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Behavioral and Voltammetric Analysis of Chronic Escitalopram Treatment to the HIV-1 Transgenic Rat: Implications for Comorbid HIV-1 and Clinical Depression

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BEHAVIORAL AND VOLTAMMETRIC ANALYSIS OF CHRONIC ESCITALOPRAM
TREATMENT TO THE HIV-1 TRANSGENIC RAT: IMPLICATIONS FOR COMORBID
HIV-1 AND CLINICAL DEPRESSION

by

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Submitted in Partial Fulfillment of the Requirements

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2018

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DEDICATION

I dedicate this thesis first and foremost to my parents, Gary and Elizabeth. Without their unconditional support and kind words, I would have never made it to this point in my life. Through their consistent support, I was able to follow my decision to become the first member of my family to pursue a PhD and an academic path. Next, I dedicate this thesis to my core group of friends at in Virginia and Tennessee. Throughout the mini vacations, late night antics and memorable moments, you kept me motivated and driven while never letting me forget to have a laugh and a good time.

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ABSTRACT

HIV-1 infection is a serious condition affecting approximately 37 million individuals. Between 30% and 60% of seropositive individuals will develop symptoms of clinical depression. These individuals are five times more likely to commit suicide than non-seropositive clinically depressed patients. Dysfunction in serotonergic and dopaminergic transmission has consistently been implicated in the pathogenesis of depression. Specifically, dysfunction in the prefrontal cortex and in the nucleus accumbens core region have been shown to be underlying factors in the trajectory of depression. Given these underlying neurological features, the present research employed behavioral testing and electrochemical recording in an attempt to elucidate the therapeutic efficacy of the SSRI escitalopram in treating HIV-1 mitigated depressive symptoms in a transgenic (Tg) rodent model of depression. The HIV-1 Tg rat contains seven of the nine genes present in the HIV viral genome and presents itself with impairments similar to those observed in human HIV-1 seropositive individuals. The HIV-1 Tg rat thus represents a non-infectious model for controlled HIV-1 exposure. Here, we report failure of the SSRI to attenuate behavioral and electrochemical alterations present in the HIV-1 Tg rat. Given the known variability of SSRI medication and previously documented individual differences to drug dosage found within the model, it is thus concluded that more research is required in order to firmly establish the global efficacy of escitalopram in treated comorbid HIV-1 clinical depression.

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CHAPTER 1

INTRODUCTION

HIV is a serious condition affecting approximately 37 million people worldwide as of 2017. Consequently, the condition is estimated to affect roughly 0.8% of adults ages 15-49 (World Health Organization, 2017). In that same year, approximately 37,000 new cases were estimated to have occurred, many of which remained unknown to affected individuals (World Health Organization, 2017). While the advent of combination antiretroviral therapy (cART) has dramatically increased the prospects of living with HIV, individuals affected still suffer from deficits associated with the condition. Adherence to cART has the potential to reduce the viral load in the periphery, though the virus still persists in the central nervous system (Bertrand et al., 2013). HIV is neuroinvasive and invades the nervous system rapidly after initial exposure, where it principally infects microglia. The neurovirulent effects of HIV infection often include neuropathy, dementia, myopathy, and myelopathy (Silvers et al., 2006).

In the United States, an estimated 30-60% of HIV-infected persons will develop some degree of clinical depression over the course of their lifetime (Bhatia and Munjal, 2014, Castellon et al., 1998). Moreover, the prevalence of suicide among such infected individuals is three to five times higher than individuals not affected with HIV, despite treatment for antiretroviral therapy (US Department of Veterans Affairs, 2009). Apathy, a distinct but highly correlated condition, also remains a common psychiatric disturbance among HIV infected individuals. This alarming comorbid prevalence is however

unsurprising given the well-documented relationship between apathy and depression (Castellon, et al., 1998; Marin, Firinciogullari, and Biedrzycki, 1993). In addition to depression and apathy, approximately 50% of seropositive individuals will develop some degree of HIV-associated neurocognitive disorder (HAND) despite adherence to cART. (Sanmarti, 2014 Bryant et al., 2015).

The development of apathy is attributed directly to the effects of viral infection, specifically to the consequent expression of the neurotoxic proteins Tat and gp120. These proteins are hypothesized to produce deleterious effects upon the dopaminergic system and the neural circuitry underlying reward pathways (McIntosh et al., 2015). The alarming degree of dopaminergic dysfunction present during HIV infection has been extensively documented in both clinical and pre-clinical literature (Aksenov et al., 2008; Bertrand et al., 2013; Ferris, Mactutus, and Booze, 2008; Fitting et al., 2008; Fitting et al., 2015; Javadi-Paydar et al., 2017; Nath et al., 2000; Paris et al., 2014; Purohit, Rapaka, and Shurtleff, 2011; Roscoe, Mactutus and Booze, 2014; Silvers et al., 2006; Zhu et al., 2009; Denton et al., forthcoming).

The dynamic relationship between HIV-1 and clinical depression has enjoyed little consideration within the literature, however, as many reports have failed to produce prospects for any effective treatment regimes. To this end, the present work sought to examine the effects of treatment with escitalopram to the HIV-1 Transgenic (Tg) rat. The HIV-1 Tg rat contains 7 of the constituent 9 genes that comprise the HIV viral genome, resulting in a non-infectious, long-term model of HIV-1 viral protein exposure, which is an effective model of HIV seropositive individuals adhering to long term cART treatment. (Reid et al., 2001; Vigorito et al., 2015 McLaurin et al., 2018). The HIV-1 Tg

rat are produced using an infectious provirus derivation following deletion of the *SphI-Ball* fragment that encompasses the *gag* and *pol* genes of the virus, rendering the model non-infectious. Production of proteins such as the deleterious *tat* and *gp120* remain under the control of the LTR promoter resulting in protein expression that is thought to undermine monoamine function (Reid et al., 2001; Bertrand et al., 2018; Denton et al., forthcoming). The HIV-1 Tg rat has previously demonstrated compromised synaptodentric connectivity in the nucleus accumbens core region, in addition to compromised rates of dopaminergic and serotonergic function in the nucleus accumbens core and prefrontal cortex, respectively (Roscoe et al., 2014; Denton et al., forthcoming).

Escitalopram is a selective serotonin reuptake inhibitor marketed under the trade name Lexapro and is commonly prescribed to individuals suffering from depression. Moreover, the Veteran's affairs administration considers escitalopram to be a safe accompaniment to cART therapy (US Department of Veterans Affairs, 2009). Thus, the present study sought to directly examine the effects of escitalopram treatment upon a behavioral testing battery comprised of five tasks commonly used to gauge depressive symptoms in rodent research, followed by examining the real-time release and reuptake kinetics of serotonin and dopamine as measured by fast-scan cyclic voltammetry (FSCV). For the present purposes, a five bottle sucrose concentration task, modified hole board task, elevated plus maze task, pre-pulse inhibition (PPI) of the visual and acoustic startle task and a social behavior task were used to evaluate the behavioral effects of escitalopram treatment in HIV-1 Tg versus F344/N rats.

Sucrose preference testing in rodents is used to gauge levels of anhedonia. Rats naturally consume sweet solutions as a preference over unsweet solutions. Decreased

consumption of a mixture of sucrose and water relative to consumption of distilled water alone is a behavioral marker indicative of anhedonic behavior in rodents (Serchov, Calker and Biber, 2016). Moreover, sucrose preference testing has previously been employed in studying the HIV-1 Tg rat (Bertrand et al., 2018). Anhedonia is classically considered a core symptom of clinical depression (Nestler, 2002). The modified hole board task gauges exploratory behavior – an indication of anxiolytic and depressive symptoms in rats (Takeda, Tsuji and Matsumiya, 1998). Such symptoms are known to be present if fewer hole pokes are performed by a rodent relative to age matched control animals. Nose pokes into the holes of the apparatus are recorded via photocells positioned underneath the platform. Pre-pulse inhibition of the acoustic and visual startle are commonly used in depression research in both human and pre-clinical populations (Perry et al., 2004; Fletcher et al., 2001). Moreover, previous research has demonstrated that the HIV-1 Tg rat possesses significant deficits in pre-pulse inhibition (Moran et al., 2013; McLaurin et al., 2017 McLaurin et al., 2018). The elevated plus maze measures exploratory behavior as a ratio of the time the rodent spends on an open and exposed arm of the maze versus time spent in closed locations of the maze . Greater time spent in closed locations of indicative of heightened levels of anxiety and depressive behaviors (Pellow, et al.,1985). Total time spent in each arm is recorded with behavioral tracking software. Social interaction is considered to be a measure of anxiety in rodents, and has previously been employed in antidepressant research (Griebel et al., 1994; Dekeyne et al., 2000; Rodriguez-Porcel, 2001).

Fast Scan Cyclic Voltammetry (FSCV) is an electrochemical technique that allows for the rapid detection of a broad range of chemical species. FSCV is a more

specialized form of cyclic voltammetry in which a considerably higher scan rate is employed allowing for the rapid acquisition of an individual voltammogram. Thus, the technique has a considerable temporal resolution. FSCV enjoys a competitive advantage over other available techniques in that concentration of the target analyte is observed in real-time, with quantification of the target analyte occurring rapidly. Moreover, positive identification of the target analyte is provided via the cyclic voltammogram produced throughout the recording period (Robinson, et. al, 2003).

FSCV involves the application of a triangular waveform within a target analyte specific voltage range to an electrode. The resting potential is held at a voltage that is insufficient to oxidize the target analyte, then rapidly increased at a high scan rate to a target potential to promote oxidation. This oxidation produces a detectable electrical current. The potential is then returned to starting potential, thus produce a detectable change in electrical current in the opposite direction. This cycle of oxidation and reduction produces time-resolved peaks that allow for the quantification of the target analyte.

The technique was initially developed by Mark Wightman in the 1980's, and first used to characterize dopaminergic transmission and reuptake in the caudate nucleus of the rat (Millar et. al, 1985). Since this initial development, FSCV has enjoyed a wide range of application to the study of dopaminergic transmission in single cells, brain slices, anesthetized animals, freely moving animals, and even awake and behaving animals (Budygin, et. al, 2001; Kelly and Wightman, 1987; Kuhr, Wightman and Rebec, 1987; Millar et al., 1985; Phillips et. al, 2003; Robinson et. al, 2003; Troyer and Wightman, 2002).

While the quantification of dopamine with FSCV enjoyed a long history of consistent replication, quantification of serotonin did not occur until much more recently. The first in-vivo recording of 5-hydroxytryptamine (5-HT or serotonin) in the mammalian brain occurred as recently as 2009 (Hashemi et. al, 2009). This was achieved through modification to the standard carbon fiber electrode with Nafion, a cation exchange polymer. While the oxidation of dopamine to dopamine-o-quinone is a relatively clean and predictable process, the oxidation of serotonin produces by-products that polymerize and permanently coat the surface of the carbon fiber microelectrode (Hashemi et. al, 2009). The Nafion coating prevents the by-products of oxidation from damaging the surface of the electrode (Hashemi et. al, 2009).

CHAPTER 2

MATERIALS AND METHODS

2.1 ETHICS STATEMENT

Experiments were conducted in accordance with the recommendations listed in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The research protocols were approved by the Institutional Animal Care and Use Committee at the University of South Carolina (assurance number: D16-00028).

2.2 SUBJECTS

Animals (n=73; HIV-1 Tg=31, F344/N=42) were obtained from Envigo, (Indianapolis, IN) and pair housed under targeted conditions of $21^{\circ}\pm 2^{\circ}$ °C, 50 % \pm 10 % relative humidity with a 12 hour light: dark cycle.. Animals were pair housed by both sex and genotype. Food (Pro-Lab Rat, Mouse, and Hamster chow # 3000) and water were available *ad libitum* throughout the duration of the experiment. All behavior tasks were conducted during diurnal hours.

2.3 DRUG TREATMENT

Escitalopram (4 mg/kg for 40 days) (Sigma Aldrich, Saint Louis, MO) or placebo pellets (Innovative Research of America, Sarasota, FL) were subcutaneously implanted in the medial neck area of each animal. Animals were anesthetized using a 2-3% concentration of sevoflurane. Fur was removed from the target area and a small (approximately 3 mm) incision was made into which the pellet was placed. Incisions were then sutured and each animal was administered a bodyweight dependent dose of

butorphanol and placed in a recovery chamber with a heating pad. Animals were returned to their home cages after full locomotor recovery occurred. Animals were monitored for one week in post-operative care before beginning behavioral testing. Animal body weight and well-being were monitored daily to ensure compliance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health.

2.4 ESTROUS CYCLE TRACKING

Vaginal lavage was performed on each day of the testing period to determine cycle stage of female rodents. Each lavage was performed with approximately 1 mL of freshly prepared phosphate-buffered solution. The solution was administered to the vagina of the rat with a standard eye dropper and quickly retracted. The solution was then evaluated under a low-power light microscope to determine cycle stage (Booze et al., 1999; Westwood, 2008).

2.5 SUCROSE PREFERENCE

Animals were individually placed in an empty testing chamber with free access to 0%, 1%, 5%, 10% and 30% concentrations of sucrose solution in 100 ml graduated cylinders equipped with stopper and drinking tube (Ancare, Bellmore, NY). Habituation to the five bottle presense occurred two consecutive days prior to testing period. Following habituation, animals were tested for 30 minutes per day for five consecutive days. Sucrose consumption was measured both with respect to meniscus volume and cylinder weight. Cylinder order was randomized daily using a Latin square design to control for any effect of cylinder position upon sucrose consumption.

2.6 MODIFIED HOLE BOARD

A custom made delrin insert equipped with 16 equidistant holes was placed inside a 40 cm³ locomotor activity chamber. Nose pokes into each hole was recorded by photocells placed below the custom insert. Each nose poke was recorded by FlexField Software (SanDiego Instruments, San Diego CA). Recording sessions occurred for 10 minutes each day for a period of 7 consecutive days following a 10 minute habituation period. The apparatus was cleaned with a 10% ethanol solution following the testing period. Testing was performed in the presence of 70db background white noise in a darkened room to encourage exploratory behavior.

2.7 ELEVATED PLUS MAZE

Each animal received a single testing session in an elevated plus maze apparatus. Behavior was recorded by a camera mounted above the apparatus. The dependent measure of interest was time spent in the open arm of the apparatus versus time spent in the closed arm of the apparatus. Overall activity was recorded with SMART tracking software (San Diego Instruments, San Diego CA). The apparatus was cleaned with a 10% ethanol solution between consecutive trials. Female animals were tested while in diestrus to control for any effect of estrus hormonal cycle upon exploratory animal. Failed trials (e.g. animal falling from apparatus) were retested one week later. This time in between trials was used to ensure no effect of learning/habituation upon subsequent plus maze trials.

2.8 PREPULSE INHIBITION OF ACOUSTIC AND VISUAL STARTLE

Animals were placed in a startle chamber (SR-Lab Startle Reflex System, San Diego Instruments) enclosed in an isolation cabinet (Industrial Acoustic Company) and

acclimated to the presence of 70dB background noise for a period of 5 minutes. The subjects were then presented with a series of six pulse-only trials at 100dB. Following this acclimation period, subjects were presented with 36 pre-pulse trials of 85dB with interstimulus intervals of 0, 8, 40, 80, 120 and 4,000 milliseconds assigned in a Latin square procedure. The stimulus occurred for 20 milliseconds. 0 and 4000 millisecond intervals were included to provide a baseline acoustic startle response. The apparatus was cleaned thoroughly with ethanol solution between each trial.

2.9 SOCIAL AND PLAY BEHAVIOR

On the day of testing, animals were habituated to the testing room for a period of 10 minutes. Animals were then placed into an empty testing chamber with a bodyweight and sex matched novel partner. Rodent interaction was recorded for a period of 10 minutes. Total interaction time was recorded as a dependent measure of social behavior. Females were tested in diestrus. Successive trials were conducted in previously cleaned cages to account for any bias due to novel smell or debris.

2.10 MANUFACTURE OF CARBON FIBER MICROELECTRODES

Carbon fiber microelectrodes were manufactured by aspirating 7 μm diameter carbon-fibers (Goodfellow Inc, Coraopolis, PA) into glass capillaries (0.6mm external diameter, 0.4mm internal diameter, A-M Systems Inc., Sequim, WA). Fibers were sealed into the capillaries with a vertical pipette puller (Narishige Group, Tokyo, Japan). The exposed fiber was trimmed to approximately 150 μm under a low-light power microscope for evaluation of serotonin and histamine and to 50 μm for evaluation of dopamine. Nafion was electrodeposited onto the exposed carbon fiber portion of

serotonin and histamine electrodes as previously described and then dried at 70° C for 10 minutes (Hashemi et al. 2009).

2.11 IMPLANTATION OF CARBON FIBER MICROELECTRODES AND STIMULATING PIN

Animals were deeply anesthetized using 2-4% sevoflurane. The animal's head was placed into a stereotaxic apparatus (David Kopf Instruments, Tujunga, CA.), with a heating pad to maintain constant body temperature. Carbon fiber microelectrodes were placed into the animal's nucleus accumbens (AP: +2.6, ML: +1.6, DV: -5.8) and CA2 region of the hippocampus (AP: -5.5, ML: +5.0, DV: -4.0), for evaluation of dopamine and serotonin, respectively (Paxinos and Watson, 2014). A stainless steel stimulating electrode (Plastics One, Roanoke VA) was implanted in the medial forebrain bundle (AP: -2.8, ML: +1.7, DV: -8.0), while a chloride electroplated silver reference electrode was placed in the hemisphere opposite the stimulating electrode. To stimulate the release of the neurotransmitters, biphasic pulse trains were applied through a NeuroLog linear constant current stimulus isolator (NL800A, Neurolog; Medical Systems Corp., Great Neck, NY). Background-subtracted cyclic voltammograms were obtained as time vs. voltage (x-axis by y-axis).

2.12 TERMINATION (SACRIFICE) OF ANIMAL

Following the conclusion of the recording session, animals were sacrificed under deep anesthesia. Brains were removed and stored in a -80°C freezer. Animals not used for voltammetry were sacrificed *via* transcardial perfusion of approximately 100 mL of freshly prepared paraformaldehyde. Brains were then removed and stored in paraformaldehyde for further analyses.

2.13 BEHAVIORAL DATA ANALYSIS

All statistical analyses were performed using IBM SPSS (version 24) where a *p*-value of less than 0.05 was considered to be statistically significant. For evaluation of sucrose preference, a mixed factorial ANOVA was utilized where transgene, sex and treatment were held as between subject factors while variable concentration of sucrose was held as a within-subjects factor. Similarly, a mixed factorial ANOVA was used for evaluation of pre-pulse inhibition where transgene, sex and treatment were held as between subject factors while variable inter-stimulus interval was held as a within-subjects factor. To evaluate elevated-plus maze performance and social behavior, a factorial ANOVA was employed to examine the effects of treatment, sex and transgene. All graphs were produced with GraphPad prism (version 5). Rodent age was held as a covariate across all analyses.

2.14 VOLTAMMETRIC DATA ANALYSIS

Recordings were obtained using customized software. LabView (Knowmad Technologies LLC). Color plots of the evoked chemicals were generated within the data analysis features of the custom software. GraphPad Prism (version 5) was used to produce current versus time plots for each neurotransmitter of interest. Peak concentrations of neurotransmitter release were analyzed using IBM SPSS (version 24) with a factorial ANOVA comparing factors of the transgene (HIV-1 Tg vs. F344/N) and sex. P-values less than or equal to 0.05 were considered statistically significant. Rates of release and reuptake of individual analytes were calculated using GraphPad prism where *K*, a nonlinear rate constant, was evaluated for both release and reuptake. Peak concentration values were obtained from the raw evoked electrical current. Female

rodents were evaluated during diestrus to control for any bias due to fluctuation of circulating hormones. All terminal sacrifices of rats were conducted during diestrus in a similar manner. This procedure was done in order to eliminate sources of variation for future analyses.

CHAPTER 3

RESULTS

3.1 SUCROSE PREFERENCE

Escitalopram was not found to alter sucrose consumption in either HIV-1 Tg animals or F344/N control animals. Linear and non-linear modeling of the response to concentration curves revealed a transgene mediated alteration of response, with a shift in response to sucrose concentration occurring in HIV-1 Tg animals independent of drug treatment. Dose-dependent responding to sucrose concentrations proceeded in a logarithmic fashion for these animals, while, in contrast, dose-dependent responding in F344/N control animals proceeded in a linear fashion. Separate models were fit to the data to illustrate this finding. (All $r^2 > 75\%$). (Figure 3.1).

3.2 MODIFIED HOLE BOARD AND ELEVATED PLUS MAZE

A mixed model ANOVA was used to evaluate the effects of genotype, sex, treatment and their interactions upon the within-subjects factor of overall exploration (hole pokes, open arm exploration) (Figures 3.2 and 3.3). A significant interaction was found for genotype and sex with respect to exploration [$F(1,71)=3.59, p \leq 0.05$]. Rats treated with escitalopram explored significantly more relative to placebo treated animals across elevated plus maze trials. [$F(1,72)=4.21, p \leq 0.05$]. While global effects of escitalopram treatment were not found across both tasks together, escitalopram significantly increased exploration in elevated plus maze trials. Males showed the lowest

response to escitalopram independent of genotype across both trials, though the effect was not statistically significant [$F(1,72)= 1.11, p=ns$]

3.3 PREPULSE INHIBITION OF ACOUSTIC AND VISUAL STARTLE

Significant main effects of genotype were reported for both visual (Figure 3.4) and auditory (Figure 3.5) PPI, with HIV-1 Tg animals demonstrating significant impairment relative to controls [visual PPI, $F(1,72)=12.12, p\leq 0.01$; auditory PPI, $F(1,72)=8.38, p\leq 0.05$]. Although escitalopram treatment did appear to alter both auditory and visual PPI in transgenic rats, no interaction effects or other main effects were found to be statistically significant. HIV-1 Tg rats treated with escitalopram demonstrated significant visual PPI impairments relative to placebo treated animals and F344/N animals, suggesting a visual effect of chronic escitalopram treatment.

3.4 SOCIAL AND PLAY BEHAVIOR

No significant main effects for between-subjects factors of genotype, sex or drug treatment. Additionally, no two-way or three way interactions were found [$F(1,72)=3.83, p=ns$]. Overall, total time in contact between rodents was stable regardless of sex, genotype or treatment condition (Figure 3.6).

3.5 DOPAMINE AND SEROTONIN VOLTAMMETRY

Decreases in peak transmission and reuptake of dopamine and serotonin were found in the HIV-1 Tg rat. Evoked dopamine and serotonin concentrations for HIV-1 Tg animals and F344 animals are displayed in figures 3.8 and 3.9 while color plots with respective cyclic voltammograms are displayed in figure 3.7. Maximal evoked concentration (C_{max}) was impaired in transgenic animals across both dopamine and serotonin recordings. [Dopamine, $F(1,9)=33.25, p\leq 0.001$; For serotonin, $F(1,16)=60.97,$

$p \leq 0.001$]. Additionally, rates of reuptake as defined by nonlinear rate constant (k) were impaired in transgenic animals relative to control animals. [For dopamine, F344/N $K=0.43$, HIV-1Tg $K=0.73$ $F(1,2634)=19.19$, $p \leq 0.001$; For serotonin, F344/N $K=0.37$, HIV-1Tg $K=0.56$ $F(1,4314)=7.308$, $p \leq 0.05$].

Increases in serotonin transmission were found in F344/N control animals treated with escitalopram, but not in HIV-1 Tg rodents (Figure 3.9). Rates of clearance (reuptake) were slower in animals treated with escitalopram ($k=0.55$) relative to animals treated with placebo ($k=0.34$) [$F(1,4074)=9.18$, $p \leq 0.05$]. While F344/N animals treated with escitalopram displayed a mild increase in peak evoked serotonergic potential, the effect was not significant [$F(1,30)=0.99$, $p=ns$] although rates of reuptake were altered for animals treated with escitalopram [$k=0.32$ vs. $k=0.20$ $F(1,7674)=23.75$, $p \leq 0.01$].

No statistically significant differences were found for dopamine transmission in HIV-1 Tg rats treated with escitalopram [$F(91,150)=1.00$, $p=ns$ (Figure 3.8). Evoked rates of maximal dopamine release were not statistically significant across a genotype by treatment analysis [$F(1,18)=0.123$, $p=ns$.], though HIV-animals treated with SSRI medication demonstrated lowest peak concentration. Rates of reuptake were not statistically different for HIV-1 Tg animals treated with escitalopram [$k=0.41$ $F(1,1414)=0.47$, $p=ns$], though F344/N animals treated with escitalopram demonstrated slower rates of reuptake than animals treated with the placebo [$k=0.49$ vs. $k=0.23$ $F(1,3834)=16.1$, $p \leq 0.001$].

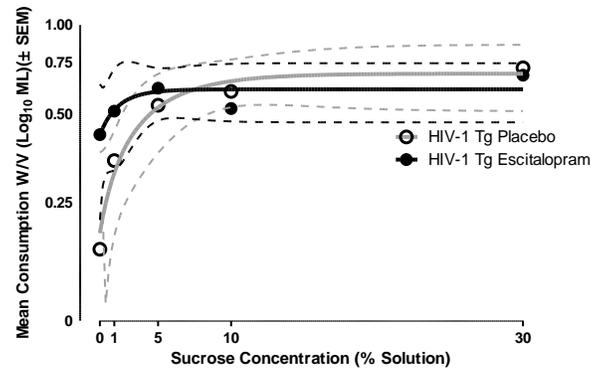
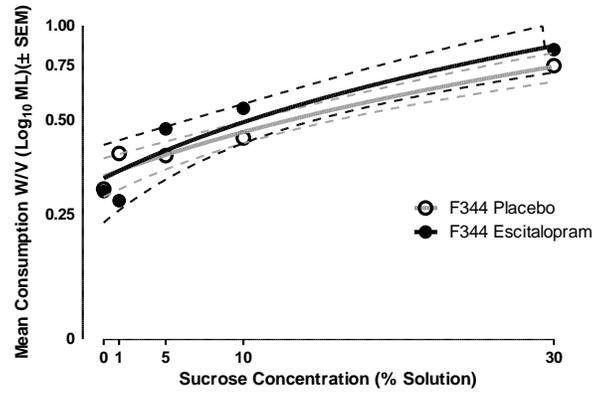


Figure 3.1: Five bottle sucrose preference test using a 0%, 1%, 5% 10% and 30% concentration. No effect of drug treatment was observed independent of animal genotype and sex. For HIV-1 Tg animals, a curvilinear shift in response occurred between 0% and 5% concentrations followed by a flattening of the dose-response curve between concentrations of 5% and 30%. F344/N animals displayed a steady linear dose response pattern. Separate models were fit to these data to illustrate this finding (all $r^2 > 0.75$).

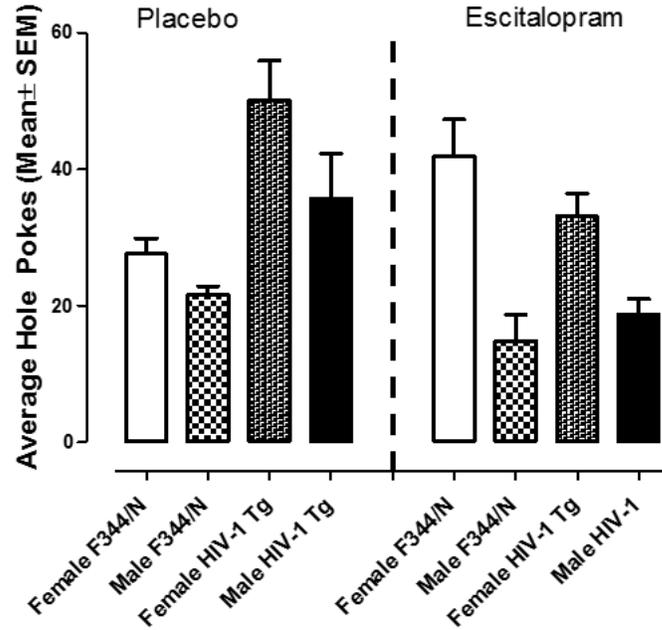


Figure 3.2: Modified hole board performance across each treatment group. Animals were tested in a custom build modified hole board apparatus across seven consecutive testing periods. Escitalopram was not found to increase number of nose pokes independently of genotype or sex. Males demonstrated lowest performance overall, though the effect was not statistically significant.

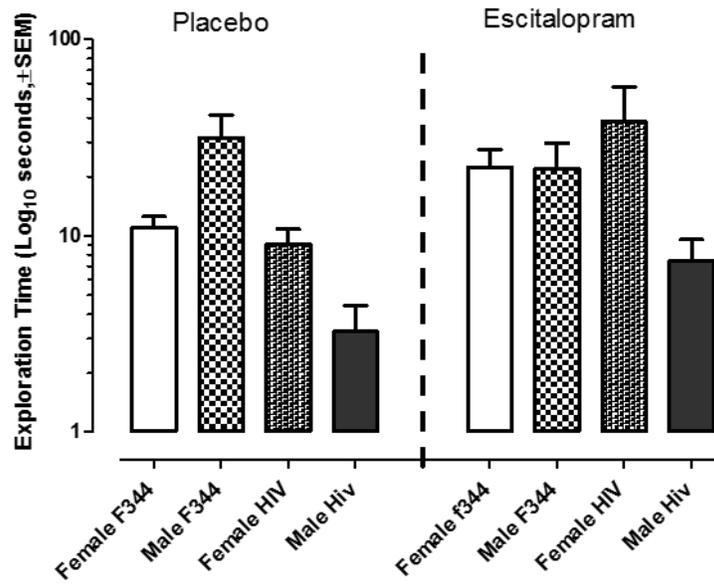


Figure 3.3: Exploration time in the open arm of an elevated plus maze apparatus. Animals were tested during one trial for a period of ten minutes. Escitalopram was found to increase time spent in the open arm independently of genotype and sex ($p < 0.05$).

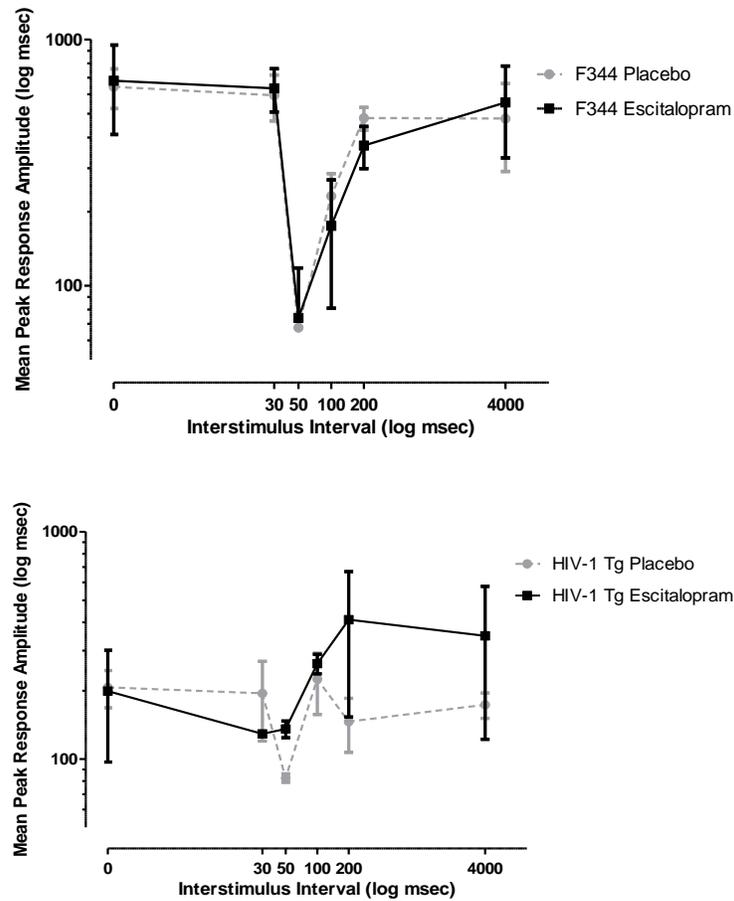


Figure 3.4: Visual prepulse inhibition. Animals were tested during one trial following a habituation trial which occurred the day before. Significant impairments were found in HIV-1 Tg animals relative to controls ($p < 0.05$). Escitalopram was not found to attenuate these deficits, however treatment did appear to alter PPI response in HIV-1 Tg rats.

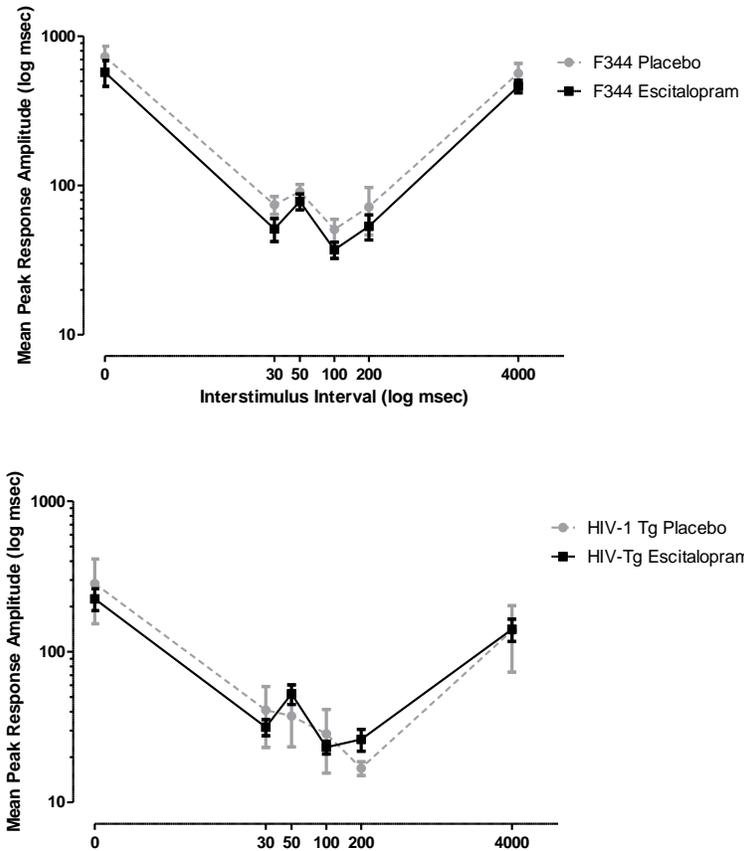


Figure 3.5: Auditory prepulse inhibition. Animals were tested during one trial following a habituation trial which occurred the day before. Significant impairments were found in HIV-1 Tg animals relative to controls ($p < 0.05$). Similarly to visual PPI, escitalopram was not found to attenuate these deficits.

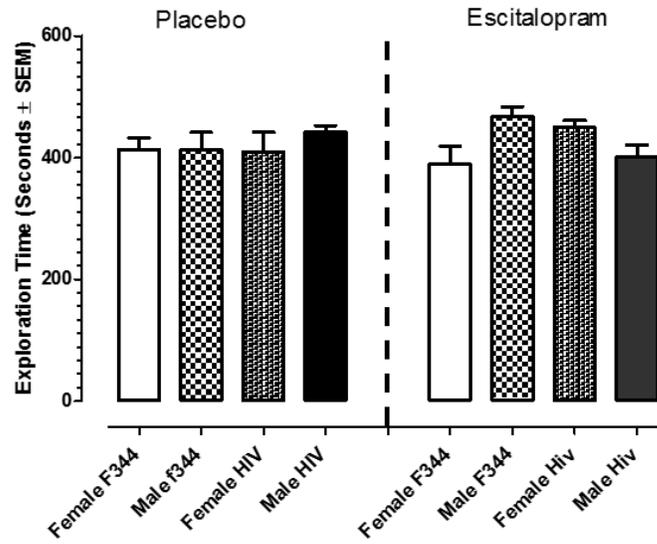


Figure 3.6: Social interaction time. Animals were tested with a novel sex and bodyweight matched partner in a novel cage across a ten minute trial period. Social interaction time was not found to be different regardless of group. No deficits in the HIV-1 Tg rat are present for social interaction time, and escitalopram was not found to alter interaction time across the trial period.

Table 3.1 Effect Sizes (partial η^2) for Behavioral Effects

3.1.1 Exploratory Behavior	
Genotype	0.04
Sex	0.17
Treatment	0.56
Genotype X Sex	0.48
Genotype X Treatment	0.04
Sex X Treatment	0.15
Genotype X Treatment X Sex	0.02
3.1.2 Visual Pre-Pulse inhibition	
Genotype	0.19
Sex	0.03
Treatment	<0.01
Genotype X Sex	<0.01
Genotype X Treatment	<0.01
Sex X Treatment	<0.01
Genotype X Treatment X Sex	<0.01
3.1.3 Auditory Pre-Pulse inhibition	
Genotype	0.14
Sex	0.04
Treatment	<0.01
Genotype X Sex	0.02
Genotype X Treatment	<0.01
Sex X Treatment	<0.01
Genotype X Treatment X Sex	<0.01
3.1.4 Social Interaction Time	
Genotype	<0.01
Sex	0.02
Treatment	<0.01
Genotype X Sex	0.02
Genotype X Treatment	<0.01
Sex X Treatment	<0.01
Genotype X Treatment X Sex	0.06

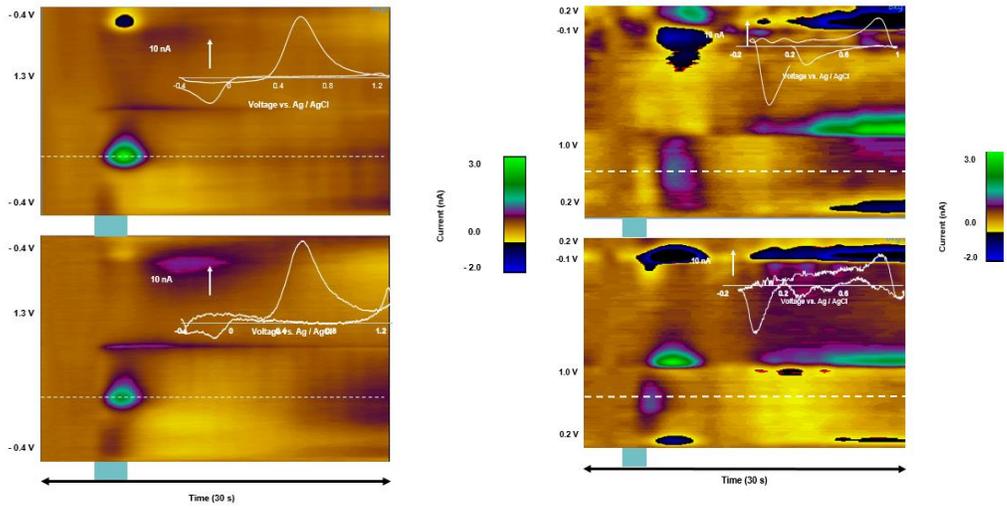


Figure 3.7: Evoked colorplots and cyclic voltammograms (inlet) for dopamine (left) and serotonin (right) in F344/N animals (top) and HIV-1 Tg animals (bottom).

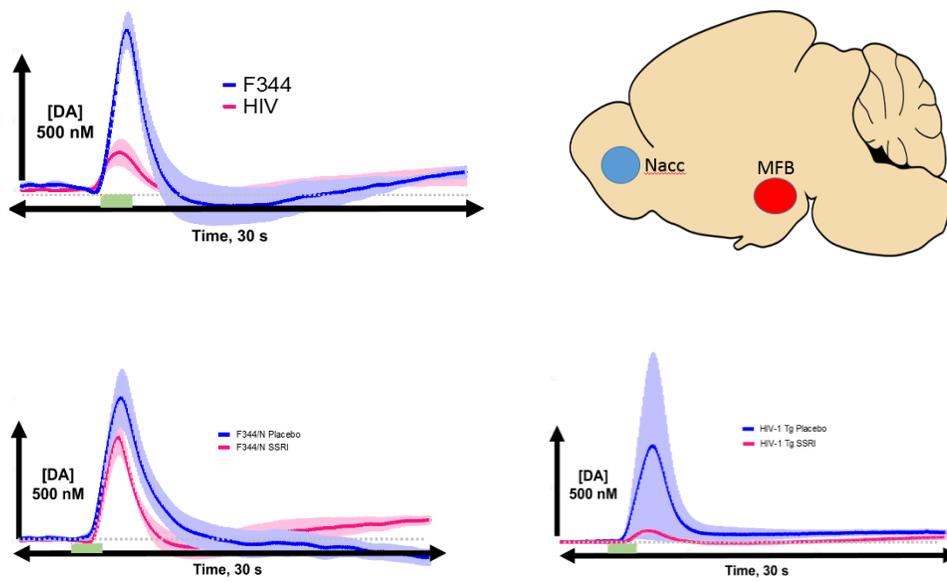


Figure 3.8: Evoked dopaminergic potentials for genotype (top) and genotype by treatment condition (bottom). Dopamine transmission was found to be impaired in HIV-1 Tg animals relative to F344 controls ($p < 0.05$). Escitalopram treatment was not found to attenuate dopaminergic deficits in HIV Tg animals.

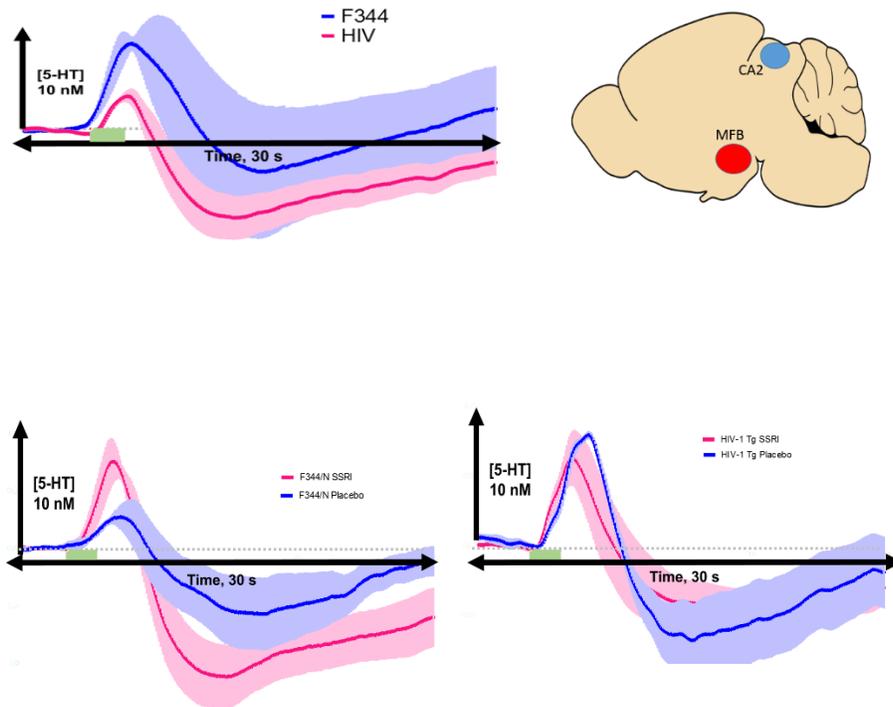


Figure 3.9: Evoked serotonergic potentials for genotype (top) and genotype by treatment condition (bottom). Serotonergic transmission was found to be impaired in HIV-1 Tg animals relative to F344 controls ($p < 0.05$). Escitalopram treatment was not found to attenuate dopaminergic deficits in HIV Tg animals, though treatment did appear to alter serotonergic function in F344 controls.

CHAPTER 4

DISCUSSION

Presently, the effects of escitalopram treatment upon the HIV-1 Tg rat were evaluated with a behavioral testing battery and *in-vivo* electrochemical analysis. Globally, escitalopram treatment did not appear to significantly alter behavioral functioning in the HIV-1 Tg rat. While treatment did indeed increase overall serotonergic transmission in F344/N controls, this effect was not found with respect to HIV-1 Tg animals, which persisted to demonstrate impairment despite treatment.

Though escitalopram did not appear to alter sucrose consumption in either HIV-1 Tg or F344/N animals, an interesting curvilinear shift in sucrose concentration responding occurred in HIV-1 Tg animals. F344/N animals displayed a steady linear relationship across increasing sucrose concentrations with higher concentrations of sucrose clearly favored. While sucrose concentrations were clearly preferred over water in HIV-1 Tg rats, responses to concentrations of 5% sucrose or higher were essentially flat-lined, with no clear preference for higher concentrations despite drug treatment. This alteration in responding is a departure from previous findings using the HIV-1 Tg rat. Indeed, Bertrand (2018) did not find a difference in response to variable sucrose concentration between F344 and HIV-1 Tg animals.

This departure from previous findings may have several alternative explanations. First, the curvilinear shift and subsequent flattening of responding in the HIV-1 Tg animals may potentially be an effect of mediation by escitalopram treatment. Previously,

Bertrand (2018) did not expose animals to any sort of treatment which might serve as a mediating factor. Secondly, the present study used a much shorter time period for testing sucrose preference. Bertrand (2018) utilized a testing period of one hour while the present experiment used a testing window of 30 minutes. This discrepancy may potentially explain the difference in findings between these studies, although this is highly unlikely.

Perhaps the most reasonable explanation for the present findings is that Bertrand (2018) performed sucrose testing on female rats that were ovariectomized and thus not subject to hormonal fluctuations. In the present experiment, female rats had intact ovaries and thus normal hormonal function. While research into the effects of hormonal cycling upon behavioral testing in rats has been rather sparse, it has been previously found that estrus cyclicity does indeed influence anxiety related behaviors (D'Souza and Sadananda, 2017) and motivated, goal-driven behaviors (Steiner et al., 1981). It is quite probable that variation in hormone levels could potentially explain this deviation from previous work.

Indeed, the present finding that sucrose consumption is impaired across variable concentrations is in line with the previous discussion of apathy in the HIV Tg rat, and fits with the narrative of apathy and HIV within the clinical literature. Here, we report a flattening of response curve across increasing sucrose concentrations. HIV-1 Tg rats did not appear to prefer 30% sucrose concentrations any more than they did 5% concentrations. These findings are highly suggestive of an apathetic response within the transgenic rat, as rodents with intact, healthy motivational systems should most clearly prefer higher sucrose concentrations to lower concentrations, with highest preference given to the highest concentration. Thus, the present findings strongly support the

argument for motivational dysregulation in the HIV-1 Tg rat, though treatment with escitalopram failed to attenuate this motivational dysregulation.

While escitalopram treatment did not appear to performance during the sucrose preference task, effects of escitalopram treatment are more clearly seen in the exploration based tasks such as the elevated plus maze. Unsurprisingly, multivariate analysis of rodent performance across both tasks revealed a significant interaction effect between genotype and sex. With respect to antidepressant treatment, it would appear across both tasks that escitalopram did indeed improve measures of exploration in both F344 controls and in HIV-1 Tg rats. With respect to elevated plus maze trials, total time spent on the open arms of the apparatus was increased in animals treated with escitalopram. In fact, exploration time for female HIV-1 Tg animals treated with escitalopram was higher than total times for each of the other groups. However, it is of note that escitalopram did not produce such robust effects in male F344 animals, as such animals treated with escitalopram explored *less* than their placebo treated counterparts.

Findings from the modified hole board task did not produce such robust effects, however, as escitalopram treated animals performed *less* average nose pokes across the trial period than placebo treated animals, with the exception of female F344 rats. HIV-1 Tg rats treated with escitalopram performed more poorly on the task than placebo treated HIV-1 Tg animals. Mirroring findings from the elevated plus maze, F344 males treated with escitalopram showed decreased activity relative to placebo treated F344 males.

While conclusions about the efficacy of escitalopram in improving performance in exploration based tasks in HIV-1 Tg rats are difficult to draw from these mixed findings, it is clear that anti-depressant treatment was less effective more males,

irrespective of genotype. This finding closely mirrors findings from clinical antidepressant trials, as males typically show decreased responsiveness to antidepressant treatment relative to females, despite the fact that females are far more at risk for the development of depression than males (Sramek et al., 2016). While many sex-difference based studies were performed for older forms of antidepressants, Khan et al., (2005) examined sex differences in response to SSRI treatment across a variety of SSRIs, including escitalopram and its r-enantiomer citalopram (tradename Celexa). In this study, while the authors argue that SSRI treatment is still a viable option for males suffering from depression, the effectiveness of this medication may be much greater for females (Khan et al., 2005).

In a 2016 review, Sramek and colleagues summarize many of the studies reporting sex differences with respect to antidepressant function, while no single mechanism is responsible for the sex dependent response to antidepressant treatment, many factors have been proposed. Of particular interest, the age of onset of depression in females is typically during adolescence and may correspond strongly to the onset of puberty. Hormonal fluctuations in puberty, menstruation and menopause may potentially affect the efficacy of treatment in addition to sex-dependent monoamine functioning. Consequently, interactions between serotonin and estrogen may potentially serve as a biological basis for variable antidepressant action (Sramek et al., 2016).

In addition to factors previously discussed Sramek and colleagues (2016) discuss factors such as body fat, weight distribution and consequent liver metabolism rates/drug clearance rates as underlying the differences between male and female response to antidepressants. Moreover, sex dependent factors such as plasma volume, enzymatic

activity, protein levels and gastric activity may underlie sex dependent responses to antidepressant treatment in addition to sociocultural factors such as adherence (Sramek et al., 2016). Overall, the present findings of decreased escitalopram efficacy in male rats independent of genotype appears to be consistent with the clinical narrative.

Here, we report significant impairment of both auditory and visual pre-pulse inhibition in the HIV-1 Tg rat. These findings follow a consistent narrative within our laboratory as such deficits in pre-pulse inhibition in the HIV-1 Tg rat have been consistently reported (Moran et al., 2013; McLaurin et al., 2016; McLaurin et al., 2017; McLaurin et al., 2017; McLaurin et al., 2018). Although the present study replicates the body of previous literature, an effect of escitalopram treatment was not found to attenuate deficits in either auditory or visual PPI for HIV-1 Tg animals. However, abnormal alterations in PPI across inter-stimulus interval for HIV-1 Tg animals were clearly observed. The cause for this disturbance remains unclear, although it was noted in the clinical literature that escitalopram treatment may induce abnormal visual experiences which may consequently interfere with visual perception (Lai 2012). Such alteration might clearly impede performance on a visual based task. However, escitalopram did not alter visual PPI performance in F344/N males. These effects may be due to individual variation of response to drug treatment in the HIV-1 Tg rat. Indeed, individual differences in the HIV-1 Tg in the variation of response to potential therapeutics has previously been described (McLaurin et al., 2018).

Deficits in pre-pulse inhibition have been previously discussed as a feature of non-psychotic major depressive disorder (Perry et al., 2004) and described as a function of serotonergic activity (Fletcher et al., 2001). Patients suffering from depression are

hypothesized to have impaired circuitry of cortico-striato-pallido-thalamic pathways which regulate PPI- a feature that is also found in schizophrenic patients (Perry et al., 2004). Although reductions in PPI are slight in depression compared with psychotic disorders such as schizophrenia, studies suggest a deficit is indeed present when compared with healthy control patients (Perry et al., 2004, Matuso et al., 2017). Pre-pulse inhibition represents a translational way to study serotonergic-based deficits which likely underlie to high incidence of comorbidity between HIV and depression. However, the effect of serotonergic agonists upon PPI is understudied and often inconclusive (Martinez and Geyer, 1997; Phillips et al., 2000; Quednow et al., 2004).

No differences were found in social interaction for any treatment group. Irrespective of genotype, sex or treatment, rodents typically spent the majority of the trail period engaged in some form of social interaction. HIV-1 Tg animals displayed no impairment in social interaction compared to controls. These findings are in line with previous work, given the context of what is known about the relatively healthy growth and progression of the HIV-1 Tg rat across its lifespan. No aberrations have been reported in healthy growth weight for the HIV-1 Tg rat (Peng et al., 2010; Moran et al., 2013). Moreover, across several studies, the HIV-1 Tg rat has repeatedly exhibited intact sensory and motor function (Peng et al., 2010; McLaurin et al., 2018). Taken together, nothing about what is known about the relatively healthy growth and function of the transgenic rat would suggest a deficit in social interaction.

Anti-depressant treatment is generally understood to produce anxiolytic properties in both preclinical (Griebel et al., 1994) and clinical studies (Kent et al., 1998). Despite this understanding, previous literature has shown r-citalopram (Lexapro) to actually

decrease social interaction in rodent models (Dekeyne et al., 2000 Rodriguez-Porcel et al., 2011). Despite the findings of these studies, here we report no global effect of escitalopram upon social interaction time. A potential explanation for these findings is that in the present study, antidepressant treatment was chronically administered to animals with healthy social interaction systems. As previously discussed, no prior literature provides an evidentiary basis for why any social impairment would exist in the HIV-1 Tg rat. Previous experiments in which antidepressant treatment was found to increase social interaction were conducted in animals which had interaction activities disrupted by procedures such as social defeat (Griebel et al., 1994). Studies which reported r-citalopram as decreasing social interaction administered the treatment to animals either as an acute dosage (Dekeyne et al., 2000) or in neonates (Rodriguez-Porcel et al., 2011). In the case of acute dosages, it is possible that an immediate increase in serotonin levels would elicit adverse effects upon initial administration (Mir and Taylor 1997; Dekeyne et al., 2000). Moreover, such acute high doses may potentially result in a loss of serotonin selectivity, and incorporate adrenergic and dopaminergic transmission, thereby producing an initial increase in anxiety behaviors (Dekeyne et al., 2000). Given that the present study utilized a chronic treatment protocol in adult rodents unlikely to have prior social impairment as a function of intervention such as social defeat, the present findings of no effects of escitalopram upon social interaction are unsurprising.

Here, we report simultaneous decreases in rates of release and reuptake in dopamine and serotonin activity in HIV-1 Tg rats relative to F344/N controls, which has previously been described by our lab (Denton et al, forthcoming). For both neurotransmitters, HIV-1 Tg animals demonstrated impaired release kinetics coupled

with a significantly decreased maximal release (C_{max}). Moreover, reuptake rates in the HIV-1 Tg rat were significantly impaired relative to controls for both dopamine and serotonin. Transgene mitigated impairments in serotonin and dopamine function may provide a neurological basis for comorbid HIV-1 and clinical depression/apathy.

The current findings of dopaminergic impairments in the HIV-1 Tg rat are consistent with our previous research (Javadi-Paydar et al. 2017), which examined reuptake times for DA in the HIV-1 Tg rat using *ex vivo* striatal brain slices. A marked extension in reuptake time for exogenously applied DA was found in the HIV-1 Tg brain slices, relative to F344/N slices, further suggesting a role of DAT dysfunction in mediating DA kinetics in the HIV-1 Tg rat. Moreover, the present findings are consistent with what was reported by Denton et al., (forthcoming), which demonstrated profound dysfunction of dopamine and serotonin transmission in the HIV-1 Tg rat using an identical FSCV protocol as what is described here. *In vitro*, the HIV-1 protein Tat inhibits vesicular monoamine transporter (VMAT2) function (Milde et al. 2012). VMAT2 plays an essential role in the packaging of dopamine and serotonin into synaptic vesicles for later release (Caudle et. al 2007, Eiden and Weihe 2011). Thus, a disruption in monoamine synthesis and packaging may play a role in present findings of dopamine and serotonergic impairments in the HIV-1 Tg rat.

Dopaminergic functioning in the HIV-1 Tg rat following long-term HIV-1 protein exposure is characterized by decreased release, lower peak concentrations, and slowed reuptake, which are consistent with *in vivo* PET imaging studies of the HIV-1 Tg rat (Sinharay et al. 2017) and findings in human HIV-1 PET imaging studies (Chang et al. 2008). In contrast, prior studies using synaptosomal preparations from HIV-1 Tg rats

(Zhu et al. 2016; Bertrand et al. 2018) reported increased DA reuptake rates. The increased efficiency of synaptosomal DAT function in HIV-1 Tg rats likely reflects a compensatory response in surviving synapses to long-term HIV-1 protein exposure. Long-term HIV-1 protein exposure (many months to years in the HIV-1 Tg rat, i.e., similar to long-term HIV-1 exposure in humans) decreases dopaminergic circuit neurotransmission, despite maximal DAT function.

The repeated finding that cortical serotonergic function is disrupted in the HIV-1 transgenic rat provides a potential biological basis for the high comorbidity instances of HIV-1 and clinical depression in the human population. An estimated 30-60% of HIV-1-infected persons in the United States will develop symptoms of clinical depression over the course of their lifetime (Bhatia and Munjal 2014, Castellon et al. 1998). Given our consistent findings of diminished serotonin in the HIV-1 Tg rat and the high rates of comorbidity of HIV-1 and depression in the clinical population, a serotonergic treatment is a logical candidate to be an effective therapeutic for individuals suffering from comorbid HIV and depression. Indeed, in a 2018 meta-analysis, Eschun-Wilson and colleagues found that antidepressant therapy was more effective than placebo treatment in combating depression across a sample of ten studies. While the authors acknowledge this trend, they emphasize that the available evidence is limited and sparse (Eschun-Wilson et al. 2018). However, before such efficacy can be reliably demonstrated, there is a clear need for more mechanistic studies of monoamines in animal models of HIV-1 and in the seropositive clinical population.

Presently, escitalopram treatment was not found to attenuate dopaminergic or serotonergic impairment in HIV-1 Tg rats, nor did it alter dopaminergic tone in F344/N

rats. However, serotonin functioning in F/344 animals was improved by escitalopram treatment, though the effect fell short of statistical significance. Treatment failed to even marginally improve serotonergic functioning in HIV-1 Tg animals. These findings suggest that proper pharmacological intervention may necessitate an individual differences based approach. Indeed, McLaurin (2018) argues the importance in assessing the individual differences that occur as a function of heterogeneity within the HIV-1 Tg rat when exploring potential treatment avenues. Given that the present findings reveal that escitalopram did not improve serotonergic tone in the HIV-1 Tg rat, but increased transmission in control rats, perhaps a similar individual differences based approach is warranted when exploring potential therapeutics for comorbid HIV and depression.

CHAPTER 5

CONCLUSIONS AND FUTURE DIRECTIONS

The present study examined the therapeutic efficacy of the SSRI antidepressant escitalopram in attenuating HIV-1 mitigated deficits in the HIV-1 Tg rat. Each of these deficits presents a strong clinical correlate to typical observations among HIV-1 patients suffering from comorbid clinical depression. Overall, escitalopram was not found to attenuate alterations in motivational response, pre-pulse inhibition or social behavior in the HIV-1 Tg rat. Escitalopram did, however, modulated exploratory based behaviors. Voltammetric analysis revealed that while escitalopram did indeed alter serotonergic function globally, it did not appear to alter transgene mediated deficits in either serotonergic or dopaminergic functioning. Thus, preliminary investigations yield little evidence in support of escitalopram as an effective therapeutic for HIV-1 comorbid depression. This is unsurprising given what is known about the widely variable nature of antidepressant action.

Given what is known about individual response to drug treatment in the HIV-1 Tg rat, future efforts will be targeted toward developing more conclusive statistical models that will be able to further elucidate the actions of escitalopram within the transgenic rat at an individual level. SSRI medication is known to have a highly variable range of efficacy with many factors contributing to its global function. Through analysis of individual variation and response to escitalopram in the HIV-1 Tg rat, targeted

therapeutics for HIV-1 seropositive patients suffering from comorbid clinical depression may perhaps be developed to attenuate depressive symptoms and hopefully restore normal emotive regulation. These treatments, in combination with adherence to cART treatment, will hopefully serve to improve overall quality of life for individuals suffering from HIV in the post-cART era. While developments in the efficacy of antiretroviral treatment have indeed served to increase the prospects of living with HIV, significant impairments such as HIV associated neurocognitive disorders and clinical depression continue to impede the prospects of living with the disease, thus necessitating the need for targeted therapeutics to treat those symptoms which remain present

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