

6-30-2016

## Selective Behavioral Alterations In The HIV-1 Transgenic Rat: Implications For Diagnosis Of Pediatric HIV-1

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SELECTIVE BEHAVIORAL ALTERATIONS IN THE HIV-1 TRANSGENIC RAT:  
IMPLICATIONS FOR DIAGNOSIS OF PEDIATRIC HIV-1

By:

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Bachelor of Arts  
Winthrop University, 2014

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Submitted in Partial Fulfillment of the Requirements

For the Degree of Master of Arts in

Experimental Psychology

College of Arts and Sciences

University of South Carolina

2016

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## **DEDICATION**

I dedicate this thesis to my parents, Preston and Kim McLaurin, who constantly encouraged me to pursue my passion and higher education. Thank you for your listening ears. Your unconditional love and support. For reminding me to laugh. But mostly, for instilling in me values of perseverance and strength that have shaped who I am today.

I want to thank the director of my thesis, Charles Mactutus, for his guidance and encouragement throughout this study. I would also like to thank my thesis committee, Rosemarie Booze, and Steven Harrod, for their valuable suggestions to improve my manuscript.

## **ACKNOWLEDGEMENTS**

This work was supported in part by grants from NIH (National Institute on Drug Abuse, DA013137; National Institute of Child Health and Human Development, HD043680; National Institute of Mental Health, MH106392) and the interdisciplinary research training program supported by the University of South Carolina Behavioral-Biomedical Interface Program.

## **ABSTRACT**

Since the advent of combination antiretroviral therapy (cART), pediatric HIV-1 (PHIV) has evolved from a fatal disease to a chronic disease with children perinatally infected with HIV-1 surviving into adulthood. The HIV-1 transgenic (Tg) rat, which expresses 7 of the 9 HIV-1 genes constitutively throughout development, was used to investigate the early development of chronic neurological impairment in PHIV. Male and female Fischer HIV-1 Tg and F344N control rats, sampled from 35 litters, were repeatedly assessed during early development using multiple experimental paradigms, including somatic growth, locomotor activity, cross-modal prepulse inhibition (PPI) and gap-prepulse inhibition (gap-PPI). A rightward shift towards later eye opening was observed in HIV-1 Tg animals in comparison to controls. HIV-1 Tg animals exhibited delays in the development of the cholinergic inhibitory system, assessed using locomotor activity. Alterations in the development of the interstimulus interval (ISI) function were observed in HIV-1 Tg rats in comparison to control animals, assessed using PPI. Presence of the HIV-1 transgene was diagnosed with 91.4% accuracy using multiple behavioral assessments on PD 20 and 21. Selective early behavioral alterations observed in the HIV-1 Tg rats provide an opportunity for the development of a clinical diagnostic screening tool, which may improve the long-term outcome for children perinatally infected with HIV-1.

## TABLE OF CONTENTS

DEDICATION.....	iii
ACKNOWLEDGEMENTS.....	iv
ABSTRACT.....	v
LIST OF FIGURES.....	vii
LIST OF ABBREVIATIONS.....	viii
CHAPTER 1: INTRODUCTION.....	1
CHAPTER 2: METHODOLOGY.....	5
CHAPTER 3: RESULTS.....	10
CHAPTER 4: DISCUSSION.....	23
REFERENCES.....	31

## LIST OF FIGURES

Figure 3.1 Eye Opening .....	15
Figure 3.2 Cumulative Locomotor Activity .....	16
Figure 3.3 Total Ambulation in Locomotor Activity .....	17
Figure 3.4 Visual Prepulse Inhibition .....	18
Figure 3.5 Prepulse Inhibition on Postnatal Day 21 .....	19
Figure 3.6 Auditory Prepulse Inhibition .....	20
Figure 3.7 Gap-Prepulse Inhibition .....	21
Figure 3.8 Discriminant Function Analysis .....	22
Figure 4.1 Hypothetical Serial Neural Circuitry of PPI.....	30



## LIST OF ABBREVIATIONS

ANOVA	Analysis of Variance
ASR	Auditory Startle Response
cART	Combination Antiretroviral Therapy
DA	Dopamine
DAT	Dopamine Transporter
Gap-PPI	Gap-Prepulse Inhibition
HAART	Highly Active Antiretroviral Therapy
HAD	HIV-1 Associated Dementia
HAND	HIV-1 Associated Neurocognitive Disorders
HDS	HIV Dementia Scale
HIV-1	Human Immunodeficiency Virus
IACUC	Institutional Animal Care and Use Committee
IC	Inferior Colliculus
IHDS	International HIV Dementia Scale
<i>M</i>	Mean
MAO-A	Monoamine Oxidase A
MTCT	Mother-to-Child Transmission
PD	Postnatal Day
PET	Positron Emission Tomography

PHE .....	Progressive HIV-1 Encephalopathy
PHIV .....	Pediatric Human Immunodeficiency Virus Type 1
PnC.....	Caudal Pontine Reticular Nucleus
PPI.....	Prepulse Inhibition
PPTg.....	Pedunculopontine Tegmental Nucleus
pTH .....	Phosphorylated Tyrosine Hydroxylase
SC.....	Superior Colliculus
<i>SEM</i> .....	Standard Error
Tg .....	Transgenic

## CHAPTER 1

### INTRODUCTION

Worldwide, approximately 39 million individuals have died from human immunodeficiency virus type-1 (HIV-1) and 35 million individuals are living with HIV-1, including over 3.2 million children ( $\leq 15$  years of age; CDC, 2013). Despite the dramatic decrease in mother-to-child transmission (MTCT), the predominant source of HIV-1 infection in children (Kourtis *et al.*, 2001), 220,000 new cases of pediatric HIV-1 (PHIV) were reported in 2014 (UNAIDS, 2015). Since the advent of combination antiretroviral therapy (cART), PHIV has evolved from a fatal disease to a chronic disease with children perinatally infected with HIV-1 surviving into adulthood (Smith & Wilkins, 2015; Crowell *et al.*, 2014). Despite decreased mortality rates, chronic neurological impairment is still commonly reported in children perinatally infected with PHIV (Franklin *et al.*, 2005; Parameswaran *et al.*, 2010). Therefore, given the prevalence of PHIV, understanding the early behavioral alterations may be vital for the development of a translational screening tool for neurological impairment in HIV-1 seropositive children.

Progressive HIV-1 encephalopathy (PHE), which is often analogous to HIV-1 associated dementia (HAD) in adults, was predominantly observed prior to the advent of cART, with prevalence rates as high as 50% (Chiriboga *et al.*, 2005; Crowell *et al.*, 2014; Shanbhag *et al.*, 2005). Common neurological manifestations of PHE include microcephaly, resulting from cerebral atrophy, developmental delays, and movement disorders (Belman *et al.*, 1985; Epstein *et al.*, 1985; Epstein *et al.*, 1986) Furthermore,

neuroimaging analyses reveal calcification in the basal ganglia, and focal white matter lesions in children with PHE (Epstein *et al.*, 1985; Epstein *et al.*, 1986; Kauffman *et al.*, 1992). Currently, in the post-cART era, the prevalence rate of PHE is between 2-15% (Chiriboga *et al.*, 2005; Shanbhag *et al.*, 2005), however, chronic neurological impairment persists.

High rates of chronic neurological impairment, including neurodevelopmental delays, are still being reported in HIV-1 seropositive children (Franklin *et al.*, 2005; Parameswaran *et al.*, 2010; review, Van Rie *et al.*, 2007). Neurological assessments, including the Bayley Scales of Infant Development and Wechsler Intelligence Scale for Children-Revised, have previously been used to assess the effect of pediatric HIV-1 on neurodevelopment (Blanchette *et al.*, 2002; Lindsey *et al.*, 2007; Van Rie *et al.*, 2008; Walker *et al.*, 2013). Despite treatment with highly active antiretroviral therapy (HAART), HIV-1 infected children exhibit significant delays in cognitive development, motor skills and language expression in both high- (Lindsey *et al.*, 2007) and low-resource countries (Van Rie *et al.*, 2008; Walker *et al.*, 2013).

Neurocognitive deficits in HIV-1 seropositive children, including disease progression, are poorly understood (Crowell *et al.*, 2014), however, there is currently a wealth of knowledge on HIV-1 associated neurocognitive disorders (HAND) in adults evidenced in both clinical and preclinical studies (i.e. Heaton *et al.*, 2010; Woods *et al.*, 2009). Neurocognitive assessments, including the Wisconsin Card Sorting Test and Stroop Color Word Test, have shown that HIV-1 seropositive individuals display significant deficits in set shifting (Carter *et al.*, 2003) and response inhibition (Hinkin *et al.*, 1999; Tozzi *et al.*, 1999). Deficits in complex problem solving and abstraction have

been demonstrated using the Wisconsin Card Sorting Test and Tower of London- Drexel Version neurocognitive assessment (Cattie *et al.*, 2012; Cherner *et al.*, 2004). Furthermore, HIV-1 seropositive individuals display a greater impulsivity than control individuals on the Iowa Gambling Test (Hardy *et al.*, 2006; Martin *et al.*, 2004).

The HIV-1 transgenic (Tg) rat, which expresses 7 of the 9 HIV-1 genes, has been used in preclinical studies to model neurocognitive deficits, including HAND, commonly observed in HIV-1 seropositive individuals (Moran *et al.*, 2013a; Moran *et al.*, 2013b; Moran *et al.*, 2014a). Specifically, adult HIV-1 Tg rats exhibit significant deficits in executive functions, including attention, inhibition, and flexibility in comparison to controls (Moran *et al.*, 2014a). Furthermore, significant alterations in temporal processing, a pre-attentive process, have been observed using prepulse inhibition (PPI) of the auditory startle response (ASR) in the HIV-1 Tg rat (Moran *et al.*, 2013a). Specifically, we have shown that, on both visual and auditory prepulse trials, HIV-1 Tg rats exhibit an insensitivity to ISI duration, suggesting a lack of perceptual sharpening with age (Moran *et al.*, 2013a). Preliminary gap-prepulse inhibition (gap-PPI) data suggest that HIV-1 Tg rats display alterations in the development of temporal processing, assessed using startle response and prepulse inhibition.

Due to high rates of chronic neurological deficits in both HIV-1 seropositive children and adults, there is a critical need for accurate screening tools for the diagnosis of HAND. Early in the HIV-1 epidemic, two screening tools, the HIV Dementia Scale (HDS; Power *et al.*, 1995) and the International HDS (IHDS; Sacktor *et al.*, 2005), were developed to screen for HAD. However, neither the HDS or the IHDS are able to accurately screen for milder forms of HAND, which are more common in the post-cART

era, affecting up to 40%-70% of HIV-1 infected individuals (Heaton *et al.*, 2010; Heaton *et al.*, 2011; Letendre *et al.*, 2009; McArthur *et al.*, 2010; Sacktor *et al.*, 2005; Zipursky *et al.*, 2013). Development of a screening tool, specifically for neurological impairment seen in PHIV has immense clinical significance and may have a significant impact on the lives of HIV-1 seropositive children and adults (Zipursky *et al.*, 2013).

Thus, the aim of the current study was to establish the early trajectory of behavioral deficits in the HIV-1 Tg rat. The HIV-1 Tg rat, which express 7 of the 9 HIV-1 genes constitutively throughout development, provides a useful model for investigating the development of neurologic impairments in pediatric AIDS (Peng *et al.*, 2010; Royal *et al.*, 2012; Vigorito *et al.*, 2015). Behavioral assessments, including locomotor activity, cross-modal PPI, and gap-PPI were conducted prior to weaning from postnatal day (PD) 12 to PD 21. It was hypothesized that HIV-1 Tg rats would exhibit selective, early alterations in somatic growth, including body weight and eye opening, and behavioral measures, including locomotor activity, cross-modal PPI, and gap-PPI compared to non-transgenic F344N controls. Understanding the early trajectory of behavioral deficits in the HIV-1 Tg rats may not only provide a translational screening tool, but is also vital to understanding the progression of neurocognitive deficits in children perinatally infected with HIV-1.

## CHAPTER 2

### METHODOLOGY

#### *2.1 Animals*

Behavioral assessments were conducted on Fischer (F344/N; Harlan Laboratories Inc., Indianapolis, IN) rats (HIV-1 Tg,  $n=19$  litters; control,  $n=16$  litters) during early development beginning at PD 12. All rats were tested for motor movement, assessed using locomotor activity (PD 12, 16, 20) and temporal processing deficits, assessed using cross-modal prepulse inhibition (PPI) of the auditory startle response (PD 14, 17, and 21). Gap-PPI was conducted on PD 18.

Animals were delivered to the facility between PD 7 and PD 9 over the course of one year. All animals were housed with their biological dam until PD 21 when animals were weaned and separated by sex. Subsequently animals were pair- or group-housed with animals of the same sex throughout experimentation. Rodent food (Pro-Lab Rat, Mouse, Hamster Chow #3000) and water were provided *ad libitum* throughout experimentation.

Animals were maintained according to the National Institute of Health (NIH) guidelines in AAALAC-accredited facilities. The targeted environmental conditions for the animal facility were  $21^{\circ}\pm 2^{\circ}\text{C}$ ,  $50\% \pm 10\%$  relative humidity and have a 12-h light:12-h dark cycle with lights on at 0700 h (EST). The Institutional Animal Care and Use Committee (IACUC) of the University of South Carolina approved the project protocol as consistent with federal assurance (# A3049-01).

## ***2.2. Somatic Growth***

### **Body Weight**

Body weight was assessed as a measure of somatic growth upon arrival at the facility between PD 8 and PD 10. Body weight was assessed periodically throughout development.

### **Eye Opening**

Eye opening was assessed as a developmental milestone on PD 13-19. Eye opening was assessed separately for the right and left eye. A scale ranging from zero to two was used with a zero score indicating a closed eye, and a score of one indicating an open eye.

## ***2.3 Motor Movement***

### **Apparatus**

Square (40 x 40 cm) (Hamilton Kinder, San Diego Instruments, San Diego, CA) activity monitors were used to assess locomotor activity. Clear Plexiglas inserts were added to convert the chambers into a round (~40 cm diameter) compartment. Free movement of animals was detected by infrared photocell (32 emitter/detector pairs) interruptions. Total locomotor activity was measured by assessing the number of photocell interruptions within a 60-minute period.

### **Locomotor Activity**

Locomotor activity testing occurred on PD 12, 16, and 20. Testing occurred for a 60-minute period between 700 and 1200h (EST) under dim light conditions, in the absence of direct overhead lighting (<10 lux). All activity monitors were located in an isolated room.



## ***2.4 Temporal Processing***

### **Apparatus**

The startle platform (SR-Lab Startle Reflex System, San Diego Instruments, Inc., San Diego, CA) was enclosed in a 10 cm-thick double-walled, 81×81×116-cm isolation cabinet (external dimensions) (Industrial Acoustic Company, INC., Bronx, NY), instead of the 1.9 cm thick ABS plastic or laminate cabinets offered with this system. Sound attenuation of 30dB(A) was provided in the isolation chamber relative to the external environment. An ambient sound level of 22dB (A) was presented in the chamber without any stimuli presented. The high-frequency loudspeaker of the SR-Lab system (Radio Shack model#40-1278B), mounted inside the chamber 30 cm above the Plexiglas animal test cylinder, delivered all auditory stimuli (frequency range of 5k-16k Hz). The animal's response to the auditory stimulus produced deflection of the test cylinder, which was converted into analog signals by a piezoelectric accelerometer integral to the bottom of the cylinder. The response signals were digitized (12 bit A to D) and saved to a hard disk. Response sensitivities were calibrated using a SR-LAB Startle Calibration System. Sound levels were measured and calibrated with a sound level meter (Kjaer Bruel 2203) with the microphone placed inside the Plexiglas cylinder.

### **Cross-modal Prepulse Inhibition**

Both visual and auditory prepulse stimuli were used to test animals for PPI of the ASR on PD 14, 17 and 21. PPI was administered using a 30-min test session, beginning with a 5-min acclimation period in the dark with 70 dB (A) background white noise, followed by 6 pulse-only ASR trials with a 10s ITI. A total of 72 trials, including an equal number of visual and auditory prepulse trials, were presented. Trials had an

interstimulus interval (ISI) of 0, 30, 50, 100, 200, and 4000 msec and were interdigitated in an ABBA order of presentation. The 0 and 4000 msec ISI trials were control trials. The intertrial interval (ITI) was variable from 15 sec to 25 sec. Inside the test cylinder, the pulse stimulus intensity was 100 dB(A) (20 msec duration). Mean peak ASR amplitude values were collected for analysis.

### **Gap-Prepulse Inhibition**

Animals were tested for gap-PPI of the ASR on PD 18. Animals were tested for gap-PPI of the ASR with a preceding gap in background white-noise as a stimulus. A 20-min test session began with a 5-min acclimation period in the dark with 70 dB(A) background white noise, followed by six pulse-only ASR trials, used for habituation, with a 10s intertrial interval (ITI). Thirty-six trials were presented using 6-trial blocks in an ABBA order of presentation. A 20-msec gap in white noise preceded a startle stimulus presented at ISIs of 30,50,100, 200 and 4000 msec. Two control trials, the 0 and 4000 msec ISI trials, were included to provide a reference ASR within gap-PPI. The startle stimulus intensity was 100 dB(A) (20 msec duration) measured inside the test cylinder. Mean peak ASR amplitude values were collected for analysis. All test sessions were conducted in the dark.

### ***2.5 Statistical Analysis***

Categorical data, including eye opening, an index of somatic growth, was analyzed using a chi-squared statistical technique. Eye opening data were assessed by individual pup. An alpha level of  $p \leq 0.05$  was considered significant.

Analysis of variance (ANOVA) techniques (SPSS Statistics 20, IBM Corp., Somers, NY) were used to analyze all continuous data. To account for the nested design

within the ANOVA analysis, individual observations were analyzed by using litter means and standard errors (Denenberg, 1984; Wears, 2002). Potential violations of sphericity of within-subjects variables (Winer, 1971) were corrected using the Greenhouse-Geisser *df* correction factor (Greenhouse & Geisser, 1959). An alpha level of  $p \leq 0.05$  was considered significant for all statistical tests.

Locomotor activity data was analyzed using a three-way mixed factor ANOVA. Cumulative photocell interruptions were used for analysis, with genotype (HIV-1 Tg vs. control) as the between-subjects factor, and time and age as the within-subjects factor.

Cross-modal PPI data were analyzed using a four-way mixed-factor ANOVA for both prepulse modalities (auditory, visual). Mean peak ASR amplitude for the 0-4000 msec ISIs were used for analysis, with genotype (HIV-1 Tg vs. control) as the between-subjects factor, and age, ISI, and trial as the within-subjects factors.

For gap-PPI, a three-way repeated measures ANOVA was performed on mean peak ASR amplitude for the 0-4000 msec ISIs, with genotype (HIV-1 Tg vs. control) as the between-subjects factor, and ISI and trial as the within-subjects factors. Gap-PPI testing began after 11 litters had arrived. The present analysis includes 24 litters (HIV-1 Tg,  $n=13$  litters; control,  $n=11$  litters).

An exploratory discriminant functional analysis was conducted to determine the diagnostic accuracy of early behavioral alterations and to determine which observed behavioral assessments were able to correctly identify animals in regard to their genotype (HIV-1 Tg vs. Control).

## CHAPTER 3

### RESULTS

#### ***3.1 HIV-1 Tg animals exhibit selective alterations in somatic growth***

Body weight measurements, obtained upon arrival (PD 8 to PD 10), were used to assess initial somatic growth. Upon arrival, there was not a significant difference in body weight between control ( $M=11.9\text{g}$ ,  $SEM=0.7\text{g}$ ) and HIV-1 Tg animals ( $M=12.5\text{g}$ ,  $SEM=0.6\text{g}$ ).

Selective alterations in somatic growth were evident in eye opening assessments, as illustrated in Figure 1. Eye opening was assessed from PD 13 to PD 19 as a measure of development. Eye opening started at PD 15 for both HIV-1 Tg and control animals. Eyes were fully open for all animals on PD 19.

A  $\chi^2$  revealed a statistically significant difference between eye opening in HIV-1 Tg vs control animals [ $\chi^2(4)=34.4$ ,  $p\leq 0.001$ ]. A significant shift in the distribution from earlier eye opening to later eye opening was observed for the HIV-1 Tg rats in comparison to control animals. Therefore, the HIV-1 Tg rat displays selective alterations in somatic growth evident in eye opening, but not in body weight.

#### ***3.2 HIV-1 Tg animals exhibit altered development of motor movement.***

Differential progression of motor movement in the HIV-1 Tg rat, relative to control animals, was assessed using cumulative frequency for locomotor activity, illustrated in Figure 2a, 2b, and 2c. The development of motor movement is significantly

altered in HIV-1 Tg animals relative to control animals. The overall ANOVA for locomotor activity revealed a significant age x time x genotype interaction [F(22,726)=6.3,  $p_{GG} \leq 0.004$ ,  $\eta_p^2 = 0.16$ ], age x time interaction [F(22,726)=77.4,  $p_{GG} \leq 0.001$ ,  $\eta_p^2 = 0.70$ ], time x genotype interaction [F(11,363)=12.1,  $p_{GG} \leq 0.001$ ,  $\eta_p^2 = 0.27$ ], and age x genotype interaction [F(2,66)=6.9,  $p_{GG} \leq 0.004$ ,  $\eta_p^2 = 0.17$ ]. Significant main effects of genotype [F(1,33)=6.1,  $p \leq 0.02$ ,  $\eta_p^2 = 0.16$ ], age [F(2,66)=103.9,  $p_{GG} \leq 0.001$ ,  $\eta_p^2 = 0.76$ ] and time [F(11,363)=607.8,  $p_{GG} \leq 0.001$ ,  $\eta_p^2 = 0.95$ ] were also observed.

Separate analyses at each age were conducted to determine the locus of these interactions. Analyses revealed a significant time x genotype interaction at PD 20 [F(11,363)=23.1,  $p_{GG} \leq 0.001$ ,  $\eta_p^2 = 0.41$ ], but not at PD 12 [F(11,363)=1.5,  $p_{GG} \leq 0.237$ ] or PD 16 [F(11,363)=1.6,  $p_{GG} \leq 0.217$ ].

Alterations in the development of motor movement, assessed using locomotor activity, are further evidenced by mean total ambulation, illustrated in Figure 3. A segmented first-order polynomial was the best fit for the total ambulation in HIV-1 Tg animals, while a second-order polynomial was the best fit for control animals. Therefore, both the time x genotype interaction at PD 20, as well as differences in the best fit for total ambulation, indicates altered development of motor movement in the HIV-1 Tg animals.

### ***3.3 HIV-1 Tg animals exhibit altered temporal processing development with a visual prepulse.***

Altered temporal processing development with a visual prepulse in the HIV-1 Tg rat, relative to control animals, is illustrated in Figure 4a and 4b. The overall ANOVA on mean peak ASR amplitude revealed a significant age x ISI x genotype interaction

[F(10,330)=5.2,  $p_{GG} \leq 0.001$ ,  $\eta_p^2 = 0.14$ ], a significant age x ISI interaction [F(10,330)=19.2,  $p_{GG} \leq 0.001$ ,  $\eta_p^2 = 0.37$ ] and a significant ISI x genotype interaction [F(5,165)=12.0,  $p_{GG} \leq 0.001$ ,  $\eta_p^2 = 0.27$ ]. Significant main effects of genotype [F(1,33)=19.8,  $p \leq 0.001$ ,  $\eta_p^2 = 0.38$ ], age [F(2,66)=48.2,  $p_{GG} \leq 0.001$ ,  $\eta_p^2 = 0.60$ ], and ISI [F(5,165)=44.6,  $p_{GG} \leq 0.001$ ,  $\eta_p^2 = 0.58$ ] were also observed.

Differences in the development of temporal processing were further examined by separate analysis of each genotype. The overall ANOVA for control animals, illustrated in Figure 3a, revealed an age x ISI interaction [F(10,140)=4.3,  $p_{GG} \leq 0.008$ ,  $\eta_p^2 = 0.23$ ]. Main effects of age [F(2,28)=6.6,  $p_{GG} \leq 0.009$ ,  $\eta_p^2 = 0.32$ ] and ISI were also observed [F(5,70)=7.6,  $p_{GG} \leq 0.001$ ,  $\eta_p^2 = 0.35$ ]. In contrast, the overall ANOVA for HIV-1 Tg animals only revealed a significant main effect of age [F(2,34)=7.0,  $p_{GG} \leq 0.004$ ,  $\eta_p^2 = 0.29$ ]. The age x ISI interaction present in the control animals, but not the HIV-1 Tg animals indicates an altered development of the ISI function.

Separate analyses at each age were also conducted, which revealed a significant genotype x ISI interaction on PD 17 [F(5,165)=4.6,  $p_{GG} \leq 0.003$ ,  $\eta_p^2 = 0.12$ ] and on PD 21, [F(5,165)=10.5,  $p_{GG} \leq 0.001$ ,  $\eta_p^2 = 0.24$ ], illustrated in Figure 5a, but not on PD 14. The genotype x ISI interactions, observed on PD 17 and PD 21, provide additional evidence for the altered development of the ISI function in the HIV-1 Tg group.

### ***3.4 HIV-1 Tg animals exhibit altered temporal processing development with an auditory prepulse.***

HIV-1 Tg animals exhibit alterations in the development of temporal processing with an auditory prepulse, as illustrated in Figure 6a and 6b. Both HIV-1 Tg and control animals exhibited a shift in maximal inhibition (from 30 msec to 50 msec) on postnatal day 21. However, HIV-1 Tg animals exhibit altered development of the ISI function in

comparison to control animals. The overall ANOVA on mean peak ASR amplitude revealed a significant age x ISI x genotype interaction [F(10,330)=3.2,  $p_{GG} \leq 0.021$ ,  $\eta_p^2 = 0.09$ ], a significant age x ISI interaction [F(10,330)=46.3,  $p_{GG} \leq 0.001$ ,  $\eta_p^2 = 0.59$ ], and a significant ISI x genotype interaction [F(5,165)=17.6,  $p_{GG} \leq 0.001$ ,  $\eta_p^2 = 0.35$ ]. Significant main effects of genotype [F(1,33)=24.2,  $p \leq 0.001$ ,  $\eta_p^2 = 0.42$ ], age [F(2,66)=34.8,  $p_{GG} \leq 0.001$ ,  $\eta_p^2 = 0.51$ ], and ISI [F(5,165)=204.1,  $p_{GG} \leq 0.001$ ,  $\eta_p^2 = 0.86$ ] were also observed.

Separate analyses at each age revealed a significant ISI x genotype interaction at all ages (i.e., PD 14, PD 17, and PD 21), indicating an alteration in the development of ISI function in the HIV-1 Tg animals. Specifically, the alterations in the development of the ISI function on PD 21 are illustrated in Figure 5b.

### ***3.5 HIV-1 Tg and control animals both exhibit significant gap-PPI.***

Both HIV-1 Tg and control animals exhibit significant inhibition with gap-PPI, illustrated in Figure 7. The overall ANOVA conducted on mean peak ASR amplitude for gap-PPI revealed that there was no genotype x ISI interaction. However, a significant main effect of genotype [F(1,22)=4.7,  $p \leq 0.05$ ,  $\eta_p^2 = 0.18$ ] and ISI [F(5,110)=9.4,  $p_{GG} \leq 0.001$ ,  $\eta_p^2 = 0.30$ ] were observed. Furthermore, comparable peak inhibition was observed in both the HIV-1 Tg and control animals at the 30 msec ISI. Therefore, the significant main effects of genotype and ISI result from a downward shift in the mean peak ASR amplitude curve and not a deficit in temporal processing.

### ***3.6 Behavioral alterations accurately diagnose the presence of the HIV-1 Transgene.***

The diagnostic utility of early behavioral alterations in the HIV-1 Tg rat was further analyzed using an exploratory discriminant function analysis to determine which

behavioral assessments and ages were best able to identify group membership. Assessment of locomotor activity on PD 20 and cross-modal PPI on PD 21 best predicted group membership, as illustrated in Figure 8. A stepwise discriminant function analysis selected four variables (Motor Movement (PD 20), Auditory Mean Peak ASR Amplitude Values at 50 msec (PD 21) and 100 msec (PD 21) and Visual Mean Peak ASR Amplitude Values at 50 msec (PD 21)) that maximally separated the HIV-1 Tg and control animals (canonical correlation of 0.831). Animals were classified (jack-knifed) with 91.4% accuracy (F approximation of Wilks'  $\lambda$  of 0.309,  $F(4, 30) = 16.8, p \leq 0.001$ ).



## Eye Opening

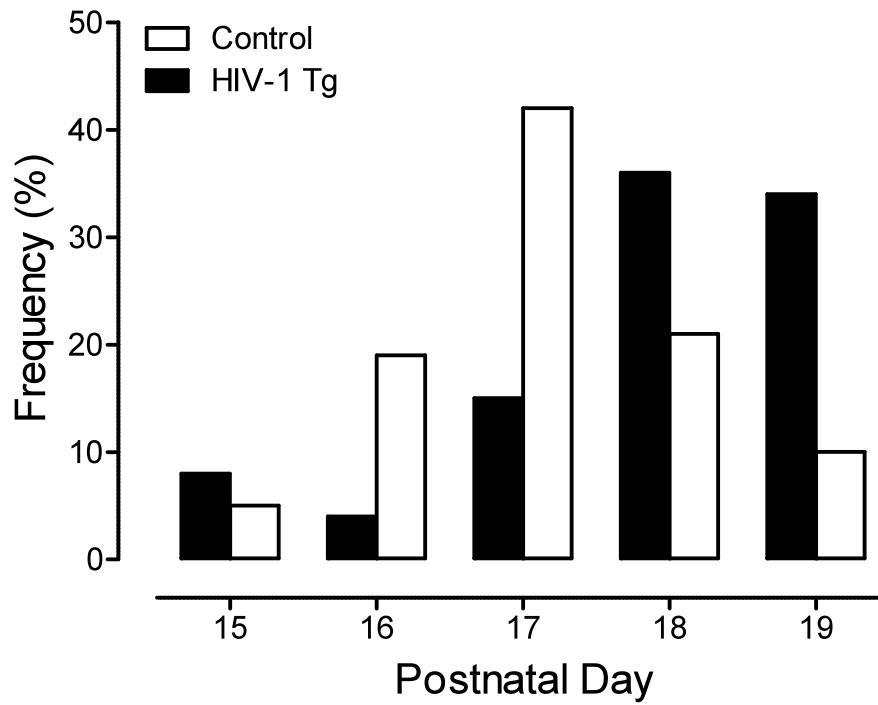


Figure 3.1 Eye Opening. A significant shift in the population of eye opening is observed; HIV-1 Tg animals open their eyes significant later than control animals ( $\chi^2(4)=34.4$ ,  $p \leq 0.001$ ), suggesting a selective alteration in somatic growth.

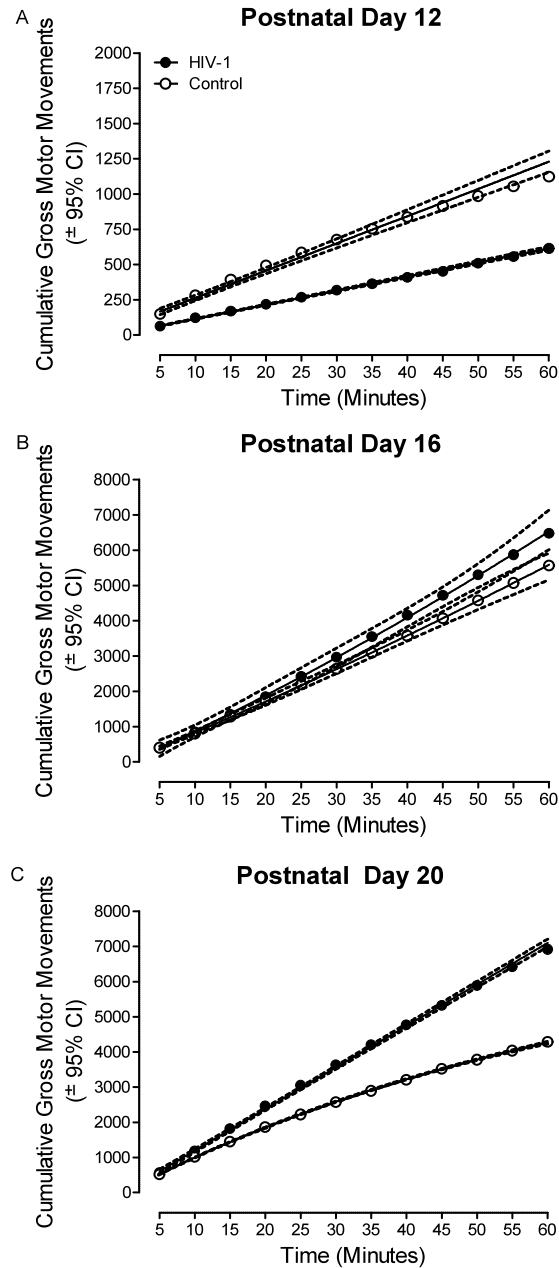


Figure 3.2 Cumulative Locomotor Activity. HIV-1 Tg animals exhibit altered development of motor movement assessed using locomotor activity. Cumulative frequencies of gross motor movement are shown on postnatal day (PD) 12 (a), PD 16 (b), and PD 20 (c). A significant age x time x genotype interaction was observed, indicating that the HIV-1 Tg animals exhibit alterations in motor movement both within a session and throughout development.

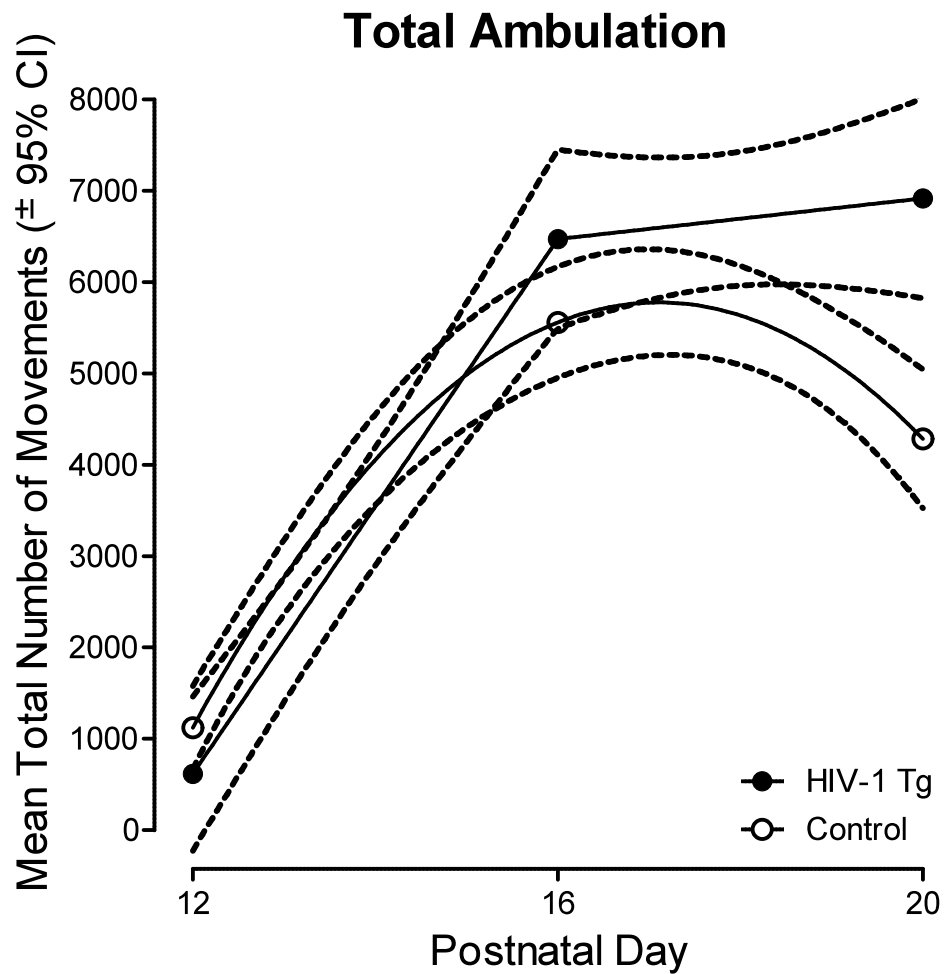


Figure 3.3 Total Ambulation in Locomotor Activity. HIV-1 Tg animals exhibit significantly altered development of motor movement, assessed using locomotor activity. A significant time x genotype interaction on PD 20 was observed, indicating altered development of motor movement in the HIV-1 Tg animals. Furthermore, a segmented first-order polynomial was the best fit for the total ambulation in HIV-1 Tg animals, while a second-order polynomial was the best fit for control animals.

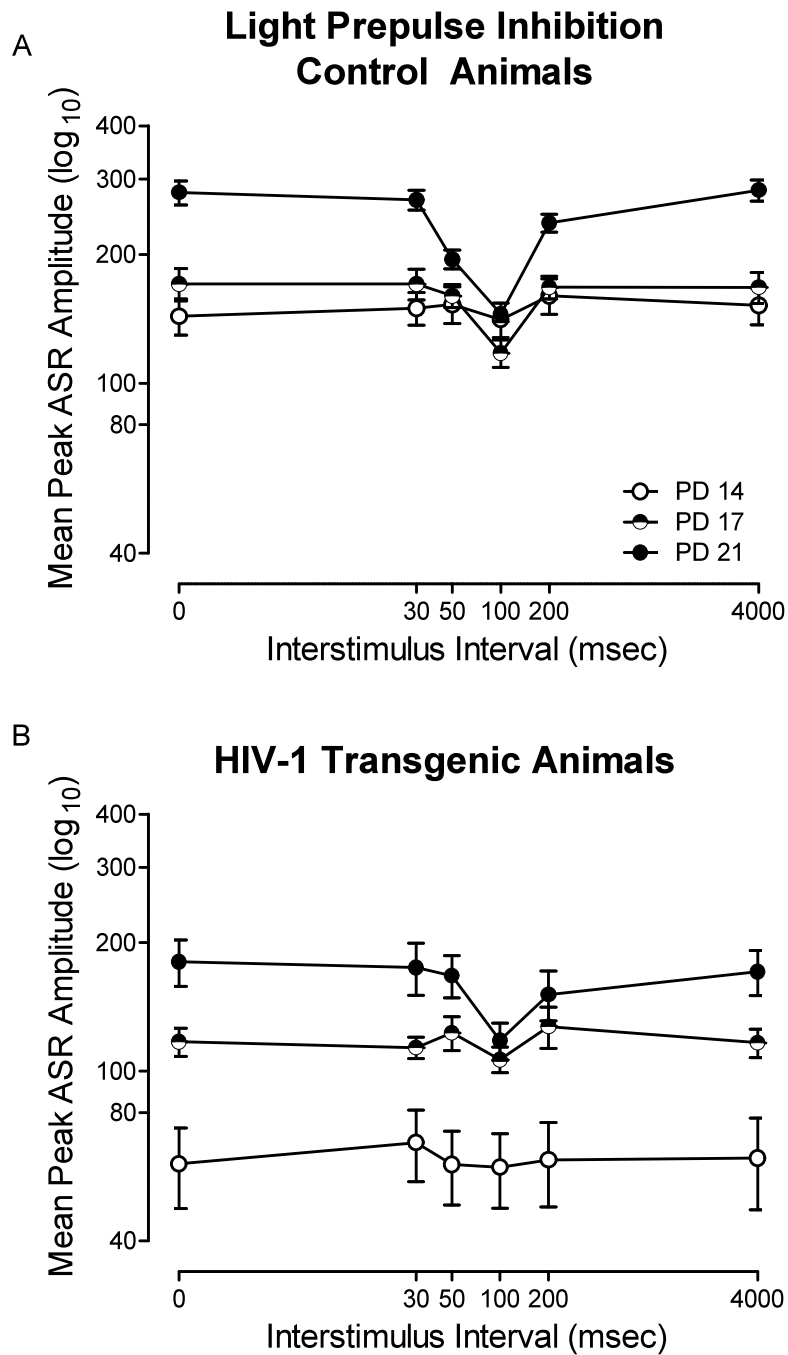


Figure 3.4 Visual Prepulse Inhibition. Prepulse inhibition (PPI) with a visual prepulse across all three test ages. A significant age x interstimulus interval (ISI) interaction was present in control animals (a), but not in HIV-1 Tg animals(b) indicating that HIV-1 Tg animals exhibit altered development of the ISI function with a visual prepulse.

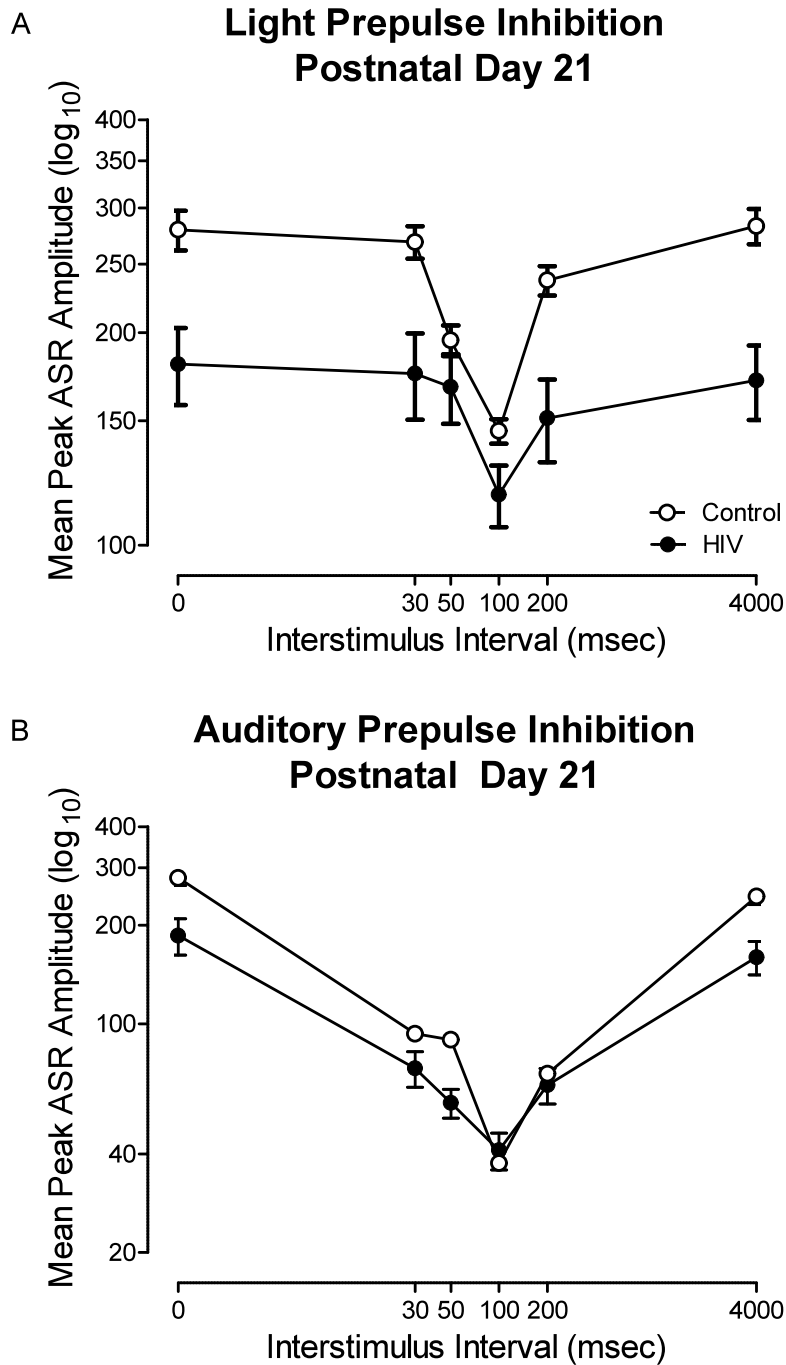


Figure 3.5 Prepulse Inhibition on Postnatal Day (PD) 21. A significant genotype x ISI interaction was observed in visual PPI at PD 17 and PD 21 (a). A significant genotype x ISI interaction was observed at all ages (PD 14, PD 17, and PD 21) in auditory PPI (b). Results are indicative of altered development of the ISI function, regardless of modality, in the HIV-1 Tg group.

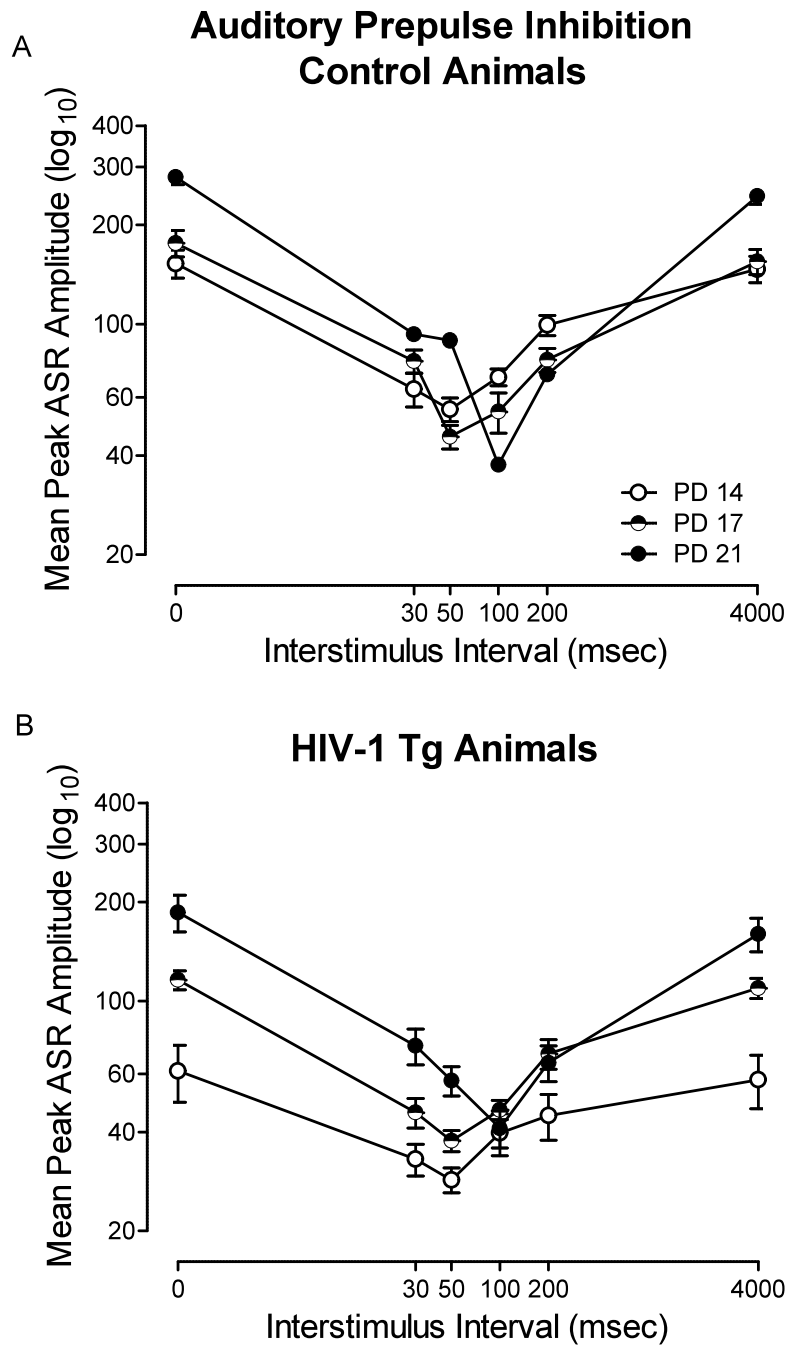


Figure 3.6 Auditory Prepulse Inhibition. Prepulse inhibition (PPI) with an auditory prepulse across all three test ages. A significant interstimulus interval (ISI) x genotype interaction was observed at all ages (i.e., PD 14, PD 17, and PD 21) indicating altered development of the ISI function in the HIV-1 Tg group.

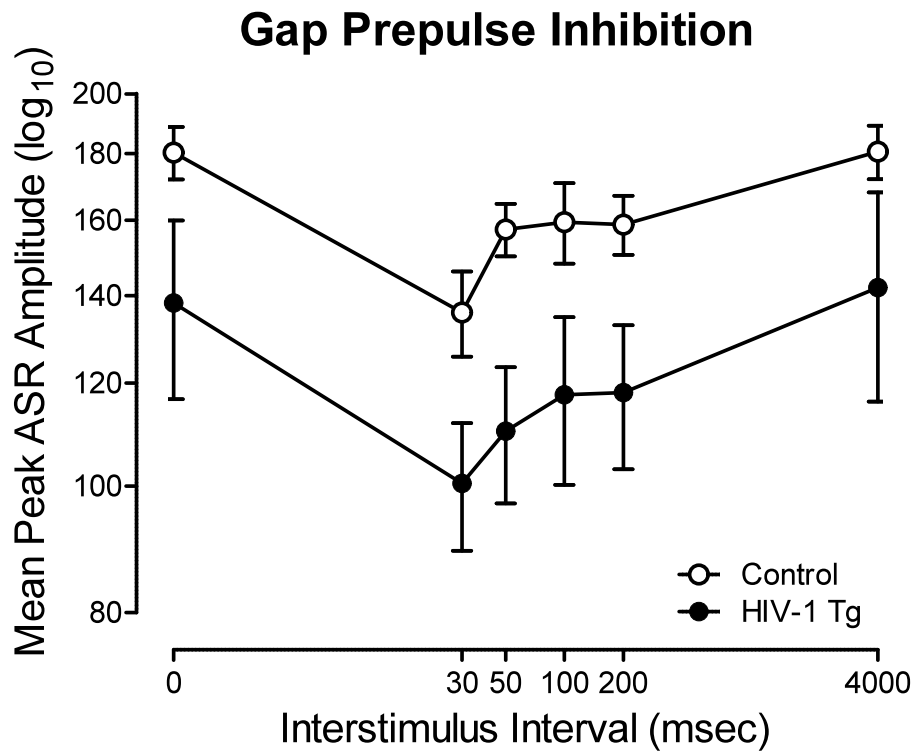


Figure 3.7 Gap-prepulse inhibition (gap-PPI). A significant main effect of age and ISI were observed. comparable peak inhibition was observed in both the HIV-1 Tg and control animals at the 30 msec ISI. Therefore, the HIV-1 Tg animals exhibit a downward shift in the mean peak ASR amplitude curve, but not a deficit in temporal processing.

## Maximal Animal Classification

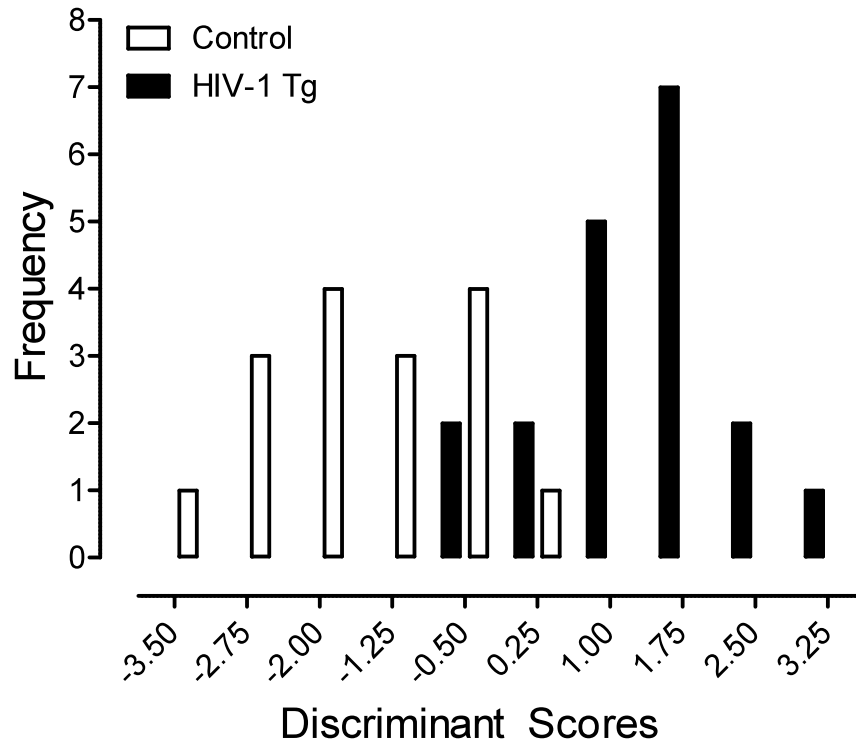


Figure 3.8 Discriminant Function Analysis. Animal classification is illustrated as a function of the canonical variable representing the simplest linear function that best separated the HIV-1 Tg and control groups (canonical correlation 0.83) and correctly identified (jackknife classification) group membership with 91.4% accuracy (93.8% of controls, and 89.5% of HIV-1 Tg animals).



## CHAPTER 4

### DISCUSSION

Selective early behavioral alterations in the HIV-1 Tg rat were observed using multiple experimental paradigms, including somatic growth, locomotor activity, cross-modal PPI, and gap-PPI. A rightward shift towards later eye opening was observed in the HIV-1 Tg animals in comparison to controls. HIV-1 Tg animals exhibit maximal spontaneous activity on PD 20, in comparison to PD 16 for control animals, indicating delayed maturation of the cholinergic inhibitory system. Alterations in the development of the ISI function were exhibited by HIV-1 Tg animals with both a visual and auditory prepulse. Presence of the HIV-1 transgene can be diagnosed with 91.4% accuracy using multiple behavioral assessments. Selective early behavioral alterations observed in the HIV-1 Tg rat resemble alterations observed in PHIV, providing the potential for developing a translational screening tool for the early diagnosis of chronic neurocognitive impairment observed in children perinatally infected with HIV-1.

#### ***4.1 Somatic Growth***

Selective alterations in somatic growth are observed in the HIV-1 Tg rat, assessed using body weight and eye opening. No statistical differences were observed in initial body weight, assessed from PD 8 to PD 10, results which replicate those previously reported in male Sprague-Dawley rats stereotaxically injected with Tat and/or gp120, HIV1 viral proteins, on PD 1 (Fitting *et al.*, 2008) and results previously reported in HIV-1 infected children (Guillen *et al.*, 2007; Parachure *et al.*, 2015). Previous reports in

children with PHIV indicate that early initiation of antiretroviral therapy, and therefore subsequent virologic control, leads to normal growth (Guillen *et al.*, 2007; Parchure *et al.*, 2015). The HIV-1 Tg rat, however, did exhibit selective alterations in somatic growth observed in eye opening measurements. Results of alterations in eye opening replicate those previously reported in male Sprague-Dawley rats stereotaxically injected with HIV-1 viral proteins (Moran *et al.*, 2014b). Therefore, the HIV-1 transgene may alter the development of selective somatic growth measurements.

#### **4.2 Motor Movement**

HIV-1 Tg rats exhibit alterations in the development of motor movement, assessed using locomotor activity. Evidence for alterations in the development of motor movement was revealed using the cumulative frequency of gross motor movements. Specifically, significant differences were evident at PD 12 and PD 20, but not at PD 16. Additional evidence for the alterations in the development of motor movement were evidenced by total ambulation. A second-order polynomial was best fit for control animals, with maximal spontaneous activity exhibited on PD 16. In contrast, a segmented first-order polynomial was the best fit for HIV-1 Tg animals. Therefore, HIV-1 Tg animals fail to exhibit decreased levels of spontaneous activity on PD 20, suggesting delayed maturation in the development of the cholinergic inhibitory system.

Alterations in the development of the cholinergic system in the forebrain may underlie early behavioral alterations in motor movement observed in the HIV-1 Tg rat. Campbell *et al.*, (1969) studied the effect of amphetamine, an indirect dopamine (DA) agonist, and scopolamine, a forebrain anticholinergic agent on locomotor activity on PD 10, 15, 20, 25, and 100. Administration of amphetamine increased activity during all

testing periods. In contrast, administration of scopolamine increased activity beginning at PD 20, suggesting that forebrain cholinergic inhibitory areas mature later than hindbrain structures (Campbell *et al.*, 1969). Furthermore, saline control groups exhibited maximum activity on PD 15, followed by a dramatic decline, implicating that forebrain cholinergic inhibitory areas may also modulate activity in a novel environment (i.e., locomotor activity; Campbell *et al.*, 1969). In the present study, control animals exhibited maximum spontaneous activity on PD 16, followed by a dramatic decline. In contrast, HIV-1 Tg animals exhibited maximum spontaneous activity on PD 20, suggesting delayed development of the forebrain cholinergic inhibitory areas.

Results of alterations in the development of motor movement in the present study replicate those previously reported in the HIV-1 Tg rat (Moran *et al.*, 2013b) and Sprague-Dawley rats stereotaxically injected with the HIV-1 viral proteins (Fitting *et al.*, 2008). In addition, developmental alterations in the HIV-1 Tg rat replicate those reported in HIV-1 infected children (Ferguson & Jelsma, 2009; Foster *et al.*, 2006; Whitehead *et al.*, 2014). Specifically, a longitudinal analysis of HIV-1 infected and sero-reverters in South Africa reported significant motor deficits in approximately 40% of HIV-1 infected children (Whitehead *et al.*, 2014).

### ***4.3 Temporal Processing***

Altered temporal processing development, assessed with cross-modal PPI, was observed in HIV-1 Tg animals compared to control animals. In PPI with a visual prepulse, the ISI functions observed in the HIV-1 Tg and control groups were not significantly different at PD 14, but subsequently changed in different ways (i.e., PD 17 and PD 21) indicating altered development of the ISI function. In addition, HIV-1 Tg

animals exhibit an alteration in the development of the ISI function observed in PPI with an auditory prepulse. Results in the present study replicate those previously reported in adult HIV-1 Tg animals (Moran *et al.*, 2013a) and Sprague-Dawley rats stereotaxically injected with the HIV-1 viral proteins on PD 1 (Fitting *et al.*, 2008; Fitting *et al.*, 2006a; Fitting *et al.*, 2006b). Both HIV-1 Tg and control animals exhibit significant inhibition in gap-PPI. Gap-PPI results in the present study are consistent with a preliminary longitudinal analysis conducted from PD 30 to PD 150.

The brain neural circuitry mediating PPI, illustrated in Figure 1, has been established using lesioning (i.e., Leitner & Cohen, 1985) and electrical stimulation studies (i.e., Li *et al.*, 1998; Li & Yeomans, 2000). Specifically, the serial circuit mediating PPI begins with auditory input relayed to the inferior colliculus (IC). Visual and tactile input, in contrast, are relayed to the superior colliculus (SC). Sensory input, regardless of modality, is then relayed from the SC to the pedunclopontine tegmental nucleus (PPTg), which ultimately triggers a cholinergic projection to the caudal pontine reticular nucleus (PnC; Fendt *et al.*, 1994; Fendt *et al.*, 2001; Koch *et al.*, 1993; Koch & Schnitzler, 1997). Activation of the PnC is relayed to motor neurons causing a startle response.

The role of dopamine in the circuit mediating PPI has been evidenced in previous behavioral and pharmacological studies (review, Geyer *et al.*, 2001; Moran *et al.*, 2009; Zhang *et al.*, 2000). Administration of direct (i.e., apomorphine) and indirect dopamine (DA) agonists (i.e., amphetamine) have been used to manipulate dopamine, subsequently disrupting PPI (review, Geyer *et al.*, 2001). An insensitivity to the manipulation of ISI, assessed using cross-modal PPI, has also been observed in rats administered

apomorphine; results that are comparable to those observed in the HIV-1 Tg rat (Moran *et al.*, 2009).

#### ***4.5 The Dopamine System and Chronic Neurological Impairment***

Clinical and preclinical studies have implicated disruptions in the DA system as an important factor in chronic HIV-1 associated neurological impairment (review, Fitting *et al.*, 2015; review, Ferris *et al.*, 2008). Significantly greater brain atrophy in HIV-1 seropositive individuals has been reported in areas rich in dopamine, including the basal ganglia (Kumar *et al.*, 2009), substantia nigra (Kumar *et al.*, 2011), and caudate nucleus (Kumar *et al.*, 2009). Furthermore, decreased DA transporter levels in HIV-1 seropositive individuals have been correlated with decreased performance on neuropsychological tests (Chang *et al.*, 2008). In vivo brain imaging studies replicate postmortem studies in HIV-1 seropositive individuals, providing further evidence for a DA system disruption in HIV-1 seropositive individuals (Purohit *et al.*, 2011). HIV-1 seropositive individuals had decreased brain volumetrics in the thalamus, hippocampus, and corpus callosum (Ortega *et al.*, 2013). Positron emission tomography (PET) scans provide evidence of a progressive striatal dopamine deficit that occurs in as the HIV-1 Tg rat ages (Lee *et al.*, 2014). Furthermore, in vitro studies have replicated the results presented in clinical and preclinical studies, which provide further evidence that the DA transporter is being targeted by Tat and gp120, two HIV-1 proteins (Aksenov *et al.*, 2008; Midde *et al.*, 2013).

Early behavioral alterations observed in the HIV-1 Tg rat may result from alterations in the development of the DA system. (review, Fitting *et al.*, 2015). Preclinical studies in the HIV-1 Tg rat have previously implicated DA system impairments as an

underlying factor in chronic neurological impairment (Moran *et al.*, 2012; Moran *et al.*, 2014b; Webb *et al.*, 2010). Specifically, pharmacological assessments were used to examine alterations in the midbrain DAergic system in the HIV-1 Tg rat (Moran *et al.*, 2012; Webb *et al.*, 2010). DAergic system dysfunction, assessed using Western blotting, was evidenced by alterations in phosphorylated tyrosine hydroxylase (pTH), dopamine transporter (DAT) mRNA, and/or monoamine oxidase A (MAO-A; Moran *et al.*, 2012; Webb *et al.*, 2010). Although multiple neural systems, including the dopaminergic and cholinergic system, may mediate the early behavioral alterations observed in the HIV-1 Tg rat, HIV-1 infection often affects DA system function, resulting in subsequent cognitive deficits (Di Rocco *et al.*, 2000; review, Fitting *et al.*, 2015; Wang *et al.*, 2004).

#### **4.6 Conclusions**

The study of selective early behavioral alterations in the HIV-1 Tg rat is vital to the development of a diagnostic screening tool for chronic neurologic impairment observed in children perinatally infected with HIV-1. HIV-1 Tg rats used in the present study are a healthier derivation of those originally described (Reid *et al.*, 2001), exhibiting no general wasting or pathological phenotypes. HIV-1 Tg litters used in the current study displayed no significant health disparities in comparison to F344 controls (i.e., similar initial body weight, similar litter size). Thus, the HIV-1 Tg rat provides a vehicle for investigating the development and underlying mechanisms involved in chronic neurologic impairments observed in PHIV (Vigorito *et al.*, 2015).

Selective early behavioral alterations observed in the HIV-1 Tg rat may provide a novel screening tool for the diagnosis of neurocognitive deficits in children perinatally infected with HIV-1. The potential utility of selective early behavioral alterations was

assessed using a discriminant function analysis, which correctly identified animals in regards to their genotype (HIV-1 Tg vs. control) with 91.4% accuracy. The presence of the HIV-1 transgene was best predicted using four variables at PD 20 and PD 21, brain development which approximates 2-3 years of age in humans (Review: Semple *et al.*, 2013). Therefore, selective early behavioral alterations observed in the HIV-1 Tg rats provide an opportunity for the development of a translational screening tool, which will allow early cART initiation, improving long-term outcomes for children perinatally infected with HIV-1 (Edwards *et al.*, 2015; Kitahata *et al.*, 2009).

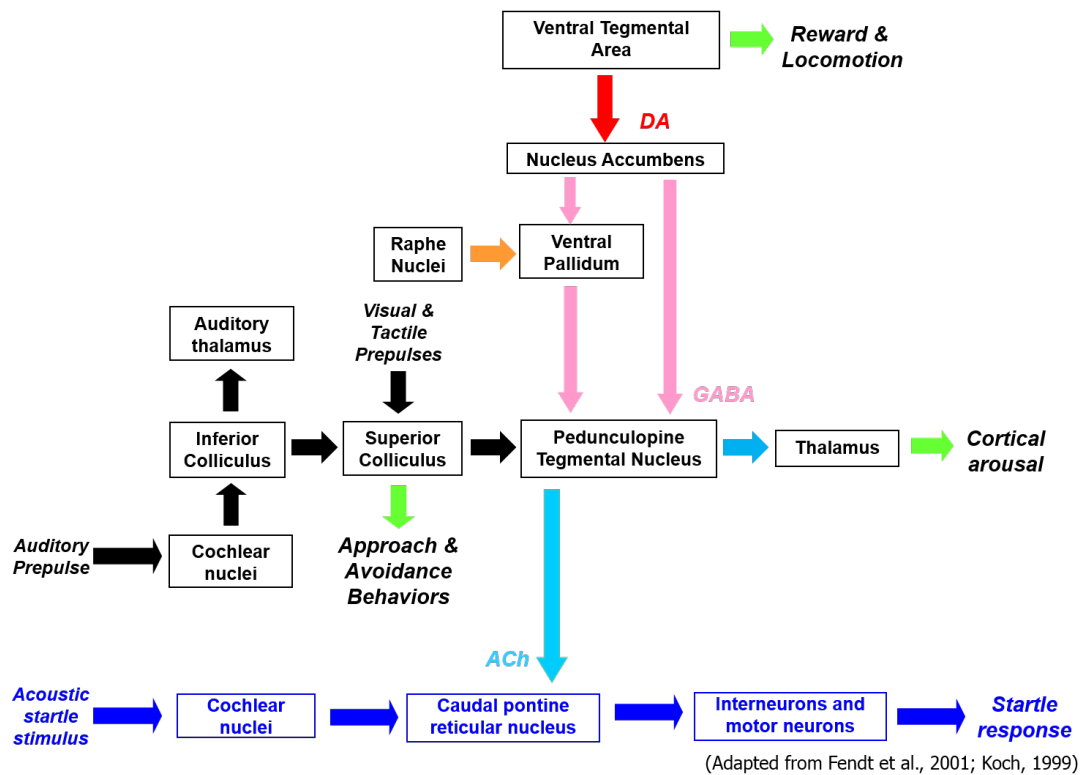


Figure 4.1. Hypothetical Serial Neural Circuitry of PPI. Adapted from Fendt *et al.* (2001) and Koch (1999).



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