreeC) was placed under the animal throughout the course of the experiment. The nucleus accumbens stereotaxic coordinates were placed at XX, YY, ZZ for the carbon-fiber recording electrode and XX YY ZZ for the stainless-steel stimulatory electrode (0.2mm diameter, Plastics One, Roanoke VA). Biphasic pulse trains applied through a linear constant current stimulus isolator (NL800A, Neurolog; Medical Systems Corp., Great Neck NY) stimulated dopamine efflux. Background-subtracted cyclic voltammograms are collected as voltage (y-axis) versus time (x-axis) and current (pseudocolor). Immediately after voltammetric recording, animals were transcardially perfused with ~100 ml of ice-cold phosphate buffered saline, prior to ~300 ml of freshly prepared 4% paraformaldehyde (Sigma-Aldrich). Brains were then stored in 4% paraformaldehyde prior to additional processing.
Data Analysis

Data were analyzed via SPSS version 22 (IBM). Analysis of FAST scan cyclic voltammetry signal amplitude (indicative of DAT activity) was analyzed by a 2 X 2 ANCOVA with genotype and gender as between subject factors with time as a covariate, evaluated at 14.5 seconds.

RESULTS

Dopamine release in the nucleus accumbens

Dopamine release in the NAcc is dependent genetic condition, F(1,8395)=239.776, p<0.001; and rat gender, F(1,8395)=37.597, p<0.001 (Fig. 1). HIV-1 Tg rats release less dopamine as a function of time during electrostimulation. In addition, a significant interaction was found F(1,8395)=7.355, p<0.01, with dopamine release being lowest in HIV-1 transgenic female rats.

MPH drug detection

Drug detection was analyzed by fitting linear regression on days of MPH addition and days without. Comparison of the fits were not significant, F(1,19)=0.05905, p=0.8083 (Fig. 2). The slopes between each regression line were not statistically significant, indicating acquisition and detection of MPH in the sucrose solution was the same between both HIV-1 Tg animals and F344 controls.

MPH Self-Administration

Piecewise linear regression analysis of MPH self-administration in the 26% sucrose solution revealed a differing acquisition rate of administration in the HIV-1 Tg
rats, F(1,269)=4.36136, p<0.05 (Fig. 3). The slope of the HIV-1 TG animals during the first 7 days is significantly increased compared to F344 controls, p<0.05.

**Locomotor behavioral sensitization**

For the MPH sensitization experiment, data was analyzed via repeated-measures ANOVA with group as a between-subject factor and day as the within-subject factor. There was a significant effect of day, F(14,518)=3.326, p<0.001. The interaction of condition and day was not statistically significant, F(14,518)=1.864, p=0.055, indicating processing differences were not temporally different in the HIV-1 Tg animals (Fig. 4). HIV-1 Tg animals obtained less rewards than control animals, F(1, 37)=15.764, p<0.001. Linear regression revealed a significant deviation from 0 for the slope of rewards for control animals, F(1,298)=5.475, p<0.05. The slope for the regression line on rewards obtained for the HIV-1 Tg animals was not significantly different from zero. Number of rewards obtained for the HIV-1 Tg group was significantly higher on the retest day compared to the first day of testing, p<0.05, which was used as the primary indicator of drug sensitization.

**Dendritic Spine Morphology**

MPH has an equalizing effect on dendritic spine morphology of medium spiny neurons in the NAcc between Control and HIV-1 Tg rats. Spine length is not significantly different in MSNs of the NAcc when compared between groups. MSN spine head diameters are significantly increased in control rats, t(109297)=2.854, p<0.01. However, spine volume is not significantly different between HIV-1 Tg and control animals. In the prefrontal cortex, pyramidal spine length is slightly increased in the HIV-1 Tg rat, t(98863)=7.065, p<0.0001, along with pyramidal spine head diameter,
DISCUSSION

The above project takes a three-pronged approach to determine dopaminergic alterations in HIV-1 transgenic rats during MPH exposure from a molecular, cellular, and behavioral perspective. While there has been a long-established understanding of dopaminergic deficits in the brain of HIV-1 transgenic rats (Lee et al., 2014; Moran et al., 2012b; Moran et al., 2013b; Wayman et al., 2015a; Webb et al., 2010; Zhu et al., 2015), no endeavors have focused on dopamine transporter activity after oral administration of methylphenidate. The absence of critical analysis of the function of this medication in the brains of HIV-1 patients, who already have an increased susceptibility to psychostimulant abuse, is alarming; especially when considering the body of work focusing on deficits caused by illegal psychostimulants.

Accurate, unbiased, computer-assisted 3D analysis of dendritic spine morphology from MSN’s in the NAcc is critical to determine synaptic alterations and functional throughput abnormalities resulting in behavioral consequences in this preclinical model of HIV-1 disease. Our lab has pioneered this area using advanced analysis techniques (DiOlistic labeling, 3D modeling software) in the animal model, and was the first to publish population analyses of morphological parameters in MSNs (Roscoe Jr. et al., 2014). Previous work in this area has relied on 2D Golgi-staining, which is biased by plane-of-view and F-actin puncta staining, which does not allow for 3D morphometry. While 2D modeling of spiny neurons has been a landmark technique that has moved the
field forward in terms of neuronal structure, it falls short of true population analysis due to biases and unsystematic sampling. DiOlistics allows for unbiased, 3D random sampling of stained neurons (a random process) resulting a quantification of spine parameters from a total population. Comparing the distributions of each parameter allows us to take the most accurate, unbiased interpretation of the data and isolate clear morphological effects of HIV-1 Tat and MPH disruption on dendritic spine formation in the NAcc. Also, we will replicate this procedure in the PFC to determine if morphometry of spines on pyramidal cells undergoes similar changes or exhibit altered structure because of previous MPH exposure.

Additionally, the authors are not aware of any study using in vivo voltammetry to determine dopamine release and reuptake in the brains of HIV-1 transgenic rats. Fast-scan cyclic voltammetry allows for subsecond, real-time analysis of dopamine release from the NAcc and represents a cutting-edge technique to analyze DAT dynamics. Our previous work using slice voltammetry has revealed altered reuptake and clearance rate of dopamine in the NAc of HIV-1 transgenic rats (Javadi-Paydar et al., manuscript under review), but inclusion of intact animals eliminates environmental variables that may be confounding these results. In vivo voltammetry for analysis of monoamine turnover (in addition to excitatory amino acid detection) represents cutting-edge methods in neuronal crosstalk analysis that creates a temporal framework of the processes necessary for the brain to adapt to the environment. Detailing DA release and clearance at a sub-second resolution portrays the most accurate and high-resolution data available in the HIV-1 transgenic rat. At the very least, the proposed effort will build upon our previous work using no-net flux microdialysis of intact HIV-1 transgenic rat brains; this previous effort
has specified the early DAT alterations caused by intra-accumbal inclusion of Tat, supporting theories of near-instant modulation of the DAT by Tat (Ferris et al., 2009c; Zhu et al., 2011a).

Increased severity of behavioral alterations in the female HIV-1 transgenic rat has been recently characterized (McLaurin et al., 2016; Roscoe Jr. et al., 2014).

Lastly, MPH self-administration has only just been reported using wild-type rat strains (Thanos et al., 2015). While Thanos et al., (2015) used very high doses of MPH in drinking water, our paradigm will use a maximum dose of 8mg/kg (corresponding to 120 rewards on an FR1 schedule) tailored to each rat’s body weight. The drug will be masked in a sucrose solution (5-26%) to prevent conditioned taste aversion to the drug’s psychostimulatory effects, and to increase motivational response for rats with low generalized responding rates. Upon completion of this experiment, our lab will be the first to report locomotor sensitization and altered consumption patterns of HIV-1 transgenic rats to self-administration of MPH.
Figure 3.1 Dopamine release is decreased in the HIV-1 Tg rat, especially in females. Dopamine release in the NAcc is dependent genetic condition, $F(1,8395)=239.776$, $p<0.001$; and rat gender, $F(1,8395)=37.597$, $p<0.001$. HIV-1 Tg rats release less dopamine as a function of time during electrostimulation. In addition, a significant interaction was found $F(1,8395)=7.355$, $p<0.01$, with dopamine release being lowest in HIV-1 transgenic female rats.
Figure 3.2 Response rates were similar between HIV-1 Tg rats and controls during the drug detection task. Drug detection was analyzed by fitting linear regression on days of MPH addition and days without. Comparison of the fits were not significant, F(1,19)=0.05905, p=0.8083. The slopes between each regression line were not statistically significant, indicating acquisition and detection of MPH in the sucrose solution was the same between both HIV-1 Tg animals and F344 controls.
Figure 3.3 Acquisition of MPH oral self-administration is altered in HIV-1 Tg rats. Piecewise linear regression analysis of MPH self-administration in the 26% sucrose solution revealed a differing acquisition rate of administration in the HIV-1 Tg rats, $F(1,269)=4.36136$, $p<0.05$. The slope of the HIV-1 TG animals during the first 7 days is significantly increased compared to F344 controls, $p<0.05$. 
Figure 3.4 Locomotor sensitization is apparent in an increased acquisition rate in both basic and fine movements in the female HIV-1 Tg rat. For the MPH locomotor sensitization experiment, data was analyzed via repeated-measures ANOVA with group as a between-subject factor and day as the within-subject factor. There was a significant effect of day, $F(14,518)=3.326, p_{agg}<0.001$. The interaction of condition and day was not statistically significant, $F(14,518)=1.864, p=0.055$, indicating processing differences were not temporally different in the HIV-1 Tg animals.
Figure 3.5 DiOlisite labeling of medium spiny neurons from the NAcc and pyramidal neurons from the PFC. Three neurons were analyzed per animal resulting in a total number of ~55,000 spines analyzed per group. MPH addition results in similar dendritic spine morphology in both HIV-1 Tg and F344 rats. F344 medium spiny neurons (a) and HIV-1 Tg medium spiny neurons (c) have similar dendritic spine morphological parameters, including length, head diameter, and volume.
A

B

C

D

E

F

Con
HIV-1 Tg
MSN Spine Length (µm)
Relative Frequency (%)

MSN Spine Length (µm)

MSN Head Diameter (µm)

Head Diameter (µm)

MSN Spine Volume (µm³)

MSN Spine Volume (µm³)

Con
HIV-1 Tg

Con
HIV-Tg

Con
HIV-1 Tg

Con
HIV-Tg

Con
HIV-1 Tg

Con
HIV-Tg
Figure 3.6 MPH has an equalizing effect on dendritic spine morphology of medium spiny neurons in the NAcc between Control and HIV-1 Tg rats. Spine length is not significantly different in MSNs of the NAcc when compared between groups. MSN spine head diameters are significantly increased in control rats, t(109297)=2.854, p<0.01. However, spine volume is not significantly different between HIV-1 Tg and control animals. In the prefrontal cortex, pyramidal spine length is slightly increased in the HIV-1 Tg rat, t(98863)=7.065, p<0.0001, along with pyramidal spine head diameter, t(93976)=8.952, p<0.0001. However, pyramidal spine volume is not significantly different between groups, p=0.707.
CHAPTER 4

GENERAL DISCUSSION
Detailing DA release and clearance at a sub-second resolution portrays the most accurate and high-resolution data available in the HIV-1 transgenic rat. The present voltammetry work builds upon Ferris et al., (2012), which used no-net flux microdialysis of intact HIV-1 transgenic rat brains. This previous effort has specified the early DAT alterations caused by intra-accumbal inclusion of Tat, supporting theories of near-instant modulation of the DAT by Tat (Ferris et al., 2009c; Zhu et al., 2011a). Tat-induced modulation of the DAT (Bertrand et al., 2013; Zhu et al., 2011; Zhu et al., 2015) results in a 33% reduction in dopamine efflux kinetics (Ferris et al., 2009b) of active transport into the cell, resulting in extracellular hyperdopaminergia. Hyperdopaminergia is thought to cause stereotypical behaviors in rodents, and effectiveness of typical antipsychotic therapeutics is modulated through dendritic spine quantity in projections to the PFC (Kim et al., 2015). The dopamine receptor-populated medium spiny neurons of the nucleus accumbens should be morphologically affected by the chronic state of hyperdopaminergia caused by Tat, and Chapter 2’s results show a shorter, stubbier spine phenotype in HIV-1 Tg rats. The decrease in slope for HIV-1 Tg male and female rats represents a decrease in dopamine release from cells of the NAcc, and the effect size is increased in female rats. Dopamine release is severely diminished in HIV-1 Tg female rats (Chapter 3) defined by a reduction in dopamine release and reuptake. The behavioral consequences are an overall reduced motivation to self-administer MPH.

The mechanism of the exacerbated decrease in dopamine release and reuptake in the female HIV-1 Tg rat is currently unknown. Female susceptibility to HIV-1 infection is increased compared to males, and women represent at least 50% of global HIV-1 infections (UNAIDS 2014). Cognitive dysfunction in HIV-1 positive females is
increased compared to HIV-1 negative females, albeit with small effect sizes (Maki et al., 2015). However, the exacerbated decrease in dopamine release and reuptake seen in the HIV-1 Tg female rat relative to males in the voltammetry experiment of Chapter 3 indicate this gender difference as a necessary area of exploration for future work. The current direction has been investigation into the ER-beta receptor and the use of phytoestrogens as a therapeutic for Tat-induced synaptic disruption in vitro (Bertrand et al., 2014; Bertrand et al., 2015). Future pursuits using microdialysis in the presence of these compounds could mark a step from an in vitro to in vivo approach to understanding the mechanism behind ER-beta modulation of Tat-induced neuronal damage. Additionally, the necessity for gender-controlled effects in NIH-funded study is evident in the results of the voltammetry findings, and greater focus on pre-clinical sex-specific effects in disease models will allow for a much more appropriate planning for future directions of study. As much of past work, both clinical and pre-clinical, have focused on males; inclusion of sex-specific effects and focus on female-specific reactions to disease pathologies are lacking in the literature compared to their male counterparts.

Over the 14-day period, HIV-1 Tg rats experienced a different procedural shift in the acquisition process to self-administer MPH. This procedural shift reveals a delayed acquisition of MPH self-administration in the control animals and, while the F344 females respond more for MPH, the HIV-1 Tg females have a sharper early-acquisition to a stable self-administration quantity over time. The data clearly shows a sharp increase in acquisition during the first week, followed by a steady period of fall in the second week for HIV-1 Tg rats. Following a two-week period of abstinence, mean self-administration was significantly increased compared to the first day of administration.
On the contrary, F344 controls experience their increase in acquisition later in time, over days 8-12, before sharply dropping off. The delayed rate-increase in F344 female rats indicates an increased susceptibility to MPH abuse in a self-administration paradigm. While HIV-1 Tg rats also experience an increased sensitivity to cocaine self-administration (McIntosh et al., 2015), abusive doses of MPH appear to cause a more temporally acute period of increased daily use followed by stabilization. The locomotor activity data follows a similar pattern, simply with reduced overall responding in the HIV-1 Tg rat. The reflection of the motivational deficit between the HIV-1 Tg and the F344 rat is therefore reflected in both MPH self-administration and corresponding locomotor activity, supporting a theory of global motivational impairment across paradigms. Future longitudinal experiments are required to determine if the temporal dynamics of acquisition and addiction stabilize in the HIV-1 Tg rat as a function of time.

The equalizing effect of MPH on dendritic spine morphology of the NAcc and PFC speaks to the efficacy of self-administered MPH as a stimulant in the female HIV-1 Tg rat. However, pyramidal spine head diameters of the PFC were altered relative to F344 control female rats. The manifestation of this effect could lie in the physical cross-talk between brain regions; i.e. an altered response from the basal ganglia to the prefrontal cortex results in altered spine head diameter on pyramidal neurons, without corresponding morphological changes in the NAcc. More work on the in vivo procedure using chemo- or opto-genetics to dissect the role of Tat in DAT modulation of synaptodendritic alterations in HIV-1 Tg rats, with or without exposure to MPH or other psychostimulants. As the formation of dendritic spines can be tracked with cytoskeletal markers (Bertrand et al., 2014), the technology is at the point where real-time in vivo
analysis of the process of synaptic formation can be visually achieved in the HIV-1 disease model. Due to the pharmacological cause-and-effect process of MPH on projections from the basal ganglia to the PFC, effects on dendritic spine morphology of the PFC would be expected, although the projected cause for an increase in spines with larger head diameters in HIV-1 Tg rats is unknown. Spine head diameters typically represent a transition to a mushroom type spine, which is a maturational peak of the spine’s functionality. As science moves to appreciate the real-time pharmacological functions of stimulants on the basal ganglia, tracking the motility of dendritic spines in drug abuse models will better describe both the function of dendritic spines and their effects on cellular communication.

MPH abuse resulted in a similar dendritic spine morphology on MSNs of the NAcc between both groups. Overall, the HIV-1 Tg rats took less MPH relative to F344 controls, but the morphological result on dendritic spines is similar. This may speak to the already hyperdopaminergic state in HIV-1 Tg rats being pushed further by MPH to match a physiological state to that of the higher-responding F344 controls. As HIV-1 Tg rats are generally smaller, the dosages were tailored to individual body weights to prevent this as a potential confound to the data. Therefore, although the HIV-1 Tg rats are physiologically ingesting less MPH than F344 controls, but the same morphological result is seen on dendritic spines of MSN’s of the NAcc. The interesting difference here is the significantly shorter head diameters seen in pyramidal neurons of the F344 controls relative to the HIV-1 Tg rats. Although a similar effect is seen in MSN’s, the physiological result in other parts of the brain are different depending on the level of extracellular dopamine which may or may not be increased due to Tat. As the prefrontal
cortex is the target of extensions from the basal ganglia, more work must be done in pyramidal neurons to determine if morphological alterations occur with other psychostimulants. Future work using a progressive-ratio schedule of reinforcement could answer the willingness of the animals to work for MPH when tasked with frustratingly increasing requirements for drug. Teasing out behavioral alterations using various schedules of reinforcement will allow for better interpretation of the morphological alterations and functional deficiency experienced in the female HIV-1 Tg rat. Additionally, using *in vivo* voltammetry while simultaneously tracking monoamine transporter activity would be the most informative in determining the behavioral processes associated with altered MPH addictive processes in the HIV-1 Tg rat. The alterations to dopamine turnover, and its possible morphological consequences, can be better understood with a greater focus on behavioral abnormalities to motivation.

These overall results are indicative of a possible fundamental difference in the acquisition of abusive psychostimulants in the human female HIV-1 population. Altered therapeutic strategies for HIV-1+ women that abuse psychostimulants may be necessary upon diagnosis to ensure a positive prognosis and proper medication adherence. Additionally, children with HIV-1 are currently being prescribed MPH, and more investigation into the possible addictive effects or subsequent alterations to the dopamine processing system of the basal ganglia are necessary to ensure therapeutic safety and efficacy of MPH in this population. As women are currently underrepresented in the literature, this work of the analysis of the female HIV-1 Tg rat and the determination of sex-specific effects in dopamine transport highlight the necessity for future pre-clinical study to ensure control for sex as a biological variable.


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APPENDIX A: PERMISSION TO REPRINT