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Modeling HIV-1-Associated Neurocognitive Disorders and the Therapeutic Effects of the Phytoestrogen Metabolite S-Equol in the HIV-1 Transgenic Rat

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MODELING HIV-1-ASSOCIATED NEUROCOGNITIVE DISORDERS AND THE THERAPEUTIC EFFECTS OF THE PHYTOESTROGEN METABOLITE S-EQUOL IN THE HIV-1 TRANSGENIC RAT

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DEDICATION

To my family: Mom, Dad, Steve, Kerri, Marty, and Lisa. Your love and support has meant everything. Thank you for always encouraging me to aim high.

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ABSTRACT

HIV-1 associated neurocognitive disorders (HAND) continue to afflict individuals with HIV-1 in the combination antiretroviral treatment (cART) era, most notably affecting executive function, as well as preattentive processing. Currently, there is no effective treatment for HAND, with only adjunctive treatment targeting symptomatic relief. There were two hypotheses in this dissertation: 1) that HIV-1 Tg rats will show disruptions in measures of executive function and preattentive processing, as well as neural network alterations in the prefrontal cortex (PFC), a brain region implicated in executive function, and 2) that administration of the phytoestrogen metabolite S-equol will improve performance as measured by executive function and attention, as well as neuronal network complexity in the PFC.

In experiment 1, using prepulse inhibition of the auditory startle response and a series of operant tasks, deficits were revealed in perceptual sharpening, sustained attention, and core components of executive function. Daily oral S-equol treatments (0, 0.05, 0.1, and 0.2 mg), administered to the animals at 6-8 months of age, improved the performance of the HIV-1 Tg animals on the sustained attention task. Assessment of neuronal networks with diOlistic labeling of pyramidal neurons in the PFC suggested that the 0.2 mg dose of S-equol ameliorated alterations in the HIV-1 Tg animals as well.

In experiment 2, treatment with S-equol (0.2 mg), begun at 2-3 months of age, significantly delayed or prevented deficits in sustained attention. HIV-1 Tg animals that

received S-equol also displayed enduring improvements in performance one month after the treatment ended, an effect not detected in any of the other groups. However, further assessments of increased demands on sustained attention as well as selective attention did not further differentiate the HIV-1 Tg and control animals.

In summary, the HIV-1 Tg rats displayed impaired performance in preattentive processing, attention, and executive function, prior to any clinical signs of wasting. S-equol was effective in both ameliorating and preventing attentional deficits, suggesting its potential use as a therapeutic for neurocognitive impairments in HAND.

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AAN ..... American Academy of Neurology

ANI .....Asymptomatic neurocognitive impairment

cART ..... Combination antiretroviral therapy

HAD .....HIV-1-associated dementia

HAND .....HIV-1-associated neurocognitive disorders

HIV-1 .....Human immunodeficiency virus

HNRC ..... HIV Neurobehavioral Research Center

MCMD ..... Minor cognitive motor disorder

MND ..... Mild neurocognitive disorder

MTCT ..... Mother-to-child-transmission

CHAPTER 1

INTRODUCTION

HIV-1-Associated Neurocognitive Disorders

The current estimate of the number of people living with HIV-1 worldwide is 34 million (UNAIDS 2012). The annual rate of new HIV-1 infections has decreased in several countries in the past decade, but many other regions, including the Middle East, North Africa, Eastern Europe, and Central Asia, have seen a continued increase in yearly incidence rates, contributing to an estimated 2.5 million new infections worldwide in 2011. Children represent a substantial proportion of these new cases; 330,000 children under 15 years of age were newly infected in 2011. Combination antiretroviral therapy (cART) has decreased the rate of mother-to-child-transmission (MTCT) of HIV-1 in the U.S. and other western countries from approximately 25% to less than 2% (Connor et al. 1994; Taha et al. 2009), but in developing countries with limited access to cART and other interventions, the MTCT rate is much higher, approximately 25-35% (Chen et al. 2009;Volmink and Marais, 2008). Although cART use substantially reduces the rate of MTCT, other factors such as high maternal viral load and preterm delivery can increase the risk of MTCT, even when cART is used (Townsend et al. 2008; Warszawski et al. 2008). Thus, HIV-1 remains a major health issue worldwide for both adults and children.

A significant and common consequence of HIV-1 infection, observed since the beginning of the HIV/AIDS epidemic (American Academy of Neurology AIDS Task

Force 1991; Antinori et al. 2007), is neurocognitive impairment, ranging from mild deficits to frank dementia. Prior to the introduction of cART, HIV-1-associated dementia (HAD) occurred in up to 20% of individuals with HIV-1 (McArthur et al. 1993; Ances and Ellis 2007). cART has dramatically reduced the prevalence of HAD to less than 5% (Heaton et al. 2010). Nevertheless, HIV-1-associated neurocognitive disorders (HAND) continue to afflict up to 50% of patients on cART (Ances and Ellis 2007; Heaton et al. 2011). Despite the increased life expectancy provided by cART, HAND is a significant health problem for millions, disrupting normal functioning of daily activities and posing an increased risk for early mortality (Vivithanaporn et al., 2010).

In the pre-cART era, neurological disorders related to HIV-1 were designated as either HAD or minor cognitive motor disorder (MCMD) according to the criteria of the American Academy of Neurology (1991). The criteria for HAD were an abnormality in at least two cognitive areas causing difficulty in everyday activities, and an abnormality in either motor function or in neuropsychiatric or psychosocial functions. MCMD was defined as cognitive and motor impairment which causes difficulty in everyday activities, which is not as severe as that meeting the criteria for HAD. The reduction in severity of cognitive dysfunction in HIV-1+ individuals on cART, as well as the lack of clear definitions of cognitive impairment in the AAN criteria, necessitated a revised nosology for the changing face of HIV-1/AIDS. The HIV Neurobehavioral Research Center (HNRC) at the University of California, San Diego, proposed diagnostic criteria of three categories of neurocognitive disorders: HIV-1-associated asymptomatic neurocognitive impairment (ANI), HIV-1-associated mild neurocognitive disorder (MND), and HIV-1-associated dementia (HAD) (Antinori et al., 2007). The HNRC criteria place emphasis on

neurocognitive impairment over motor impairment or psychosocial problems, and list the following as cognitive domains that should be evaluated: verbal/language, attention/working memory, abstraction/executive function, memory (learning/recall), speed of information processing, sensory-perceptual ability, and motor skills. A diagnosis of ANI is based on performance that is at least one standard deviation below the mean in at least two cognitive areas. Individuals with neurocognitive impairment that falls into the category of ANI do not experience difficulty in daily functioning as a result of the impairment. MND is defined by performance that is at least one standard deviation below the mean in at least two different cognitive domains, with the additional requirement being some degree of difficulty in everyday functions as a result of neurocognitive impairment. Lastly, HAD is diagnosed when the individual performs at least two standard deviations below the mean in at least two different cognitive domains with difficulty in performing everyday tasks. In each category, the impairment does not meet criteria for delirium, and there are no comorbid conditions which may underlie the neurocognitive impairment.

Currently, there is no effective treatment for HAND, despite a number of strategies that have been attempted or that are presently being tested (Alfahad and Nath, 2013). cART is limited as a neurotherapeutic due to low penetration across the blood-brain barrier (Thomas 2004), leaving only adjunctive treatments targeting symptomatic relief (Chung et al. 2008; Dewhurst et al. 2007; Eilers et al. 2008; Zink et al. 2005).

Executive Function and HIV-1

Before the introduction of cART, HIV-1+ individuals with HAND showed deficits primarily in information processing speed, sensory-perceptual skills, motor skills,

and language (Heaton et al. 1995; Heaton et al. 2011). Current studies on HAND, which represent individuals on cART, demonstrate that attention, memory, and executive function are the cognitive domains more readily affected in HAND (Cysique et al., 2004; Garvey et al., 2009; Heaton et al., 2011). Executive function, in particular, shows the greatest decline as a function of HIV-1 disease progression (Reger et al., 2002), which can have significant effects on the ability to perform important daily activities (Scott et al., 2011; Cattie et al., 2012). Executive function is a combination of higher-order cognitive processes that include planning, decision-making, problem-solving, and judgment (Spreen and Straus 1998). Attention, flexibility, response inhibition, and set-shifting are core components forming the foundation of higher-order processes of executive function (Keeler and Robbins, 2011).

A common clinical assessment of executive function is the Wisconsin Card Sorting Test (WCST), in which the participant is instructed to match cards based on one of several possible categories (e.g., color, number, shape), which is not made explicitly known to the participant. The participant determines the sorting criterion, which changes after a certain number of correct responses, based on feedback from the test administrator or computer. With these task demands, the WCST targets flexibility, set-shifting or shifting between sorting criterion, and inhibition of prepotent responses based on the previously correct sorting criterion. It has been used extensively to characterize the deficits in executive function in individuals with HIV-1 (Tozzi et al. 1993; Basso and Bornstein, 2003; Heaton et al., 2011). Another typical test of executive function that has been employed in studies of HAND is the Stroop Color Word Test (Chang et al., 2002; Maki et al., 2009). The participant is instructed to state the color in which a word is

presented, which, in the case of a word that is itself a color, requires inhibition of the prepotent response to read the word aloud as the answer. The use of the Stroop Color Word Test thus assesses inhibition, but not flexibility or set-shifting. Flexibility as an executive function has been measured in terms of category fluency, revealing deficits in switching subcategories while producing a list of words in a given category (Iudicello et al., 2008).

In some cases, even when HIV-1+ individuals perform as well as HIV-1- individuals on executive function tasks, significant differences may nevertheless be found in brain function. Using fMRI, HIV-1+ participants have decreased BOLD signal change in frontal regions, diminished functional frontostriatal connectivity, and a potential compensatory activity increase in the parietal attentional networks (Chang et al. 2001; Melrose *et al.*, 2008). In conjunction with reduced activity, volume loss in the medial frontal and premotor cortices, as well as thinning of the prefrontal cortex, are found in HIV-1+ individuals, reflecting the worsening of executive function which is associated with these brain regions (Thompson *et al.*, 2005).

Preattentive Processing and HIV-1

One of the most consistent neurological impairments that has been observed in the early stages of HIV-1 infection is an alteration in temporal processing, as assessed with amplitude and latency measures of auditory evoked potentials (AEPs), both without (Castello et al. 1998; Fein et al. 1995; Gil et al. 1992; Koralnik et al. 1990; Pagano et al. 1992; Schroeder et al. 1996; Vigliano et al. 2000), and after, cART initiation (Chao et al. 2004; Matas et al. 2010). Simian and feline immunodeficiency virus models have also

demonstrated alterations in evoked potentials (Phipps et al. 2000; Prospero-Garcia et al. 1999; Raymond et al. 1998; Riazi et al. 2009). Alterations in AEPs become more apparent with disease progression in HIV-1+ patients (Goodwin et al. 1996; Lalwani and Sooy 1992; Ollo et al. 1991; Schroeder et al. 1994), as is more generally observed in the progression from mild cognitive impairment to dementia (Golob et al. 2007).

More recently, temporal processing in HIV-1+ individuals has been assessed using prepulse inhibition (PPI) of the startle response. PPI is an operational measure of sensorimotor gating, a preattentive process in which the startle reflex is reduced when the startle stimulus is preceded 30–500 msec by a weak prepulse (Hoffman and Ison 1980). In HIV-1+ individuals with HAND, PPI is significantly reduced compared to cognitively unimpaired HIV-1+ individuals (Minassian et al. 2013). Alterations in PPI have also been observed in HIV-1 transgenic rats as well as in rats stereotaxically injected with the HIV-1 viral proteins Tat and gp120. Specifically, female Sprague-Dawley HIV-1 transgenic (Tg) rats have peak inhibition to an auditory prepulse at the 40 msec interstimulus interval (ISI) when tested at 5, 6, and 7 months of age, which was a leftward shift from the control group's peak inhibition at the 80 msec ISI (Moran et al. 2013). A leftward shift has also been demonstrated in the inhibition function in 30- and 60-day old male Sprague–Dawley rats following neonatal Tat injection (Fitting et al. 2006a) and in 9-month old male and female Sprague–Dawley rats given neonatal gp120 injections (Fitting et al. 2006b). Assessment of PPI in HIV-1 Tg rats prior to adulthood, which may provide information regarding subtle early-onset neurological alterations analogous to those reported in HIV-1+ humans, has not been conducted. Such information would be especially valuable in its potential for diagnostic and therapeutic applications in HAND.

Neuropathology of HIV-1

Central dopamine (DA) system dysfunction often results from HIV-1 infection and is associated with subsequent cognitive deficits (Chang et al. 2008; diRocco et al. 2000; Kumar et al. 2011; Wang et al. 2004). HIV-1 entry to the brain occurs early in the progression of the infection (An et al. 1999; Sinclair et al. 1994). The CNS is a main target of HIV-1 due to the presence of the CD4 and CCR5 receptors on macrophages and microglia of this system, to which the virus binds, subsequently allowing the virus to enter the cell (Clapham and McKnight, 2001). Macrophages infected with HIV-1 cross the blood-brain barrier, further infecting macrophages and microglia throughout the brain. As a result, viral load in the brain increases and neurotoxic HIV-1 proteins, such as Tat and gp120, are released, causing cell excitotoxicity and apoptosis (Ensoli et al. 1993; Tardieu et al. 1992). Thus, HIV-1 does not directly infect neurons, yet it initiates a chain of events resulting in neuronal damage and death that can persist even after viral suppression (Nath et al. 1999).

The DA system is particularly vulnerable to neuronal damage caused by the HIV-1 proteins, with DA-dependent signaling identified as one of the main mechanisms of its neurotoxicity (Aksenova et al. 2006; Silvers et al. 2007; Wallace et al. 2006). The dopamine transporter (DAT) is targeted by HIV-1 proteins Tat and gp120, resulting in transporter impairment (Aksenov et al. 2008; Ferris et al. 2009; Midde et al. 2013; Zhu et al. 2009; Zhu et al. 2011), as a function of direct protein-protein interactions (Zhu et al. 2009) involving an allosteric modulation of DAT by the Tat protein (Zhu et al. 2011). Impairment of DAT subsequently elevates synaptic concentrations of dopamine (DA). Dopamine (DA) thus binds to DA receptors on microglia, setting off several processes: HIV-1 replication, release of Tat and gp120, and production of inflammatory mediators,

such as cytokines (TNF-alpha) and chemokines. The inflammatory mediators and HIV-1 proteins induce apoptosis of DA neurons, further impairing the DA systems (Purohit et al. 2011).

Another distinguishing feature of HIV-1 neuropathology is the reduction of neuronal network complexity, which may be evident in dendritic spine density and morphology. Post-mortem neuropathological analyses on individuals with HIV-1 have revealed dendritic abnormalities, including vacuolation (Wiley, 1991), dilation, reduced branching density and dendritic length, and reduced dendritic spine density (Masliah et al., 1997). Dendritic abnormalities found during autopsies have been correlated with poor performance on neuropsychological tests, especially in tests of learning, perceptual-motor skills, abstraction and verbal abilities (Masliah et al., 1997). An in vitro study of prolonged exposure to lymphotropic HIV-1, macrophage-tropic HIV-1, or recombinant gp120 from HIV-1 found a decrease in microtubule and neurofilament protein expression in human fetal brain-derived neurospheres, inhibiting postmitotic neuronal dendrite development (McCarthy, Vidaurre, & Geffin, 2006). HIV-1 protein Tat-treated neuronal cultures from mice (Lu et al. 2011) and rats (Bertrand et al. 2013) exhibit significant loss of dendrites and dendritic spine structures. Dendritic pathology including beading and reduced spine density is also observed in the HIV-1 Tat transgenic mouse (Fitting et al. 2010).

Effects of Estrogenic Compounds on Cognition and Neuroanatomy

The effects of estrogen on cognition have long been studied, mostly to determine appropriate treatments for postmenopausal women who experience a decline in cognitive functions. Several studies have demonstrated improvements in cognitive function in

those receiving estrogen treatments (Carlson and Sherwin, 1998; Duff and Hampson, 2000; Keenan et al. 2001; Smith et al. 2001). However, because of the potential side effects of estrogen therapy, isoflavones have been explored as a safer, alternative treatment for neurocognitive impairment; beneficial effects of isoflavones have been reported (Casini et al. 2006; Duffy et al. 2003; File et al. 2005). Isoflavones, such as genistein and diadzein, are nonsteroidal plant-derived compounds that bind to estrogen receptors (Folman and Pope, 1966; 1969).

Findings from animal studies have also supported the use of isoflavones as a therapy for neurocognitive impairment. Improved performance in rats has been observed on hippocampal-based cognitive tasks such as the Morris water maze and the radial arm maze, following daily oral soy isoflavone treatment (Monteiro et al. 2008; Pan et al. 2000; Sarkaki et al. 2008), although there are examples of isoflavone treatment either having no effect or resulting in impairments (Neese et al. 2010; 2012). In the study of HAND, improving performance on executive function tasks that engage the PFC is of greater interest, primarily because executive function is more severely affected in HAND than are other cognitive domains. In addition, soy isoflavones bind preferentially to the estrogen receptor beta (ERβ) (Kuiper et al. 1997; Turner et al. 2007), which is highly expressed in the PFC. If soy isoflavones can improve cognitive performance that relies on the hippocampus, where a smaller concentration of ERβ is found, then the potential improvements in executive function by soy isoflavones could possibly be even more pronounced by their action in the PFC.

The beneficial effects of phytoestrogens on neurocognitive performance may derive from their actions on the dopamine (DA) system. The soy isoflavone genistein can

attenuate damage to DA neurons via inhibition of microglial activation and proinflammatory factor generation, as occurs in Parkinson's disease (Chen et al. 2007; Wang et al., 2005). Rats administered daily oral genistein treatment show an increase in DAT expression in the PFC (Neese et al. 2010). Stimulation of ERβ in ovariectomized rats with the ERβ agonist diarylproprionitrile causes increases in norepinephrine (NE) and DA activity in the PFC as indexed by their respective metabolites, as well as enhanced recognition memory (Jacome et al. 2010).

Estrogen and estrogenic compounds also impact neuronal network complexity. In female rats, spine density on pyramidal neurons of the primary sensory and motor cortices are higher during proestrous, when estrogen levels are at their highest, than during estrous or diestrous (Chen et al., 2009). Ovariohysterectomy reduces spine density on these neurons, whereas treatment with an estrogen pellet implanted subcutaneously for 14 days increases spine density to levels approximating those of the estrous and diestrous periods. Furthermore, ovariectomized rats that were given a phytoestrogen-enhanced diet (810 micrograms/g) for 7-9 weeks showed a significantly higher spine density in hippocampal and prefrontal cortex (PFC) pyramidal neurons (Luine et al., 2006). An increase in dendritic spine density in the PFC has also been observed in monkeys following intramuscular estradiol injections (Hao et al. 2006). Treatment of hippocampal slices with 17beta-estradiol promoted new spine formation without altering the pre-existing networks (Mendez et al., 2011). In addition to their benefits on neuronal growth, estrogenic compounds including soy isoflavones improve performance in maze tasks in ovariectomized female rats, suggesting an effect on memory processes (Monteiro et al., 2008; Sarkaki et al., 2008). In regard to HIV-1-induced neuronal injury, 17B-estradiol

treatment significantly reduces neurotoxic effects of Tat in rat neuronal cultures (Adams et al., 2010), and the phytoestrogens daidzein and liquiritigenin can both reduce synaptodendritic damage as well as restore neuronal networks affected by Tat (Bertrand et al. 2013). Clearly, the promise of phytoestrogen treatment for neurocognitive impairment requires in vivo studies of the effect of such compounds on neurocognitive function in an animal model of HIV-1, followed by measures of neuronal network complexity.

Equol is a metabolite produced by intestinal bacteria following ingestion of the soy isoflavone daidzein (Setchell et al. 1984). S-equol (the S-enantiomer) has selective affinity for ERβ, and shows an even greater affinity for ERβ than 17β-estradiol. S-equol is also the only enantiomer of equol that is produced by humans (Setchell et al. 2005). The benefits of a soy food-based diet, such as a reduced risk for certain cancers and increased bone density, are most typically found in adults who produce equol (Jackson et al. 2011; Lampe 2009; Setchell et al. 2002), who represent only 25-35% of the Western population (Rowland et al. 2000; Setchell and Cole, 2006). Therefore, equol, rather than its precursor, may be a more efficient and effective potential therapeutic for neurocognitive impairment of HAND.

The HIV-1 Transgenic Rat

The HIV-1 transgenic (Tg) rat is produced by microinjecting the HIV-1 provirus, minus the *gag-pol* genes, into fertilized one-cell eggs. In this way, the rat expresses 7 of the 9 genes for HIV-1, regulated by the viral promoter, Tat. It provides several advantages for its use compared to other animal models of HIV-1: 1) as a small animal

model, it is less expensive and more easily handled and housed than nonhuman primate subjects; 2) with the deletion of the HIV-1 genes *gag-pol*, it is non-infectious; 3) it expresses viral genes in lymph nodes, spleen, thymus and blood, similar to infected humans; 4) it develops neurological abnormalities that resemble those observed in humans with HIV-1, including proinflammatory responses and the expression of HIV proteins in the brain (Royal et al. 2012). The use of the HIV-1 Tg rat provides the opportunity to evaluate the progression of cognitive deficits over time, determine the relationship between cognitive deficits and neuroanatomical alterations, and identify appropriate treatments for neurocognitive impairment and the most effective time course for these treatments.

In addition to the specific benefits of studying the HIV-1 Tg rat in terms of modeling the expression of the virus, the rat can also provide a useful vehicle for the study of cognitive function and its underlying neurobiology. Several complex behaviors that rely on PFC function have been demonstrated in the rat. Homologous subregions of the PFC in the rat, monkey, and human are associated with parallel cognitive functions as well, suggesting that rat can be a useful and valid model of complex cognitive functions (Kesner and Churchwell 2011). Thus, executive function and attention, as the most affected domains in HAND, may be assessed with translatable results in the rat, with the use of measures selected to model specific components of cognitive function such as flexibility, inhibition, set-shifting, and sustained and selective attention.

Experimental Goals

HAND is a serious health issue that afflicts millions of people. The most notable deficit observed in individuals with HAND is the deterioration of executive function, which relies on several other core components of cognition, including attention. The preattentive process of sensory gating is also disrupted in individuals with HAND, prior to the development of deficits in higher level cognitive domains. Dopamine systems, which are a major neural substrate for executive function and preattentive processing, are particularly vulnerable to the neurotoxic effects of HIV-1. There is currently no effective treatment for HAND, although cART can attenuate the severity of the disorder. Estrogen-based compounds are a promising source of potential therapeutic use, given their neuroprotective properties, even in the presence of the HIV-1 protein Tat. In order to investigate potential therapeutics, the cognitive deficits and the underlying neuroanatomical abnormalities of HAND need to be modeled in preclinical studies; accordingly, the HIV-1 Tg rat was selected. The HIV-1 Tg rat provides an excellent vehicle to study HIV-1, expressing 7 of the 9 genes for HIV-1, and further resembles HIV-1+ humans with regard to the proinflammatory response and the presence of HIV-1 proteins in the brain. Little is known, however, of the cognitive deficits of the HIV-1 Tg rat as they relate to the current profile of HAND.

Thus, the goals of the present experiments were twofold: 1) determine the profile of cognitive deficits and neuroanatomical alterations present in the HIV-1 Tg rat; and 2) assess the utility of the phytoestrogen metabolite S-equol as a therapeutic for cognitive deficits and neuroanatomical alterations in the HIV-1 Tg rat.

Experimental Questions

The major experimental questions of this dissertation are the following: How are measures of attention, executive function and (pre)attentive processes, as well as neural network complexity, altered in the HIV-1 Tg rat (i.e., Can we model neurocognitive impairments across the progression of HAND?)? Can these cognitive, behavioral, and neuroanatomical alterations be ameliorated (Experiment 1) or delayed (Experiment 2) with the phytoestrogen metabolite S-equol?

The following hypotheses are proposed: 1) HIV-1 transgenic rats will exhibit deficits in attention and executive function, as measured with signal detection, and in preattentive processing, as measured with prepulse inhibition of the auditory startle response, as well as neural network alterations in the medial prefrontal cortex, an area implicated in executive function; and 2) S-equol will improve neurocognitive performance as measured as by executive function and attention.

CHAPTER 2

EXPERIMENT 1

The purpose of Experiment 1 was threefold: 1) to model the neurocognitive impairments of HAND using measures of preattentive processing and executive function, 2) to determine if treatment with S-equol at doses comparable to human soy consumption can ameliorate the neurocognitive impairments, and 3) to explore neural network alterations in the PFC as a function of chronic expression of the HIV-1 transgene. Executive function and preattentive processing are deficient in individuals with HIV-1, and reduced neural network complexity is associated with symptoms of HAND. *In vitro* studies show that HIV-1 viral proteins alter dendritic spine measures. Phytoestrogens enhance spine density, which should reflect improved cognitive function. Phytoestrogens have also been shown to improve cognition in preclinical studies (Monteiro et al. 2008; Pan et al. 2000; Sarkaki et al. 2008), but in some cases, appear to be without effect or impair it (Neese et al. 2010; 2012).

The prepulse inhibition (PPI) test and a series of operant tasks tapping attention and core components of executive function were conducted on HIV-1 Tg and control rats in experiments 1A and 1B. After assessing differences in performance between the two groups, sucrose or S-equol pellets (90 mg) were administered to the animals for 45 days while they continued to be tested on operant tasks, to determine if the S-equol treatment ameliorated deficits in performance in experiment 1C. The animals were divided into 4

dose groups, vehicle (sucrose) or one of three doses of S-equol (0.05, 0.1, and 0.2 mg), which allowed for a between-groups comparison of the vehicle group, and low, medium, and high dose groups, and a determination of the lowest dose to produce a detectable effect for use in experiment 2. Brains were then extracted for assessment of alterations in neuronal network complexity. Sample size for the experiment was determined by a power analysis in previous studies with this procedure.

The following hypotheses were proposed: 1) HIV-1 transgenic rats will exhibit deficits in attention and executive function, as measured with signal detection, and in preattentive processing, as measured with prepulse inhibition of the auditory startle response, as well as neural network alterations in the medial prefrontal cortex, an area implicated in executive function; and 2) phytoestrogens will improve neurocognitive performance as measured as by executive function and attention.

Experiment 1A: Preattentive Processing

Preattentive processing was assessed by measuring PPI of the auditory startle response (ASR) using both auditory and visual prepulses. Alterations in PPI, as measured by the amplitude of the ASR, have been demonstrated in adult HIV-1 Tg rats; in the present experiment, animals were tested starting at 2 months of age to determine if alterations in PPI can be observed early in the expression of the transgene, and were subsequently tested at later time points to determine the development of PPI with age and continued expression of the transgene. One of the readily apparent phenotypes of the HIV-1 Tg rat (and mouse, see Mozes et al. 2002) is cataracts, which present potential limitations in behavioral assessments that require a functional visual system. Therefore, during the first test session, the animals were tested with visual prepulses of different

intensities, to determine if the HIV-1 Tg animals were capable of detecting a visual prepulse, as evidenced by PPI of the ASR. If the HIV-1 Tg animals can display PPI with a visual prepulse, then the generality of any potential alterations in PPI across prepulse modality (visual and auditory) can be determined. It was hypothesized that the HIV-1 Tg animals would display alterations in PPI, given the evidence that HIV-1+ individuals are impaired in preattentive processing as measured with AEPs and PPI.

Animals

Two-month-old ovariectomized female Fischer (F344/N; Harlan Laboratories) rats (HIV-1 Tg, n=41; control, n=42) were delivered to the facility in groups of 3-14 animals every week for 10 weeks. All animals were group- or pair-housed throughout the experiments. Rodent food (2020X Teklad Global Extruded Rodent Diet (Soy Protein-Free)) and water were available *ad libitum* throughout the first PPI test period. The animals were under food restriction (85% body weight) beginning one week prior to and throughout operant testing, which coincided with the last two PPI test periods. The animals were maintained according to the National Institute of Health (NIH) guidelines in AAALAC-accredited facilities. The animal facility was maintained at 21° ± 2°C, 50% ± 10% relative humidity and had a 12-h light:12-h dark cycle with lights on at 0700 h (EST). Rats were handled for one week prior to any behavioral testing procedures. The Institutional Animal Care and Use Committee (IACUC) of the University of South Carolina approved the project protocol.

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, 81x81x116-cm isolation cabinet (external dimensions) (Industrial Acoustic Company, INC., Bronx, NY), rather than the 1.9 cm thick ABS plastic or laminate cabinets offered with this system. This double-walled isolation chamber provides over 30dB(A) of sound attenuation relative to the external environment. The ambient sound level in the chamber without any stimuli presented is 22dB(A). The high-frequency loudspeaker of the SR-Lab system (Radio Shack model#40-1278B) that was used to deliver all auditory stimuli (frequency range of 5k-16k Hz) was mounted inside the chamber 30 cm above the Plexiglas animal test cylinder. Three white LED lights were mounted inside the chamber, one on the wall behind the test cylinder (22 lux) presented as the visual prepulse at each of the three test periods, and two on opposite walls on either side of the cylinder (100 lux combined) presented together as the visual prepulse during a session in the first test period. The animal's whole body startle 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 (Bruel & Kjaer 2203) with the microphone placed inside the Plexiglas cylinder.

All animals were tested for PPI starting at 2 months of age, approximately one week after arrival.

ASR Habituation

One week after arrival, the animals were administered a 36-trial auditory startle test session to habituate them to the auditory stimulus and test procedures. Each session began with a 5-min acclimation period of 70dB(A) background white noise, followed by 36 trials of a 100dB(A) white noise stimulus with a 20-msec duration and a 10-sec intertrial interval (ITI). All test sessions were conducted in the dark.

Prepulse Inhibition Test

One day after ASR habituation, animals were tested for PPI of the ASR with both visual and auditory prepulse stimuli. All animals were administered a 30-min test session, which began with a 5-min acclimation period in the dark with 70dB(A) background white noise, followed by 6 pulse-only ASR trials with a 10-sec ITI. An equal number of visual and auditory prepulse trials [72 trials total; Interstimulus intervals (ISI) of 0, 8, 40, 80, 120, and 4000 msec, 6-trial blocks, Latin square design] were interdigitated in an ABBA order of presentation. The 0 and 4000 msec ISI trials were control trials used to calculate percent inhibition on PPI trials. The pulse stimulus intensity was 100dB(A) (20 msec duration) measured inside the test cylinder. A variable 20-sec ITI was used (range=15-25 sec). All animals were tested on two consecutive days. On the first day, one LED light (22 lux at the level of the test cylinder) was presented as the visual prepulse; on the second day, two LED lights (100 lux at the level of the test cylinder) were presented. On both test days, the auditory prepulse was an 85dB(A) white noise stimulus (20 msec duration) measured inside the test cylinder. Mean peak ASR amplitude values were collected for analysis. Percent PPI was derived from mean peak ASR amplitude data as the difference between average peak amplitude at 0 and 4000 msec ISIs and at the ISI at

which peak inhibition was observed, divided by average peak amplitude at 0 and 4000 msec ISIs, multiplied by 100.

Animals were tested similarly a second and third time with a minimum of 2 months of age separating each of the 3 tests for each animal. Only the 22 lux visual prepulse was used in the second and third test sessions, as it was determined after the first test that this stimulus was sufficient to cause robust inhibition of the ASR.

Statistics

All data were analyzed using analysis of variance (ANOVA) techniques (IBM SPSS Statistics 20). For each prepulse type (auditory and visual), a four-way mixed-factor 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 age, ISI, and trial as the within-subjects factors. A four-way mixed-factor ANOVA was also performed on mean peak ASR amplitude during visual prepulse trials at 2 months of age, with genotype as the between-subjects factor, and light intensity, ISI, and trial as the within-subjects factors. A similar analysis was conducted on mean peak ASR amplitude during auditory prepulse trials at 2 months of age, to determine if the intensity of the light stimulus used in the interdigitated visual prepulse trials differentially affected auditory PPI. Mean peak ASR amplitude during the habituation session was also analyzed with linear regression analyses. An alpha level of $p \leq 0.05$ was considered significant for all statistical tests. Sample sizes were chosen with the goal of sufficient statistical power (> 0.80) to maximize the likelihood of detecting subtle early alterations of expression of the HIV-1 transgene.

Experiment 1A: Results

Body Weight.

The HIV-1 Tg group weighed significantly less than the control group across the 6-month period during which they were tested, $F(1, 80)=92.4$, $p\leq0.001$ (Figure 2.1). Both groups increased significantly in body weight across this period [HIV-1 Tg: $F(21, 798)= 128.0$, $p\leq0.001$; control: $F(21, 882)=121.6$, $p\leq0.001$]. Through PD 91, both groups increased in weight according to a one-phase association curve fit. After attaining adulthood, and with the implementation of food restriction, both groups increased in weight in a linear function. There was no significant difference in the slope of these lines, indicating that the groups did not differ in their rates of growth.

ASR Intrasession Habituation.

Mean peak ASR amplitude data from the habituation session are illustrated in Figure 2.2. With the exception of the first 6 trials (during which the HIV-1 Tg group showed a sharp decrease in the ASR), linear regression analysis revealed that there was no difference in the rate of habituation across the test session for the HIV-1 Tg and control groups (Regression line slopes: HIV-1 Tg, -4.24+/-1.24; Control: -2.7+/-0.91), with no difference in overall ASR between groups, $F<1.0$.

PPI with an Auditory Prepulse (in Both 22 and 100 lux Visual Prepulse Contexts).

Mean peak ASR amplitude during auditory prepulse trials assessed at 2 months of age is illustrated in Figure 2.3. This first test period included a comparison of auditory prepulse trials that were interdigitated with 22 lux visual prepulse trials, and auditory prepulse trials that were interdigitated with 100 lux visual prepulse trials. Most importantly, there was no 3-way interaction between genotype, light intensity, and ISI,

nor an interaction between genotype and light intensity, suggesting that the brightness of the visual prepulse did not differentially affect PPI of either group during auditory prepulse trials. In fact, the ISI functions of the HIV-1 Tg and control groups changed in a similar manner with the increased light intensity of the prepulse, reflected by a significant light intensity x ISI interaction in each group [Control: $F(5,205)=5.0$, $p\leq0.001$; HIV-1 Tg: $F(5,200)=3.1$, $p\leq0.05$]; with both groups, the ISI functions sharpened and the point of peak inhibition shifted from the 80 msec ISI to the 40 msec ISI. The HIV-1 Tg and control groups also both displayed quadratic trends for ISI during auditory prepulse trials in each visual prepulse context, characteristic of the fundamental temporal domain of PPI [22 lux visual prepulse: Control, $F(1,41)=76.3$, $p\leq0.002$; HIV-1 Tg, $F(1,40)=69.6$, $p\leq0.001$; 100 lux visual prepulse: Control: $F(1,41)=122.0$, $p\leq0.001$; HIV-1 Tg: $F(1,40)=110.7$, $p\leq0.001$]. However, there was a genotype x ISI interaction for mean peak ASR amplitude during auditory prepulse trials that were interdigitated with the 100 lux visual prepulse trials, $F(5,405)=6.3$, $p\leq0.001$, indicating a relative insensitivity to manipulation of ISI duration in the HIV-1 Tg group. There was no genotype condition x ISI interaction during trials interdigitated with 22 lux visual prepulse trials.

PPI with an Auditory Prepulse Across Age (in the 22 lux Visual Prepulse Context).

The ANOVA conducted on mean peak amplitude during auditory prepulse trials interdigitated with 22 lux visual prepulse trials across all three test periods revealed a significant genotype x age x ISI interaction, $F(10, 790)=8.1$, $p\leq0.001$, as well as a genotype x ISI interaction, $F(5,395)=23.3$, $p\leq0.001$, an age x ISI interaction, $F(10,$

790)=14.3, $p \leq 0.001$, and a genotype x age interaction, $F(2,158)=16.6$, $p \leq 0.001$. Additional analyses were conducted to identify the locus of these interactions.

Separate analyses of each group revealed a main effect of age, $F(2,82)=21.3$, $p \leq 0.001$, and an age x ISI interaction, $F(10,410)=18.4$, $p \leq 0.001$, in the control group, illustrated in Figure 2.4. These effects were not observed in the HIV-1 Tg group, suggesting that the expression of the HIV-1 transgene interfered with the age-dependent development of perceptual sharpening.

Complementary results were obtained after separate analyses at each age, which revealed significant genotype x ISI interactions during the 2-month retest [$F(5,395)=10.5$, $p \leq 0.001$] and the 4-month retest [$F(5,395)=26.1$, $p \leq 0.001$], but not during the initial test (2 months old). The genotype x ISI interactions at the later ages indicate, as does the age x ISI interaction in the control group but not the HIV-1 Tg group, altered development of the ISI function in the HIV-1 Tg group; they did not exhibit the same sharpening of the ISI function with age that is apparent with the control group. Changes in percent PPI across age also reflect the differential development of the ISI function. The HIV-1 Tg group increased in percent PPI from 30.0%±5.5 during the initial test to 52.8%±4.4 during the 4-month retest, whereas the control group showed a much greater relative increase, from 15.2%±7.8 to 64.0%±3.9.

The overall genotype x ISI interaction reflects not only the relative insensitivity of the HIV-1 Tg group to manipulation of ISI duration, but also the differential peak inhibition of the two groups, observed at the 80 msec ISI during the initial test and the 120 msec ISI during the 2-month and 4-month retests for the HIV-1 Tg animals, and at the 80 msec ISI during the initial test and the 40 msec ISI during the 2-month and 4-

month retests for the control animals. Both groups showed significant quadratic trends for ISI, characteristic of the fundamental temporal domain of PPI [Control: $F(1,41)=261.9$, $p\leq0.001$; HIV-1 Tg: $F(1,38)=108.6$, $p\leq0.001$].

PPI with a Visual Prepulse (22 and 100 lux).

The ANOVA conducted on mean peak ASR amplitude during 22 and 100 lux visual prepulse trials at 2 months of age revealed that there was no interaction between genotype, light intensity, and ISI. There was also no genotype x light intensity or light intensity x ISI interaction, nor a significant main effect of light intensity. The effect of the 22 lux light on the ASR was robust and sufficient to demonstrate visual PPI in the HIV-1 Tg as well as the controls (percent PPI at the 40 msec ISI: Control, 72.2±2.6%; HIV-1-Tg, 74.9%±2.5); therefore, only the 22 lux visual prepulse was used for the subsequent test periods. Both groups displayed quadratic trends for ISI in each visual prepulse condition [22 lux: Control, $F(1,41)=148.3$, $p\leq0.001$; HIV-1 Tg, $F(1,40)=110.7$, $p\leq0.001$; 100 lux: Control, $F(1, 41)=138.4$, $p\leq0.001$, HIV-1-Tg: $F(1,40)=54.9$, $p\leq0.001$], with comparable peak inhibition at the 40 msec ISI (100 lux visual prepulse trials, percent PPI: Control, 73.9%±3.1; HIV-1-Tg, 66.2%±3.8). However, there was a significant genotype x ISI interaction during 22 lux visual prepulse trials, $F(5,405)=2.4$, $p\leq0.05$, as well as during 100 lux visual prepulse trials, $F(5,405)=9.0$, $p\leq0.001$, reflecting the relative insensitivity of the HIV-1 Tg group to manipulation of ISI duration (see Figure 2.5).

PPI with a Visual Prepulse Across Age (22 lux only).

Mean peak amplitude during 22 lux visual prepulse trials at each test period is illustrated in Figure 2.6. There was a significant genotype x age x ISI interaction, $F(10, 790)=7.7$, $p\leq0.001$, as well as a genotype x ISI interaction, $F(5,395)=31.1$, $p\leq0.001$, an

age x ISI interaction, $F(10, 790)=5.5$, $p\leq0.001$, and a genotype x age interaction, $F(2,158)=15.7$, $p\leq0.001$. Additional analyses were conducted to identify the locus of these interactions.

Separate analyses of each group revealed a main effect of age, $F(2, 82)=24.2$, $p\leq0.001$, and an age x ISI interaction in the control group, $F(10,410)=10.4$, $p\leq0.001$, but neither effect was present in the HIV-1 Tg group, suggesting that the expression of the HIV-1 transgene interfered with the age-dependent development of perceptual sharpening.

Significant genotype x ISI interactions were detected at each test period [initial test: $F(5,405)=2.4$, $p\leq0.05$; 2-month retest: $F(5,400)=18.7$, $p\leq0.001$; 4-month retest: $F(5,400)=29.3$, $p\leq0.001$]. Each group showed a significant quadratic trend for ISI [Control: $F(1,41)=381.3$, $p\leq0.001$; HIV-1 Tg: $F(1,38)=184.7$, $p\leq0.001$], but the genotype x ISI interactions indicated that the HIV-1 Tg group was relatively insensitive to manipulation of ISI duration at each test period, providing further evidence that the HIV-1 Tg group did not develop normal perceptual sharpening.

The HIV-1 Tg and control groups each displayed peak inhibition at the 40 msec ISI at each test period. The HIV-1 Tg group showed a quadratic trend for percent PPI across age, $F(1,38)=13.0$, $p\leq0.005$, whereas the control animals displayed similar percent PPI at each age. The quadratic trend reflects the reduction in percent PPI during the 2-month retest in the HIV-1 Tg group (58.2%±4.2, compared to 74.9%±2.5 during the initial test period and 72.1%±2.7 during the 4-month retest), which can be attributed to their lower ASR amplitude at the 0 and 4000 msec ISIs; amplitude at the point of peak

inhibition, 40 msec, was almost identical for the two groups (HIV-1 Tg = 85.0±15.3; Control = 83.7±8.2).

Experiment 1A: Discussion

The present experiment demonstrated alterations in preattentive processing as assessed with PPI of the ASR in the HIV-1 Tg rat early in the expression of the HIV-1 transgene and prior to any documented neurological symptoms or signs of wasting. In the absence of any difference in overall ASR or rate of habituation to the startle stimulus, the HIV-1 Tg group exhibited a flatter ISI function during PPI trials, which did not sharpen with age, as it did with control animals. Furthermore, the flatter ISI function was observed in both auditory and visual prepulse conditions, demonstrating the generality of sensorimotor gating deficits across prepulse modality. Over time, auditory prepulses precipitated a temporal shift in peak inhibition in HIV-1 Tg animals relative to controls, whereas with visual prepulses, both groups displayed peak inhibition at the 40 msec ISI. The observed alterations in PPI indicate a lack of perceptual sharpening with age and a relative insensitivity to the temporal dimension of sensorimotor gating in the HIV-1 Tg rat, resembling the temporal processing deficits reported in HIV-1+ individuals early in the disease course.

Perceptual sharpening is a developmental process in which responses are evoked by more specific stimuli, i.e., stimulus discrimination (Ganz 1968; Gibson 1969; Tees 1976; Werner 1948). Younger subjects (Rubel and Rosenthal 1975) and sensory-deprived subjects (Kerr et al. 1979) exhibit significantly flatter stimulus generalization gradients. Thus, experience, often a function of age, is a crucial element in normal perceptual

sharpening. In rats, the heart rate orienting response has been used to measure the ontogeny of perceptual sharpening. Rats at 16-17 days of age that have been habituated to an auditory stimulus will generalize the habituated response to a wide range of auditory stimuli; by day 20, stimulus discrimination is apparent with a much sharper generalization gradient (Campbell and Haroutunian 1983). We have previously observed perceptual sharpening of PPI with auditory, visual, and tactile prepulses in Long-Evans rats, at PD 18, 35, and 90 (Hord et al. 2008). These animals showed gradually sharper ISI functions for PPI with an auditory or tactile prepulse across age. As the visual system is the last system to develop in the rat (Gottlieb 1971), PPI with a visual prepulse was not apparent until PD 90, when peak inhibition was exhibited at the 40 msec ISI. At PD 18 and 35, the ISI functions were flat, reflecting the immaturity of the visual sensory system. The HIV-1 Tg and control rats in the present study exhibited robust PPI with a visual prepulse at two months of age, which suggests that the visual system and its afferents to PPI circuitry are well-developed at this age. However, the point of peak inhibition in the ISI functions of the HIV-1 Tg group under both prepulse conditions did not become clearly defined across age as was observed in the control group. Although the HIV-1 Tg rats appear to have functional auditory and visual systems, a more specific deficit in the development, or perceptual sharpening, of temporal sensitivity in the context of PPI was exhibited.

In the present experiment, the ISI functions of the HIV-1 Tg and control groups were most similar at two months of age, and then changed in different ways across age. For PPI with an auditory prepulse, both groups had peak inhibition at the 80 msec ISI at two months of age. At the later ages, however, the HIV-1 Tg group displayed peak

inhibition at the 120 msec ISI, representing a rightward shift from the control group's peak of inhibition at the 40 msec ISI. Differences in the point of peak inhibition have previously been observed in female Sprague-Dawley HIV-1 Tg and control rats as well, between 5-7 months of age (Moran et al. 2013). Shifts in peak inhibition have also been demonstrated in rats administered HIV-1 viral protein injections. Leftward shifts were observed in 30- and 60-day old male Sprague-Dawley rats following neonatal Tat injection (Fitting et al. 2006a) and in 9-month old male and female Sprague-Dawley rats given neonatal gp120 injections (Fitting et al. 2006b).

Despite the differences in sensorimotor gating observed between the HIV-1 Tg and control groups, they displayed similar inhibition to a visual prepulse, which is particularly notable given the presence of cataracts in the HIV-1 Tg animals. It is clear from this finding that the HIV-1 Tg group could detect the 20 msec visual stimulus despite their cataracts, and thus, a visual stimulus greater than or equal to the intensity and duration used in this experiment would have utility in other experimental paradigms with HIV-1 Tg rats, especially measures of executive function and other cognitive domains relevant to the study of HAND. The use of visual stimuli permits utilization of a variety of methods to test cognition in HIV-1 Tg rats.

The utilization of a visual prepulse in the PPI paradigm also affords the opportunity to determine the generality of any alterations in sensorimotor gating independent of prepulse stimulus modality. Comparable alterations in auditory and visual PPI, as observed in the present experiment, are consistent with a deficit in sensorimotor gating. Differential alterations in auditory versus visual PPI would, alternatively, be consistent with a more restricted sensory system impairment.

In summary, the present experiment demonstrates that HIV-1 Tg rats exhibit neurological deficits early in the expression of the HIV-1 transgene, prior to clinical signs of wasting, which progress with age, bearing a marked resemblance to the temporal processing deficits observed in individuals with HIV-1. Both the relative insensitivity to the temporal dimension of sensorimotor gating and the lack of development of perceptual sharpening with age suggest clear evidence of temporal processing deficits in the HIV-1 Tg rat.

Experiment 1B: Executive Function

The animals were trained on a series of operant tasks tapping attention and core components of executive function, modeling the analogous functions in humans, specifically the fundamental components of flexibility, inhibition, and set-shifting. It was hypothesized that the HIV-1 Tg animals would exhibit deficits in attention and the core components of executive function, which are the cognitive domains that are most typically impaired in individuals with HAND. Parallel components of executive function supported by homologous subregions of the PFC in the rat and human have been widely demonstrated (Kesner and Churchwell, 2011). Thus, for the present experiment, executive function and attention may be assessed with translatable results in the rat, with the use of measures selected to model specific cognitive functions.

Apparatus

Behavioral training and testing was conducted in 22 operant chambers located inside sound-attenuating chambers (Med Associates). One wall of each chamber consisted of two retractable levers, a pellet dispenser (45 mg) between the two levers, and a panel light (20±2 lux) above each lever and one above the pellet dispenser. A house

light was located on the rear wall. Signal presentation, lever operation, reinforcement delivery, and data collection were controlled by a PC and Med-PC for Windows software (V 4.1.3; Med Associates).

Signal Detection Task

Starting at 3-months of age, animals were initially trained to press both levers on an FR-1 schedule of reinforcement for sucrose pellets (45 mg). To prevent side-bias, subjects were not rewarded for more than five consecutive presses on a single lever. After the animals achieved at least 40 reinforcers during the 42-min sessions for three consecutive days, with less than 20% variance across days, they were trained on the signal detection task.

Each signal detection session began with a 5-min habituation period. The house light was off for the duration of the session. The presentation of signals (central panel light illumination) and non-signals (no illumination) was randomized over the 160 trial-session, with intertrial intervals of 9 ± 3 sec, during which time the levers remained retracted. Levers were extended 2 seconds after each trial (signal or non-signal) began and remained extended for 6 seconds for the animal to make a response. During signal trials, the light stimulus remained illuminated until the animal made a response, or 6 seconds elapsed, whichever occurred first. For half of the animals, lever presses on the left lever during signal trials and on the right lever during non-signal trials were rewarded (hits and correct rejections, respectively). The reverse set of rules was used for the other half of the subjects. Incorrect responses during signal trials (misses) and non-signal trials (false alarms) were not rewarded. An incorrect response was proceeded by up to three

repetitions of the trial. Finally, failure to respond appropriately to the correction trials resulted in a forced-choice trial in which the same stimulus type was repeated (signal or non-signal) but only the correct lever was extended and remained extended until a response was made or 2 minutes elapsed, whichever occurred first. Each animal was trained on the task each day until it achieved 70% or greater accuracy on three consecutive sessions. Accuracy was calculated as the total number of hits and correct rejections divided by the total number of correct and incorrect responses in a session.

Discrimination Task

Each discrimination task session began with a 5-min habituation period. The house light was off for the duration of the session. Left panel light and right panel light trials were presented randomly in a 160-trial session, with intertrial intervals of 9 ± 3 sec, during which time the levers remained retracted. The light stimulus during each trial was presented for 1 second, followed by the presentation of both levers for 6 seconds or until the animal made a response, whichever occurred first. Animals were rewarded for pressing the lever underneath the light stimulus. Following an incorrect response, animals were presented with three correction trials. If the animal again responded incorrectly, it was given a forced trial. Each animal was trained on the task until it achieved 70% or greater accuracy on three (non-consecutive) sessions. Accuracy was calculated as the total number of correct responses divided by the total number of correct and incorrect responses in a session.

Reversal Task

Each discrimination reversal task session began with a 5-min habituation period. The houselight was off for the duration of the session. Left panel light and right panel light stimulus trials were presented randomly in a 160-trial session, with intertrial intervals of 9 ± 3 sec, during which time the levers remained retracted. The light stimulus during each trial was presented for 1 second, followed by the presentation of both levers for 6 seconds or until the animal made a response, whichever occurred first. The opposite set of response rules from the discrimination task was used in the reversal task; the animals were rewarded for pressing the lever on the opposite side of the light stimulus. Following an incorrect response, animals were presented with three correction trials. If the animal again responded incorrectly, it was given a forced trial. Each animal was trained on the task until it achieved 70% or greater accuracy on three (non-consecutive) sessions. Accuracy was calculated as the total number of correct responses divided by the total number of correct and incorrect responses in a session.

Extradimensional Set-Shifting Task

Each extradimensional set-shifting task session began with a 5-min habituation period. The houselight was off for the duration of the session. Left panel light and right panel light stimulus trials were presented randomly in a 160-trial session, with intertrial intervals of 9 ± 3 sec, during which time the levers remained retracted. The light stimulus during each trial was presented for 1 second, followed by the presentation of both levers for 6 seconds or until the animal made a response, whichever occurred first. Half of the

animals were rewarded for pressing the left lever, and the other half were rewarded for pressing the right lever, regardless of the position of the light stimulus. Following an incorrect response, animals were presented with three correction trials. If the animal again responded incorrectly, it was given a forced trial. Each animal was trained on the task until it achieved 70% or greater accuracy on five consecutive sessions. Accuracy was calculated as the total number of correct responses divided by the total number of correct and incorrect responses in a session.

Statistics

All data were analyzed using SPSS Statistics 20 (IBM Corp., Somers, NY). For the signal detection task, percent accuracy, and hits, misses, correct rejections, and false alarms were analyzed with independent samples T-tests. For the discrimination, reversal, and extradimensional set-shifting task, a two-way mixed-factor analysis of variance (ANOVA) was used to analyze correct responses and errors, with genotype (HIV-1 Tg or control) as the between-groups factor, and response type (correct responses vs. errors) as a within-subjects factor. Each animal's performance on each measure was averaged across the first three days at which the animal performed with 70% accuracy, to provide the data for the analyses. Trials and errors to criterion were analyzed with independent sample T-tests, and sessions to criterion were analyzed with curve-fitting to assess the temporal process of acquisition. An alpha level of $p \leq 0.05$ was considered significant for all statistical tests. Sample sizes were chosen with the goal of sufficient statistical power (> 0.80) to maximize the likelihood of detecting subtle early alterations of expression of the HIV-1 transgene.

Experiment 1B: Results

Signal Detection Task.

All controls and all but 2 HIV-1 Tg animals met the criterion within one month of daily testing. The HIV-1 Tg and control groups followed similar rates in achieving the criterion, but the HIV-1 Tg group showed an initial 7-day lag before any of the animals met the criterion, reflected by their significantly greater number of trials [$t(80)=-2.1$, $p\leq0.05$] and errors to criterion [$t(80)=-2.4$, $p\leq0.05$] compared to the control group. In addition, a one-phase association regression analysis on the cumulative proportion of animals meeting the criterion over sessions revealed that a different curve was best fit to each group, $F(3,27)=245.2$, $p\leq0.01$, further illustrating the gap between groups in the number of sessions needed to reach criterion (see Figure 2.7). Once the animals met the criterion, percent accuracy was not significantly different between groups (3-day average: HIV-1 Tg: 77.8+/-0.43; Control: 77.8+/-0.42). However, the HIV-1 Tg animals responded significantly less than the control animals during the signal detection task, $F(1,80)=8.2$, $p\leq0.01$. A significant group x response type (correct vs. error) interaction [$F(1,80)=8.6$, $p\leq0.01$] suggested that the HIV-1 Tg animals displayed a different response profile than the control group. The HIV-1 Tg animals had fewer hits than the control animals, $t(80)=2.6$, $p\leq0.05$, misses, $t(80)=2.5$, $p\leq0.05$, and correct rejections, $t(80)=2.9$, $p\leq0.005$, than the control group, consistent with a lapse of attention. There was no significant difference in the number of false alarms, the index of response inhibition.

Discrimination Task.

All controls and 29 HIV-1 Tg animals met the criterion within 40 days of daily testing. There was no significant difference between HIV-1 Tg and control animals in errors or trials to criterion ($t \leq 1$, $p > 0.8$). However, regression analyses revealed that the rate at which each group achieved the criterion followed different functions; the HIV-1 Tg animals exhibited a linear function ($r^2 = 0.99$) whereas the control group showed a curvilinear one-phase association function ($r^2 = 0.98$), illustrating the slower acquisition of the task by the HIV-1 Tg animals over sessions (see Figure 2.8). Of the animals that met the criterion, there was no significant difference in percent accuracy (3-day average: HIV-1 Tg: 74.9+/-0.52; Control: 74.1+/-0.51). Despite attaining the same accuracy, the HIV-1 Tg animals that met the criterion made significantly fewer overall responses, $F(1,70) = 6.8$, $p \leq 0.05$. A significant interaction between group and response type, $F(1,70) = 6.8$, $p \leq 0.05$, indicates that there was a smaller difference between the number of correct responses and the number of errors in HIV-1 Tg group compared to control group.

Reversal Task.

Of the subjects that met the criterion for the discrimination task and continued to the discrimination-reversal task, 22 control animals and 8 HIV-1 Tg animals met the discrimination-reversal task criterion within one month of daily testing. There was no significant difference between HIV-1 Tg and control animals in errors or trials to criterion ($t \leq 1$, $p > 0.4$). However, linear regression analysis on the cumulative proportion of animals that achieved the criterion over sessions revealed that each group met the

criterion at different rates, demonstrated by significantly different slopes for each group's linear function, $F(1,30)=31.8$, $p\leq 0.001$ (see Figure 2.9). Of the animals that met the criterion, there was no difference in percent accuracy (3 day average: HIV-1 Tg, 73.5+/-0.66; Control, 73.9+/-0.43). Similarly, there was no significant difference between groups in the total number of responses, and there was no interaction between group and response type.

Extradimensional Set Shifting Task.

All control animals and all but 4 HIV-1 Tg animals met the criterion for extradimensional set-shifting within 8 days of testing (see Figure 2.10). The HIV-1 Tg and control animals did not differ in the number of trials or errors to criterion. Linear regression of the cumulative proportion of animals achieving the criterion over sessions revealed that the slopes and intercepts for each group were not significantly different; thus, the HIV-1 Tg and control animals did not meet the criterion at different rates. Of the animals that met the criterion within 8 days, there was no significant difference between groups in percent accuracy (5 day average; HIV-1 Tg, 90.1+/-0.60; Control, 90.2+/-0.56). The groups were not significantly different in the total number of responses and there was no interaction between group and response type.

Experiment 1B: Discussion

A series of operant tasks tapping attention and core components of executive function revealed prominent cognitive deficits in the HIV-1 Tg rat, prior to any documented neurological symptoms or clinical signs of wasting (Peng et al. 2010).

Chronic low level exposure to HIV-1 proteins in the HIV-1 Tg rat, which resembles the suppression of infection with brain proinflammatory immune response in HIV-1 positive individuals under cART, primarily impairs sustained attention as well as inhibition and flexibility as core components of executive function. Thus, the neurocognitive impairments that define HAND in the cART era can be modeled and demonstrated in the HIV-1 Tg rat, providing opportunities to develop therapeutics for HAND.

In a signal detection task tapping sustained attention and inhibition as a core component of executive function, all of the HIV-1 Tg rats attained the criterion of three consecutive sessions at 70% or greater accuracy, but did so after a significantly greater number of trials than the control animals. Despite achieving the same level of accuracy as the control group, the HIV-1 Tg animals made significantly fewer responses. Specifically, the number of hits, misses, and correct rejections, but not false alarms, was lower in the HIV-1 Tg group. The ability of the HIV-1 Tg animals to maintain a high level of accuracy demonstrates that they can acquire the task, albeit more slowly and despite a reduced response rate. In addition, the HIV-1 Tg animals demonstrated response inhibition comparable to that of the control animals, as indicated by the low number of false alarms in both groups.

In a discrimination task which served to provide a baseline for the subsequent reversal and extradimensional set-shifting tasks, the HIV-1 Tg rats again exhibited a slower rate of acquisition of the criterion, which required three sessions at 70% or greater accuracy, as well as a significantly lower response rate compared to the control animals. As in the signal detection task, they were able to maintain a high level of accuracy despite a difference in the number of correct responses and errors compared to the control

animals. Not all of the HIV-1 Tg animals acquired the task; however, those that did took longer to achieved the criterion level of accuracy, suggesting an impairment in attentional processes.

Like the signal detection task, the discrimination task required the animals to attend to a visual stimulus; in contrast, the critical factor in determining the correct response was the position of the stimulus rather than its presence or absence. Although both tasks tap into sustained attention, the discrimination task was more challenging for the animals in the present study compared to the stimulus detection task. In the signal detection task, the animals were presented with the visual stimulus until they made a response on a lever; the duration of the stimulus was contingent on their response, for a maximum of six seconds. This contingency was implemented after the animals failed to learn the task when only a brief one second stimulus was employed. In the discrimination task, the animals were presented with the visual stimulus for only one second, after which the levers were extended for them to make the response. Therefore, the demands on attention were greater in the discrimination task, as the stimulus was turned off before they could make a response on a lever. The difference in task demands may explain why fewer HIV-1 Tg animals were able to meet the criterion on the discrimination task. However, this task was not the animals' first exposure to stimuli of shorter durations. Prior to the discrimination task, the animals were trained on another sustained attention task, in which the visual stimulus was presented for 100, 500, or 1000 ms, prior to the presentation of the levers (data not shown). Not all of the animals were able to maintain accuracy on this task above 70%, as the much shorter stimulus durations as well as their variable presentation throughout each session increased the task difficulty. The

subsequent discrimination task was expected to be an easier test for the animals, given the consistent 1000 ms stimulus duration. The change in the stimulus-response contingency, by which the position of the visual stimulus, rather than its presence or absence, indicated the correct response, apparently was difficult for the HIV-1 Tg animals to learn and respond to consistently.

It is notable and worthy of acknowledging that despite the evidence for comparable levels of accuracy, lower rates of responding, compared to the control rats, were displayed by the HIV-1 Tg rats in both tasks. Typically, a lower response rate is indicative of attenuated motivation. In the face of greater cognitive demand, the subject may become less likely to be motivated to work for the reinforcer. A lower number of responses was observed in the HIV-1 Tg group during the signal detection and discrimination tasks, but in these and all other tasks, the HIV-1 Tg animals that met the criteria performed with the same high level of accuracy as the controls. Therefore, it is unlikely that the animals were not sufficiently motivated. In addition, all of the animals were maintained at 85% of the normal body weight for female F344 rats of their age in order to instill motivation to work for the sucrose reinforcers.

In the discrimination-reversal task, which taps flexibility and inhibition, only a quarter of the HIV-1 Tg animals that were tested with reversal (i.e., those that did not meet the criterion for the discrimination task were not tested with reversal) were able to meet the criterion of three sessions at 70% or greater accuracy, and those animals reached the criterion with a slower rate of acquisition compared to the control animals. The animals that met the criterion performed at the same accuracy level and did not show any difference in the total number of responses or in the number of correct responses or errors

compared to the control animals. Impairments in flexibility and inhibition as core components of executive function are clearly observed in the HIV-1 Tg group with the reversal task, although the deficits are not uniform across all of these animals. Most of the HIV-1 Tg animals were not able to acquire the reversal task, and those that did acquired it significantly more slowly than the control animals, consistent with a major impairment in the population of HIV-1 Tg rats. Thus, as in many cognitive disorders, there are varying degrees of impairment, but impairment nonetheless.

In the extradimensional set-shifting task which taps set-shifting, inhibition, and flexibility, the HIV-1 Tg animals acquired the criterion of five consecutive sessions at 70% or greater accuracy at the same rate as the control group, performing with the same high level of accuracy. The HIV-1 Tg animals also responded at the same rate as the control animals throughout sessions, with no difference in the number of correct responses or errors compared to the control group. The extradimensional set-shifting task was undoubtedly the least difficult task for both groups, as all but 4 animals met the criterion within 8 days, as opposed to the other tasks which required up to 40 days to acquire. It is also the one task that did not demonstrate any difference in performance between the HIV-1 Tg and control animals. Set-shifting, a component of executive function which was uniquely targeted by the extradimensional set-shifting task, thus appears to be intact in the HIV-1 Tg animals. In contrast, as noted above, HIV-1 Tg rats were impaired in acquiring the discrimination or reversal tasks, which place demands on cognitive flexibility and inhibition, but not set-shifting.

DA plays a critical role in the processes of attention and executive function. In animal studies, the function of DA in these cognitive processes has been primarily tested

by administering DA agonists and antagonists. For example, disruptions in sustained attention assessed with the five-choice serial reaction time task have been reported following mPFC infusions of D1 antagonists; in contrast, mPFC infusions of D1 agonists produce improvements in sustained attention (Granon et al. 2000; Chudasama and Robbins, 2004). In addition, DA receptor activity in the nucleus accumbens regulates cortical ACh efflux (Moore et al., 1999; Zmarowski et al. 2005), another important process underlying sustained attention (McGaughy et al. 1996; Himmelheber et al. 2000). Executive function processes also rely on the integrity of DA systems; performance on the reversal task is disrupted by D2 receptor antagonists (Lee et al., 2007; Ridley et al., 1981) and DA depletion in the striatum (Clarke et al. 2011; O'Neill and Brown 2007), and is also correlated with D2 receptor activity in the striatum (Clatworthy et al, 2009; Kellendonk et al, 2006; Groman et al., 2011). Increases in DA in the rat PFC improve performance on the extradimensional set-shifting task (Tunbridge et al. 2004), whereas depletion of PFC DA impaired performance on the task in monkeys (Crofts et al., 2001). Rodent models of schizophrenia often incorporate the extradimensional set-shifting task to demonstrate deficits in executive function, which are attenuated by D2 antagonist antipsychotics (McLean et al. 2008; Rodefer et al., 2008; Tait et al., 2009).

In experiment 1A, the HIV-1 Tg group demonstrated temporal processing deficits as measured with prepulse inhibition. The HIV-1 Tg animals, compared to controls, showed an insensitivity to interstimulus interval and a lack of development of perceptual sharpening in relation to the interstimulus interval with age. As in the present experiment, the deficits were observed prior to the onset of clinical symptoms or wasting. The temporal processing deficits that are displayed early in the expression of the HIV-1

transgene may underlie other cognitive impairments such as those observed in the present experiment. Fundamental to an assessment of temporal processing in attention and executive function is the manipulation of temporal aspects, such as signal duration, which is explored in Experiment 1C.

In summary, the HIV-1 Tg rats displayed impaired performance in a series of cognitive tasks, prior to any clinical signs of wasting, that implicate specific deficits in the processes of attention as well as flexibility and inhibition as core components of executive function. Deficits in attention and core components of executive function may reflect an underlying impairment in temporal processing in HAND.

Experiment 1C: Amelioration of Attentional Deficits with S-equol

The previous experiments demonstrated that the HIV-1 Tg animals have deficits in temporal processing, as measured with PPI, and that they have deficits in attention and executive function as assessed with the signal detection tasks. In the present experiment, animals were again tested with the signal detection task, with the additional manipulation of the temporal domain, i.e., signal duration. Three hypotheses were proposed: 1) shorter signal durations will increase demands on sustained attention for both the HIV-1 Tg and control animals; 2) the HIV-1 Tg group will display a greater impairment in performance relative to the control group; and 3) daily treatment with S-equol will ameliorate the observed deficits in the HIV-1 Tg group relative to the control group.

Materials and Methods

S-equol.

S-equol was obtained from Cayman Chemical Company (Ann Arbor, MI) and incorporated into 90 mg sucrose pellets by Bio-Serv (Frenchtown, NJ), to produce pellets containing 0.05 mg S-equol. Plain 90 mg sucrose pellets were also obtained from Bio-Serv to provide to the sucrose-only dose group.

Design.

Animals were first trained with the signal detection task with varying signal durations. After achieving the criterion for acquisition (70% accuracy on three consecutive days), the animals were subsequently tested with operant tasks tapping executive functions, the results of which are described in Experiment 1B. Following these tests, the animals began daily treatment with sucrose or S-equol. After 1 week of treatment, the animals resumed testing with the signal detection task that they had previously learned in Experiment 1B. Once each animal achieved the criterion of 3 consecutive days of 70% accuracy, it was tested with the 100, 500, and 1000 ms signals as they had been trained on previously. Daily treatment with S-equol or sucrose continued throughout behavioral testing.

All animals were assigned to one of 4 dose groups (vehicle, 0.05, 0.1, and 0.2 mg S-equol) using a randomized-block design, with percent accuracy on the signal detection task as the blocking factor, such that animals of all accuracy levels were distributed equally across dose groups. Each S-equol pellet contained 0.05 mg; thus, the 0.05 mg dose group received one pellet per day, the 0.1 mg dose group received 2 pellets per day,

and the 0.2 mg dose group received 4 pellets per day. The vehicle group received 4 sucrose pellets per day. The doses selected for this experiment yielded a daily amount of 0.25-1.0 mg/kg, which is equivalent to a 2.5-10 mg dose in a 60 kg human. Animals were given their treatments in separate cages at least one hour after behavioral testing, and typically consumed their pellets within seconds.

Procedure.

Prior to testing with varying signal durations, animals were again trained on the signal detection task as described in Experiment 1B. After reaching the criterion of 70% accuracy on three consecutive days, animals began daily signal detection test sessions with varying signal durations. Each session began with a 5-min habituation period. The houselight was off for the duration of the session. Signal and nonsignal trials were presented randomly and equally in a 160-trial session, with intertrial intervals of 9 ± 3 sec, during which time the levers remained retracted. Signals were presented for 100, 500, or 1000 ms; each signal duration was randomly and equally presented throughout the session. Signal offset was followed by the presentation of both levers for 6 seconds or until the animal made a response, whichever occurred first. For half of the animals, lever presses on the left lever during signal trials and on the right lever during non-signal trials were rewarded (hits and correct rejections, respectively). The reverse set of rules was used for the other half of the subjects. Incorrect responses during signal trials (misses) and non-signal trials (false alarms) were not rewarded. Each animal was trained on the task until it achieved 70% or greater accuracy on three consecutive sessions. Percent accuracy was calculated as the number of hits during 1000 ms signal trials and the

number of correct rejections divided by the number of hits and misses during 1000 ms signal trials, false alarms, and correct rejections, multiplied by 100. The same operant chambers as described in Experiment 1B were used in the present experiment. signal detection task that they had previously learned. Once each animal achieved the criterion of 3 consecutive days of 70% accuracy, it was tested with the 100, 500, and 1000 ms signals as they had been trained on previously. Daily treatment with S-equol or sucrose continued throughout behavioral testing. The same operant chambers as described in Experiment 1 were used in the present experiment.

Statistics.

All data were analyzed using SPSS Statistics 20 (IBM Corp., Somers, NY). For the initial signal detection task with varying signal durations, independent samples t-tests were performed on percent accuracy, false alarms, and correct rejections. A two-way mixed-factor ANOVA was conducted on hits and misses, with genotype (HIV-1 Tg vs. control) as the between-subjects factor, and signal duration (100, 500, or 1000 ms) as a within-subjects factor. A mixed-factor ANOVA was also conducted on performance at the end of the 45-day S-equol treatment period, with genotype and S-equol dose (0, 0.05, 0.1, or 0.2 mg) as between-subjects factors, and signal duration as a within-subjects factor for hits and misses. A separate ANOVA was also conducted to compare performance on the first three days of S-equol treatment to performance at the end of the 45-day treatment period. Each animal's performance on each measure was averaged across three consecutive days at the respective time points to provide the data for the analyses. An alpha level of $p \leq 0.05$ was considered significant for all statistical tests.

Sample sizes were chosen with the goal of sufficient statistical power (> 0.80) to maximize the likelihood of detecting subtle early alterations of expression of the HIV-1 transgene.

Experiment 1C: Results

When the animals were first trained on the signal detection task with varying signal durations, both the HIV-1 Tg and the control groups showed significant linear trends for signal duration for hits [HIV-1 Tg: $F(1, 32)=61.1$, $p\leq0.001$; Control: $F(1, 41)=53.2$, $p\leq0.001$] and for misses [HIV-1 Tg: $F(1, 32)=60.1$, $p\leq0.001$; Control: $F(1, 41)=59.6$, $p\leq0.001$], indicating that all animals performed worse as a function of shorter signal durations. However, the HIV-1 Tg group, relative to controls, failed to reliably detect the signal at shorter signal durations. The HIV-1 Tg animals had approximately equal numbers of hits and misses to the 500 ms signal, and more misses than hits during 100 ms signal trials (see Figure 2.11). The control animals, in contrast, performed with accuracy during 500 ms signal trials; a major decrement in accuracy was only apparent with the 100 ms signal. The HIV-1 Tg animals also had significantly lower percent accuracy than the control animals, $t(73)=2.4$, $p\leq0.05$, even when only the animals that achieved 70% accuracy on 3 consecutive days were included, $t(35)=2.1$, $p\leq0.05$. There was no difference in the number of hits, misses, false alarms, or correct rejections between groups.

After 45 days of S-equol or sucrose treatment, the HIV-1 Tg animals had significantly more misses [$F(1,70)=6.6$, $p\leq0.05$] and correct rejections [$F(1,77)=6.8$, $p\leq0.05$] than the control animals, with no difference in hits, false alarms or percent

accuracy. Both the HIV-1 Tg and control animals exhibited a linear trend for signal duration for both hits [$F(1,70)=155.1$, $p\leq0.001$] and misses [$F(1,70)=148.3$, $p\leq0.001$], as observed when the animals first learned the task prior to S-equol or sucrose treatment. There was no significant effect of S-equol treatment, nor a condition x S-equol treatment interaction, on any measure.

Additional analyses on the top performing third of all animals (determined by percent accuracy) were conducted to further explore the nature of the difference in the number of misses between groups. When performance was compared between the first week of S-equol treatment and after 45 days of treatment, the HIV-1 Tg animals given the 0.2 mg dose of S-equol and control animals collapsed across all doses showed a significant decrease in the number of misses [HIV-1 Tg: $F(1,3)=20.6$, $p\leq0.05$; Control: $F(1,3)=76.3$, $p\leq0.005$]. The HIV-1 Tg animals also displayed differential responding at the 500 ms signal length, with more hits than misses, as the controls consistently show (see Figure 2.12). There were significant group x pre-post equol treatment interactions at the 500 and 1000 ms signal lengths [500 ms: $F(1,6)=7.2$, $p\leq0.05$; 1000 ms: $F(1,6)=26.9$, $p\leq0.005$], but not at the shortest signal (100 ms). The HIV-1 Tg animals showed a greater decrease in misses at the 500 and 1000 ms signal durations compared to the control animals (see Figure 2.13).

Experiment 1C: Discussion

In experiment 1A, the HIV-1 Tg group demonstrated temporal processing deficits as measured with prepulse inhibition. In experiment 1B, HIV-1 Tg animals exhibited deficits in attention and core components of executive function. To test the possibility

that temporal processing deficits also underlie the observed impairment in attention, the animals were again tested with the signal detection task in the present experiment, with the additional manipulation of the temporal domain, i.e., signal duration.

Both the HIV-1 Tg and control animals were sensitive to reduced signal duration, expressed as fewer hits and more misses with the shorter signals, as is expected with the manipulation of this parameter. However, the HIV-1 Tg animals exhibited a greater impairment in performance, with an inability to reliably detect the signal at the 500 and 100 ms durations. The control group, in contrast, maintained a greater number of hits than misses with the 500 ms signal. The HIV-1 Tg group also had lower percent accuracy, reflecting their performance with the 1000 ms signal trials (hits and misses) as well as non-signal trials (false alarms and correct rejections).

Performance on this task was again assessed after daily oral treatment with S-equol, to determine its potential therapeutic effects in ameliorating the observed deficits in the HIV-1 Tg group. An effect of S-equol was not detected in the initial analyses. When only the top third of the animals were included in the analysis, a significant decrease in the number of misses from the beginning to the end of the treatment period was demonstrated by the HIV-1 Tg animals that received 0.2 mg of S-equol. The control animals did not show an effect of S-equol treatment; control animals in all dose groups had significantly fewer misses by the end of the treatment period. However, the magnitude of the control group's improvement was only approximately half of that exhibited by the HIV-1 Tg animals that received the 0.2 mg dose of S-equol. Thus, S-equol was an effective treatment for the attentional deficits of the HIV-1 Tg animals. The

treatment did not improve performance for all of the HIV-1 Tg animals; S-equol appears to be most effective for subjects that have milder neurocognitive impairments.

Sustained attention is the process by which one detects and responds to infrequent and unpredictably occurring target stimuli over long periods of time, and is an integral component underlying the higher order cognitive processes of executive function. It has been previously characterized in both humans and rats, with analogous changes in performance related to varying task parameters, such as signal duration, intensity, and frequency (McGaughy and Sarter, 1995; Bushnell, 1998; Parasuraman and Davies, 1977). For the present experiment, the use of a range of signal durations revealed significant deficits in sustained attention in the HIV-1 Tg group, giving further evidence of a temporal processing deficit. Treatment was S-equol was effective in ameliorating these deficits.

Experiment 1C: Anatomical Assessment

For the second part of Experiment 1C, brains were extracted from the animals to assess neuronal network complexity in the PFC. It was hypothesized that 1) the HIV-1 animals treated with sucrose only would exhibit altered neuronal networks compared to the control animals, and 2) S-equol would ameliorate alterations in the HIV-1 Tg animals.

Methods

Preparation of tissue.

Twenty-four hours after their last behavioral test session, animals were anesthetized using sevoflurane (Abbot Laboratories, North Chicago IL) and transcardially perfused with

100ml of 100mM PBS wash followed by 100-150ml of 4% paraformaldehyde buffered in PBS (Sigma-Aldrich, St. Louis, MO). Brains were dissected and post-fixed in 4% paraformaldehyde for 30-60 minutes. After post-fixation, 200µM thick coronal sections were cut using a rat brain matrix (ASI Instruments, Warren, MI). Slices were washed in PBS 3 times following slicing and placed in a 24-well cell culture plate (Corning, Tewksbury MA).

Preparation of diOlistic cartridges.

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

Preparation of tefzel tubing.

Tefzel tubing (IDEX Health Sciences, Oak Harbor, WA) was cut into three 1.7M lengths. Polyvinylpyrrolidone (100mg; PVP, Sigma-Aldrich, St. Louis, MO) was dissolved in 10ml ddH2O, briefly vortexed, then passed through each length of the tubing. The purpose of this step is to enhance binding of the tungsten beads to the tubing. While vortexing, the 3ml bead/dye solution was slowly drawn into the tubing and placed in the

tubing prep station (Bio-Rad) for 5 minutes. After slowly draining the water from the tube using a syringe and tubing adapter, the tubing was spun in the prep station for approximately 10 minutes. Following this step nitrogen gas flow was adjusted to 0.4-0.5LPM and the tubing was further spun for 50-60 minutes to ensure the tubing was fully dry. Once dry, tubing was cut into 13mm segments and stored under anhydrous conditions until use.

DiOlistic labeling using the helios gene gun.

The Helios gene gun system (Bio-Rad, Hercules, CA) was loaded with the previously prepared cartridges containing DiI-coated tungsten beads. He gas flow was adjusted to 80 PSI. The particles were delivered through 3uM pore filter paper directly onto the slice with the barrel placed approximately 2.5cm away from the sample. After washing 3 times in PBS, slices were stored overnight at 4˚ C to allow dye diffusion across the cell membrane. Following diffusion, slices were mounted using Pro-Long Gold Antifade reagent (Invitrogen, Carlsbad CA), cover slipped with Fischer #1 size coverslips (ThermoFischer Scientific, Waltham, MA), and stored in the dark at 4˚ C.

PFC dendritic analysis and varicosity quantification.

Z-stack images of pyramidal neurons from the infralimbic cortex of the anterior cingulate cortex, located approximately 3.2mm to 1.7mm anterior to bregma (Paxinos and Watson, 2005), were obtained with intervals in the z-plane at 0.15 µm with a Nikon TE-2000E confocal microscope and Nikon's EZ-C1 software (version 3.81b). A green helium-neon (HeNe) laser with an emission of 533nm was used to capture the epiflorescence of DiI. Varicosities were manually counted while viewing images with Neurolucida version 10.52.

Criteria for sample inclusion in the data analysis were axon length of 50-150 µm and a varicosity/axon width ratio of 1.5-3:1.

Peripheral effects of S-equol.

To assess potential peripheral effects of S-equol, the uterine horn was dissected from the peritoneal cavity of all rats by separating the uterine horns from the underlying tissue, and excising the uterine body. Wet weights of the uterine horns were taken immediately following removal.

Statistics.

All data were analyzed using SPSS Statistics 20 (IBM Corp., Somers, NY). An ANOVA was conducted on the number of varicosities, with genotype and S-equol dose as the between-subjects factors. An ANOVA was also conducted on relative uterine weight, with genotype and S-equol dose as the between-subjects factors.

Experiment 1C: Anatomical Assessment Results

Neuronal network complexity.

Brain tissue slices from 5 control animals and 5 HIV-1 Tg animals yielded images with clearly quantifiable varicosities. Representative samples are shown in Figure 2.14. Of these samples, 2 HIV-1 Tg animals and 3 control animals had received the daily sucrose treatment, and 3 HIV-1 Tg animals and 2 control animals had received the daily 0.2 mg S-equol treatment. There was no overall effect of genotype or S-equol treatment on the number of varicosities per 10 microns. However, the HIV-1 Tg animals that received 0.2 mg of S-equol daily had significantly fewer varicosities than the HIV-1 Tg

animals that received sucrose, $t(3)=3.4$, $p \leq 0.05$ (see Figure 2.15). There was no effect of S-equol treatment on the control animals, nor a difference in the number of varicosities between the control and HIV-1 Tg animals in either drug group.

Peripheral effects of S-equol.

As illustrated in Figure 2.16, there was no significant effect of genotype or S-equol on uterine weight, and no significant interaction between genotype and S-equol.

Experiment 1C: Anatomical Assessment Discussion

Alterations in synaptodendritic networks caused by HIV-1 may underlie the observed neurocognitive impairments in HAND. Executive functions rely heavily on the integrity of frontostriatal circuits (Mega & Cummings, 1994), which are commonly affected by HIV-associated neuropathologies. Reductions in synaptodendritic complexity (Masliah et al. 1997; Everall et al., 1999) in frontal regions have been associated with HIV-1 associated neurocognitive impairments, even in less severe cases. Axonal damage in frontostriatal fiber bundles as measured with diffusion tensor imaging has also been observed in HIV-1+ individuals, prior to any symptoms of dementia (Pfefferbaum et al., 2009). Neuronal abnormalities such as these may precede the onset of functional deficits, necessitating the study of neuronal networks in the HIV-1 Tg rat in conjunction with assessments of cognitive function.

The goal of the anatomical assessment of the present experiment was to determine if there were differences between the HIV-1 Tg and control groups in neural network complexity of the PFC, a region implicated in executive function. Dendritic spine counts

could not be obtained from these subjects, because the DiI did not sufficiently diffuse throughout the spines in the PFC. Axonal varicosities in the PFC were readily visible, however; thus, the varicosity density was determined for each sample that had clearly defined axons and varicosities.

Varicosities appear as spherical enlargements along the axon, releasing neurotransmitters. Varicosities containing catecholamines are widely found in the PFC (Fuxe et al. 1968). The development of immunohistochemistry for dopamine β-hydroxylase, which converts dopamine to norepinephrine, has allowed for selective quantification of noradrenergic varicosities, which is predominantly found in the PFC, relative to other brain regions (Agster et al. 2013). Norepinephrine (NE) thus plays a significant role in mediating PFC function, regulating attention and executive function. Noradrenergic stimulation of α_{2A}-receptors on dendritic spines of pyramidal neurons in the PFC strengthens synaptic connections by closing the ion channels on the spines, increasing the efficacy of synaptic inputs (Wang et al. 2007). Without noradrenergic stimulation of these α_{2A}-receptors, ion channels remain open and synaptic inputs are weakened.

As described earlier, sustained attention depends on DA systems in the PFC, as evidenced by the differential effects of PFC-infused DA agonists and antagonists on sustained attention (Granon et al. 2000; Chudasama and Robbins, 2004). Noradrenergic systems are also implicated in sustained attention. Nonhuman primates exhibit phasic release of NE in the PFC while successfully performing sustained attention tasks, and tonic release of NE during periods of high distractability (Aston-Jones and Cohen 2005). Nearly complete loss of cortical NE, following infusions of catecholamine-selective

neurotoxins 6-hydroxydopamine or anti-dopamine-beta hydroxylase-saporin into the trajectory of the dorsal noradrenergic ascending bundle, significantly affects attention only with unpredictable presentation of target stimuli, with the presentation of distractor stimuli (Carli et al. 1983), and with higher event rate (Milstein et al. 2007). Thus, NE may play a more important role in attention processes when attentional load is greater. Further study of neural network structures implicating NE as well as DA in the PFC will be important in clarifying alterations underlying neurocognitive impairments of HAND.

The development of an effective estrogenic compound therapeutic for neurocognitive impairment necessitates the assessment of potential side effects that may be mediated by stimulation of ERs in the periphery. Peripheral effects of S-equol were assessed by obtaining the uterine weight of each animal. The lack of difference in uterine weight between those that received S-equol and those that received sucrose indicates that S-equol did not exert any significant peripheral effects on estrogen receptors. S-equol preferentially binds to the ERβ receptor, which is located in greater concentration in the brain, specifically the PFC, compared to peripheral organs such as the uterus. The popular use of hormone replacement therapy for menopausal symptoms was halted by the discovery that it can exert detrimental effects, such as an increased risk for cancer. Phytoestrogens and their metabolites like S-equol have been recognized as a safer alternative treatment in part due to their greater selectivity for the ERβ receptor, which is not present in high amounts in peripheral organs.

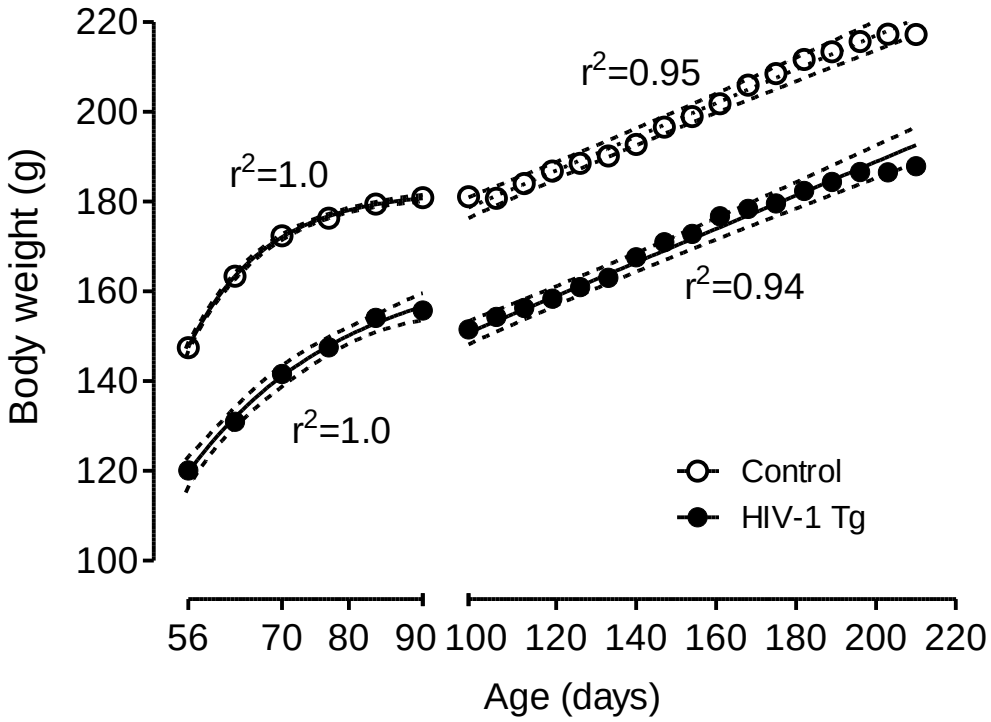

Figure 2.1. Mean body weight of the HIV-1 Tg animals and control animals across age with the best fit nonlinear regression for each group (±95% CI). The HIV-1 Tg group weighed significantly less than the control group across the 6-month period during which they were tested. Both groups increased significantly in body weight across this period did not differ in their rates of growth. The x-axis break at 100 days indicates the point at which animals began food restriction, prior to testing.

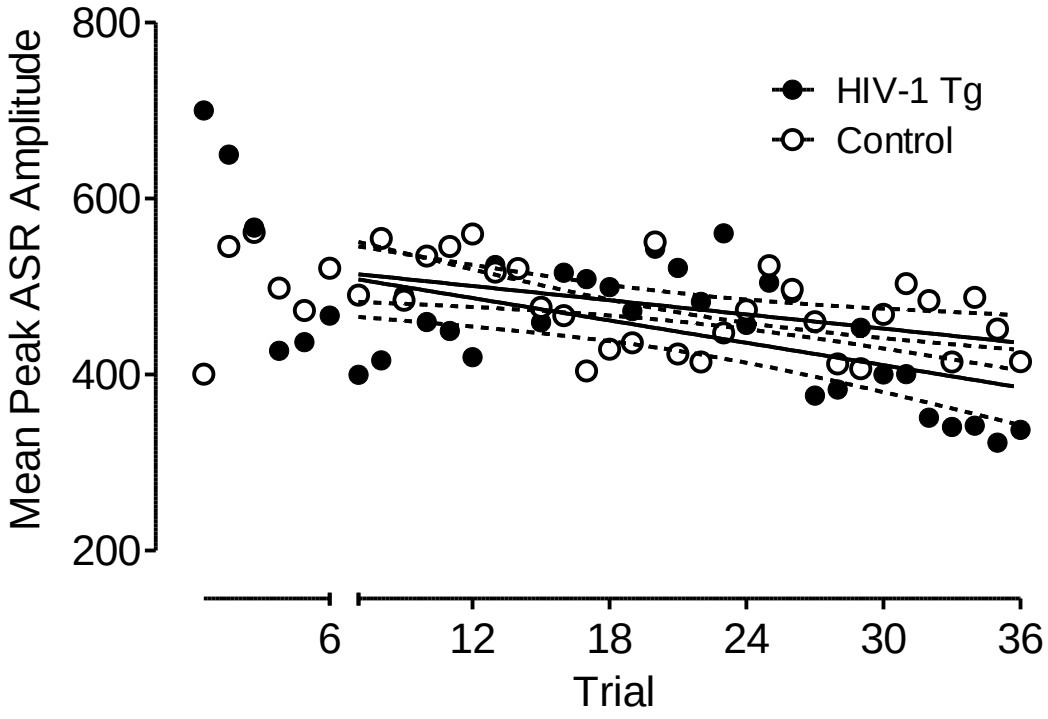

Figure 2.2. Mean peak ASR amplitude during the habituation session (±95% CI). After the initial 6 trials, the HIV-1 Tg and control groups showed no difference in overall ASR (F=0.17), and did not differ in rate of habituation to the auditory startle stimulus. Regression line slopes: HIV-1 Tg, -4.24+/-1.24; Control, -2.7+/-0.91.

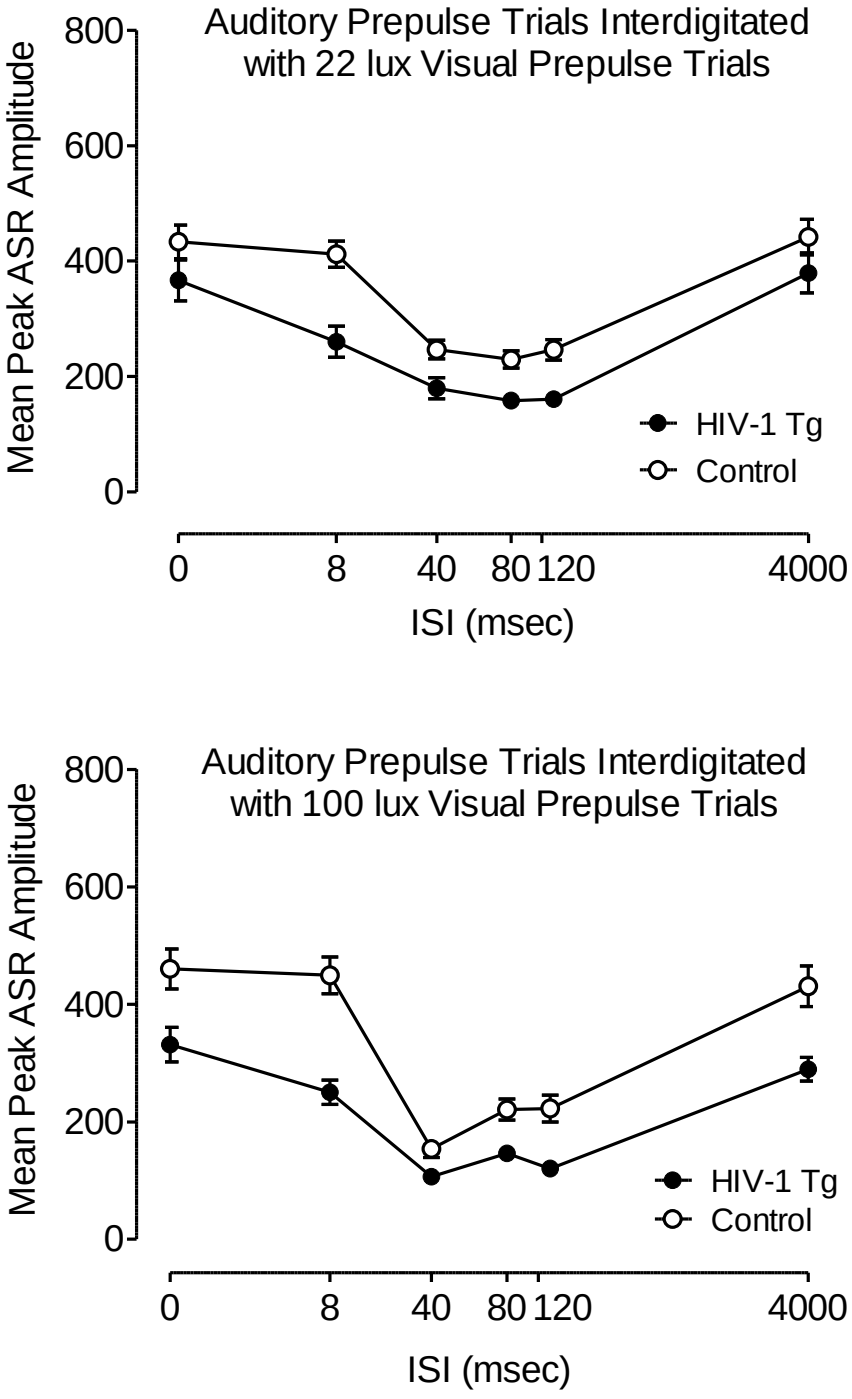

Figure 2.3. Mean (+/- SEM) peak ASR amplitude on PPI trials with an auditory prepulse during sessions interdigitated with 22 lux (top panel) and 100 lux (bottom panel) visual prepulse trials conducted at 2 months of age.

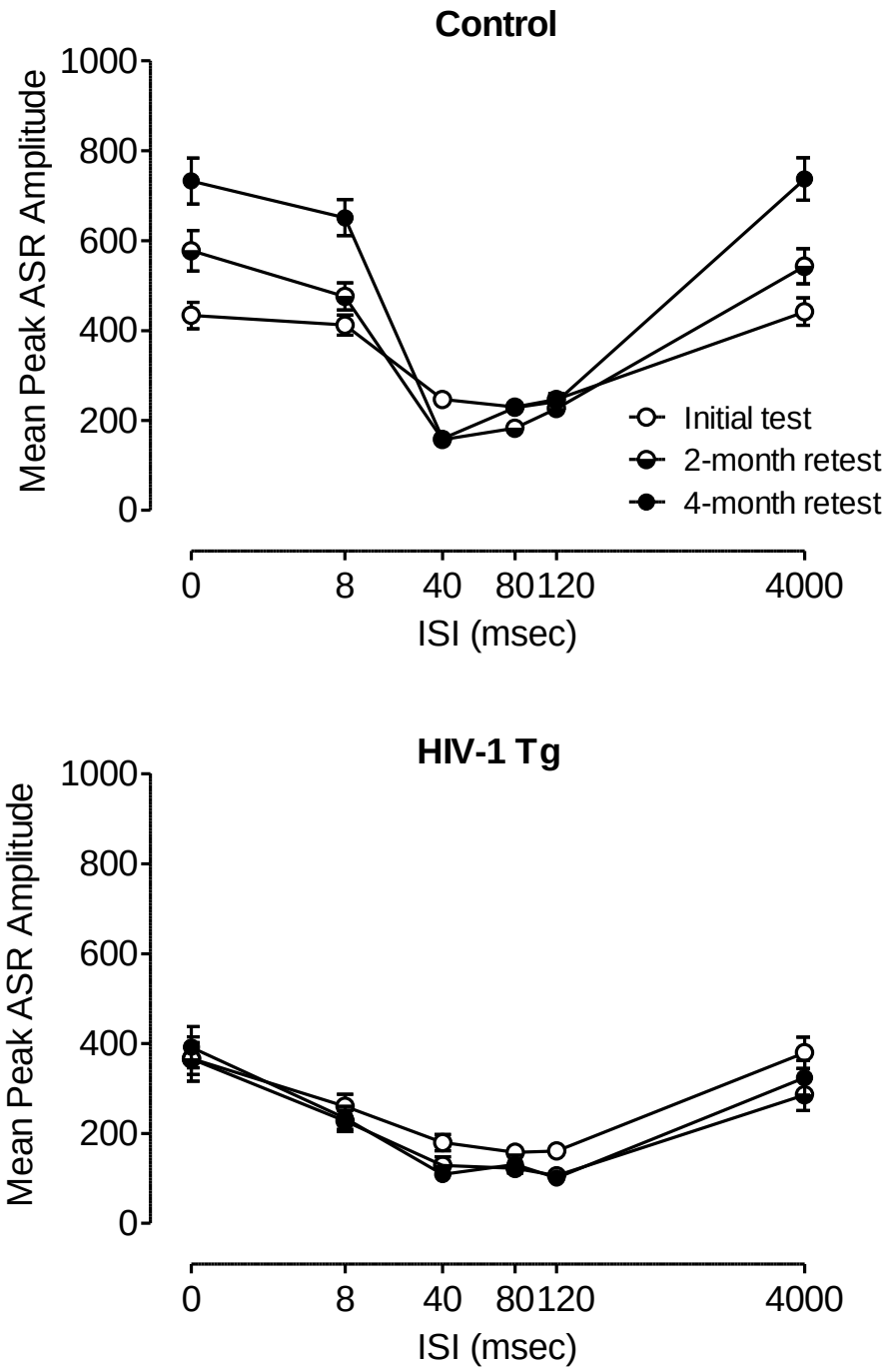

Figure 2.4. Mean (+/- SEM) peak ASR amplitude on PPI trials with an auditory prepulse interdigitated with 22 lux visual prepulse trials across all three test ages.

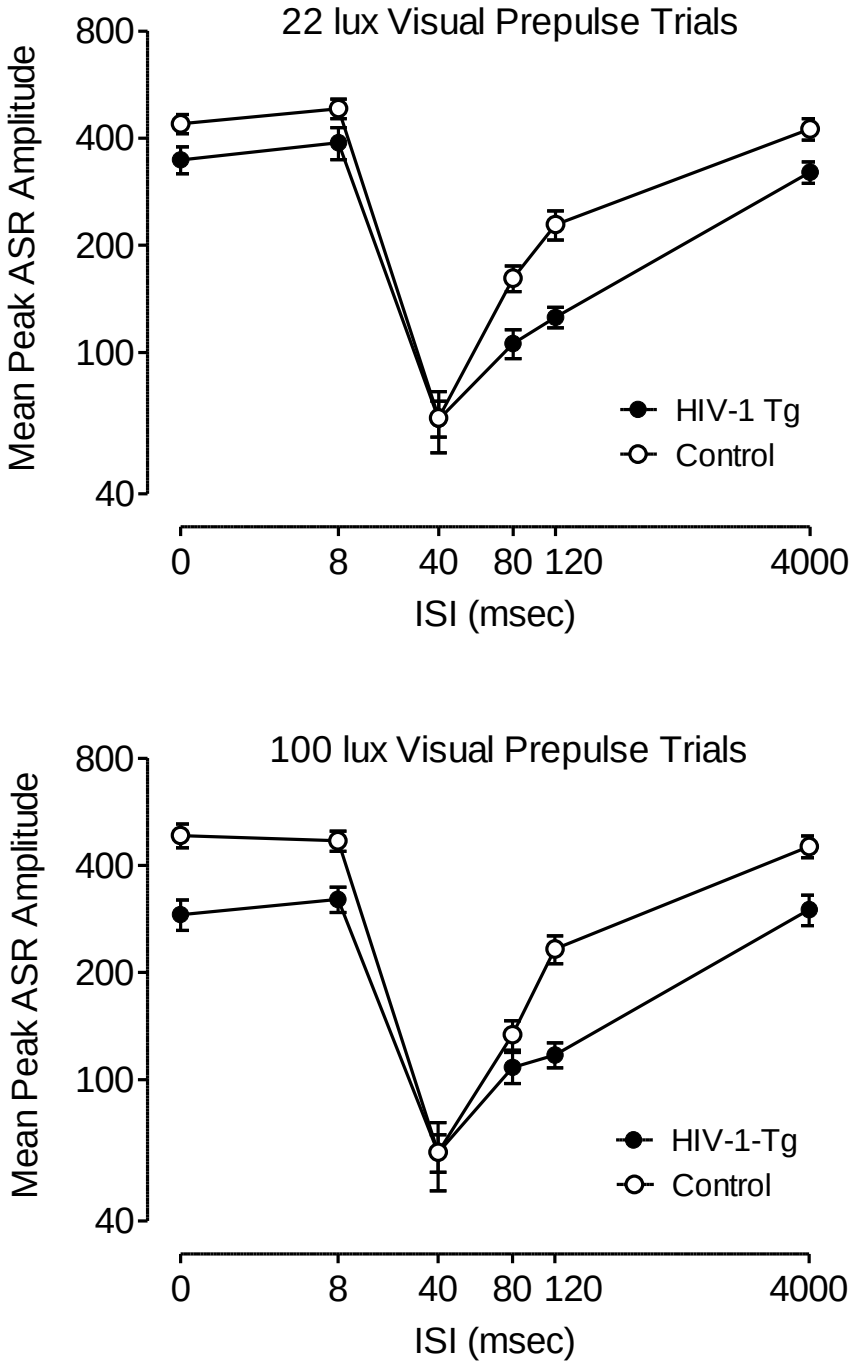

Figure 2.5. Mean (+/- SEM) peak ASR amplitude with 22 lux (top panel) and 100 lux (bottom panel) visual prepulses across all three test sessions conducted at 2 months of age.

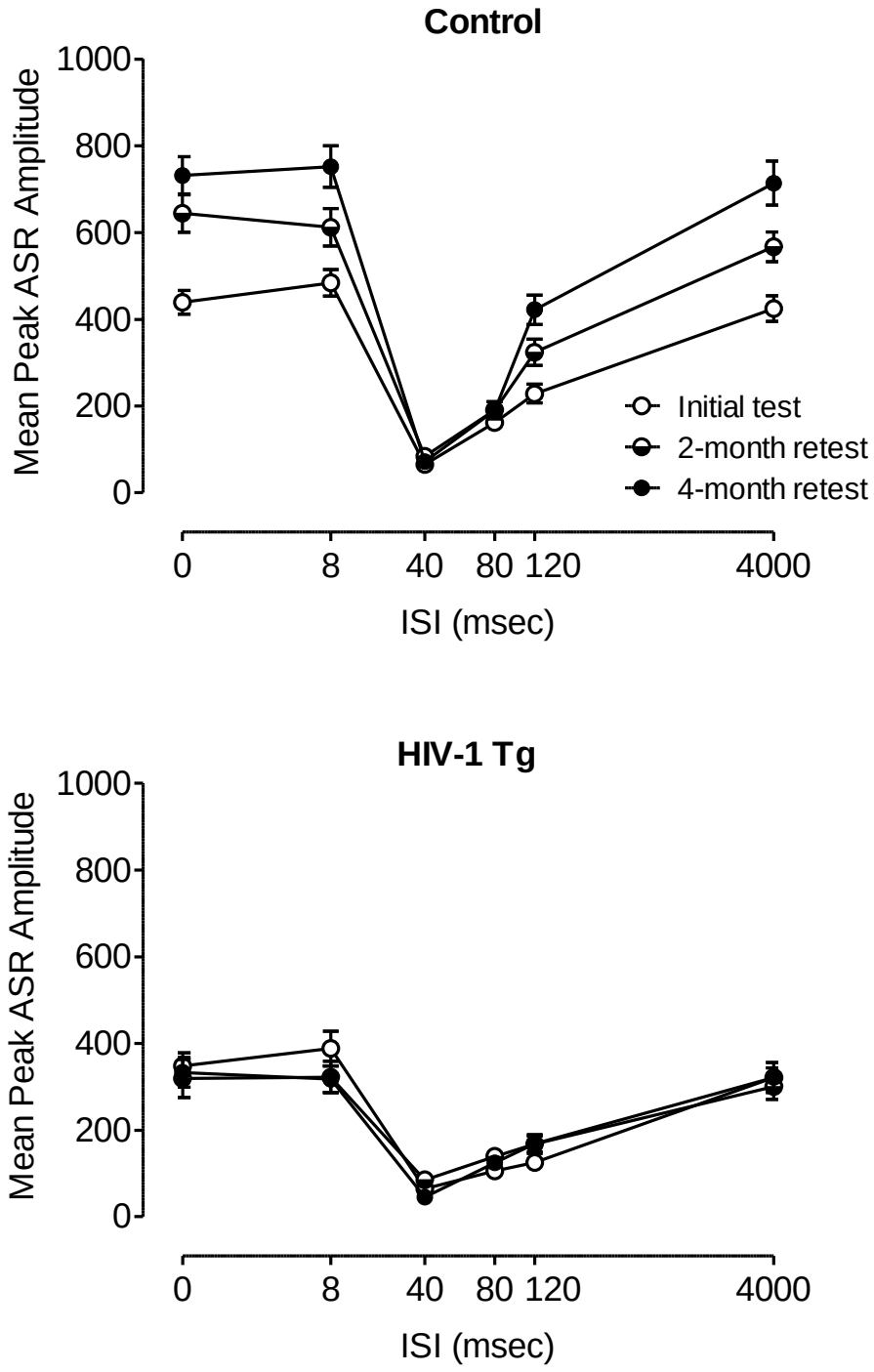

Figure 2.6. Mean (+/- SEM) peak ASR amplitude with a 22 lux visual prepulse across all three test sessions.

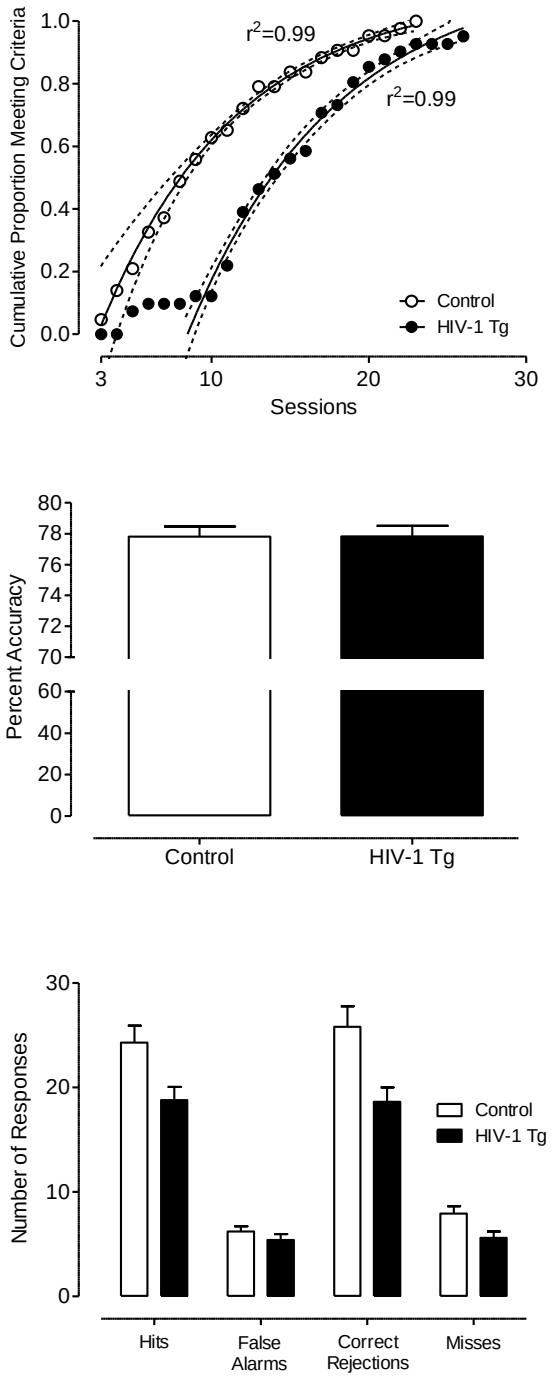

Figure 2.7. Performance on the signal detection task. Cumulative proportion of animals achieving the signal detection task criterion of 70% accuracy on 3 consecutive sessions (±95% CI) (top panel). Middle panel: Mean (+/- SEM) percent accuracy after meeting the criterion. Bottom panel: Mean (+/- SEM) number of hits, false alarms, correct rejections, and misses after meeting the criterion.

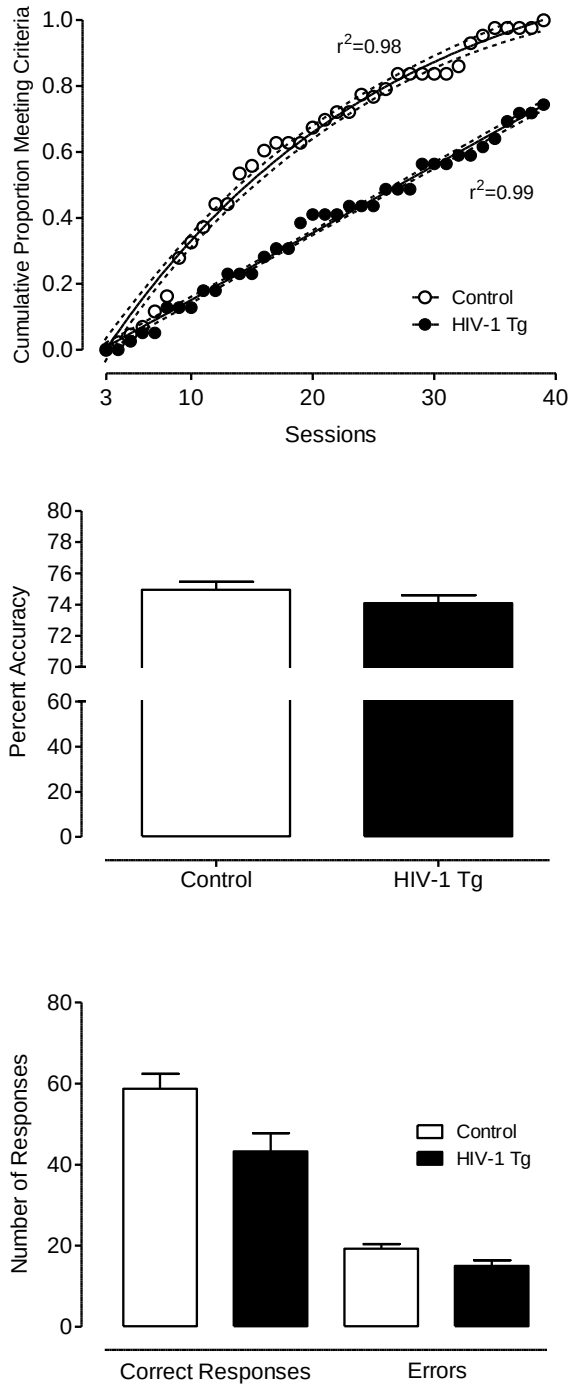

Figure 2.8. Performance on the discrimination task. Cumulative proportion of animals achieving the discrimination task criterion of 70% accuracy on 3 sessions (±95% CI) (top panel). Middle panel: Mean (+/- SEM) percent accuracy after meeting the criterion. Bottom panel: Mean (+/- SEM) number of correct responses and errors after meeting the criterion.

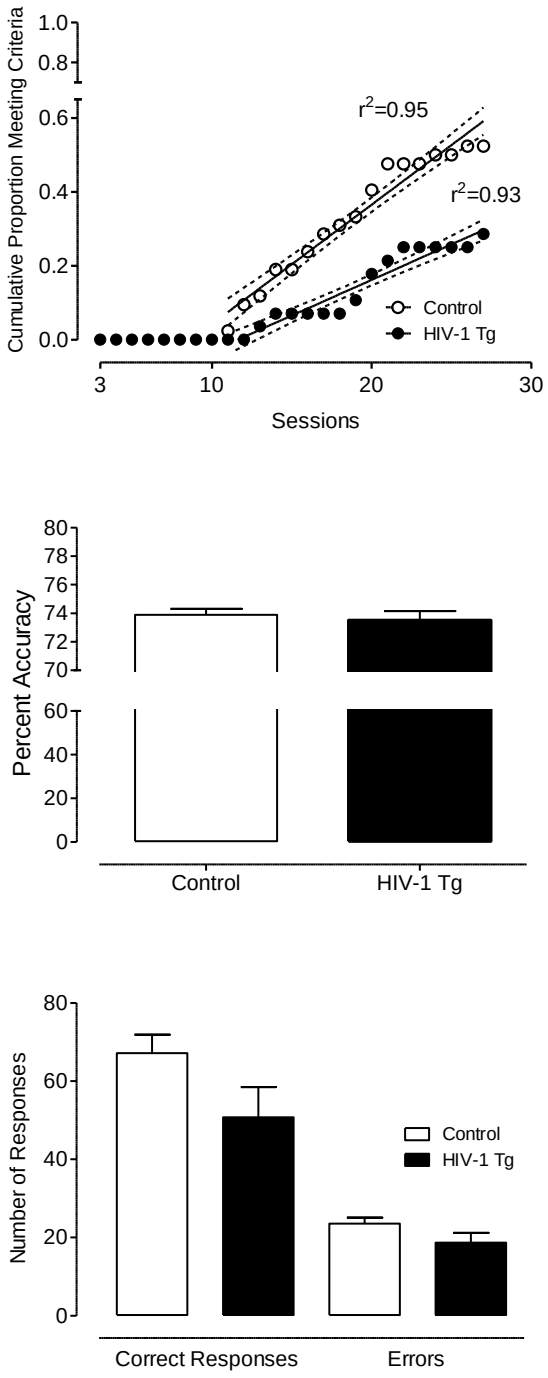

Figure 2.9. Performance on the reversal task. Cumulative proportion of animals achieving the reversal task criterion of 70% accuracy on 3 sessions (±95% CI) (top panel). Middle panel: Mean (+/- SEM) percent accuracy after meeting the criterion. Bottom panel: Mean (+/- SEM) number of correct responses and errors after meeting the criterion.

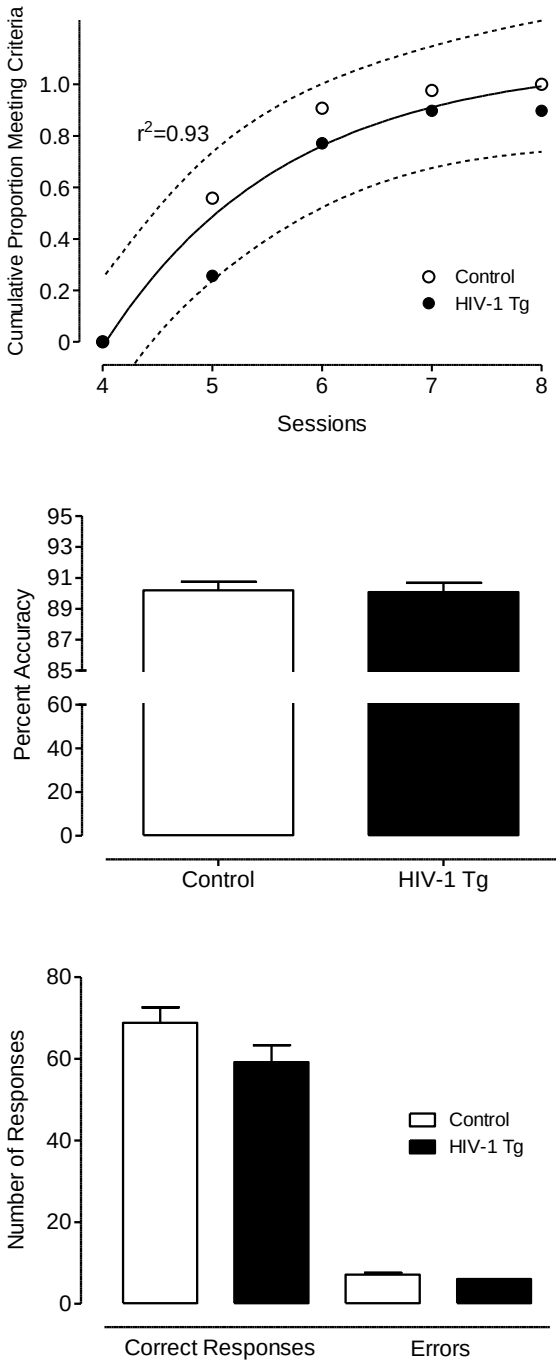

Figure 2.10. Performance on the extradimensional set-shifting task. Cumulative proportion of animals achieving the task criterion of 70% accuracy on 3 sessions (±95% CI) (top panel). Middle panel: Mean (+/- SEM) percent accuracy after meeting the criterion. Bottom panel: Mean (+/- SEM) number of correct responses and errors after meeting the criterion.

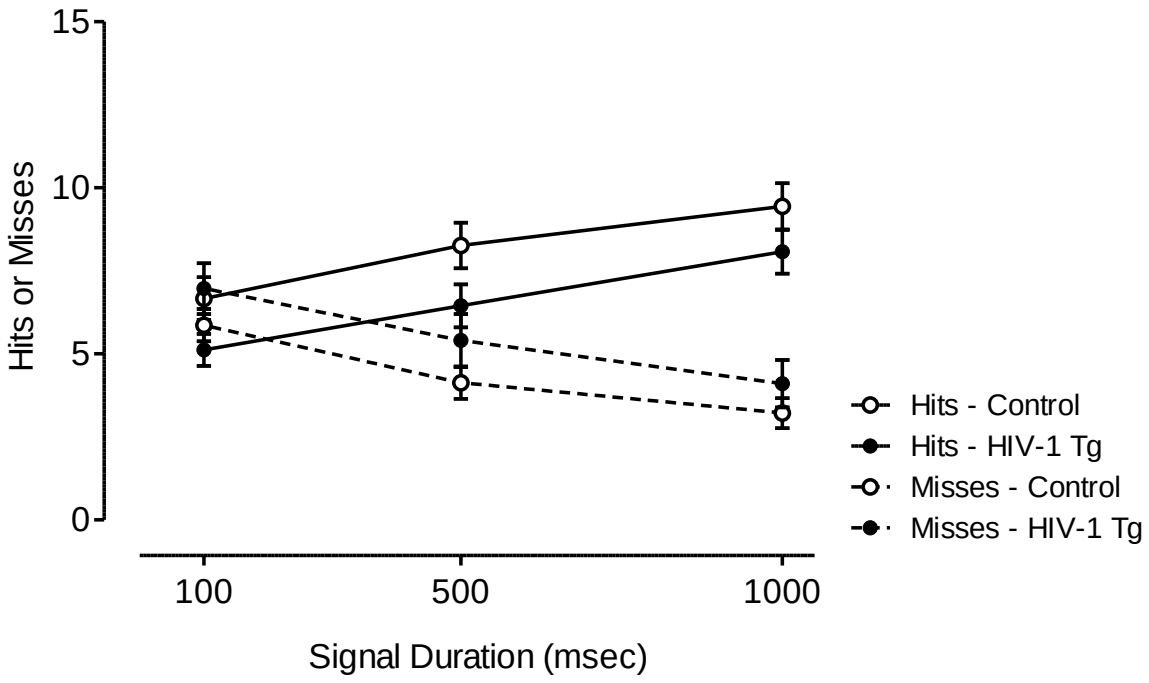

Figure 2.11. Mean (+/- SEM) number of hits and misses across signal duration on the signal detection task after approximately 2 weeks of daily training.

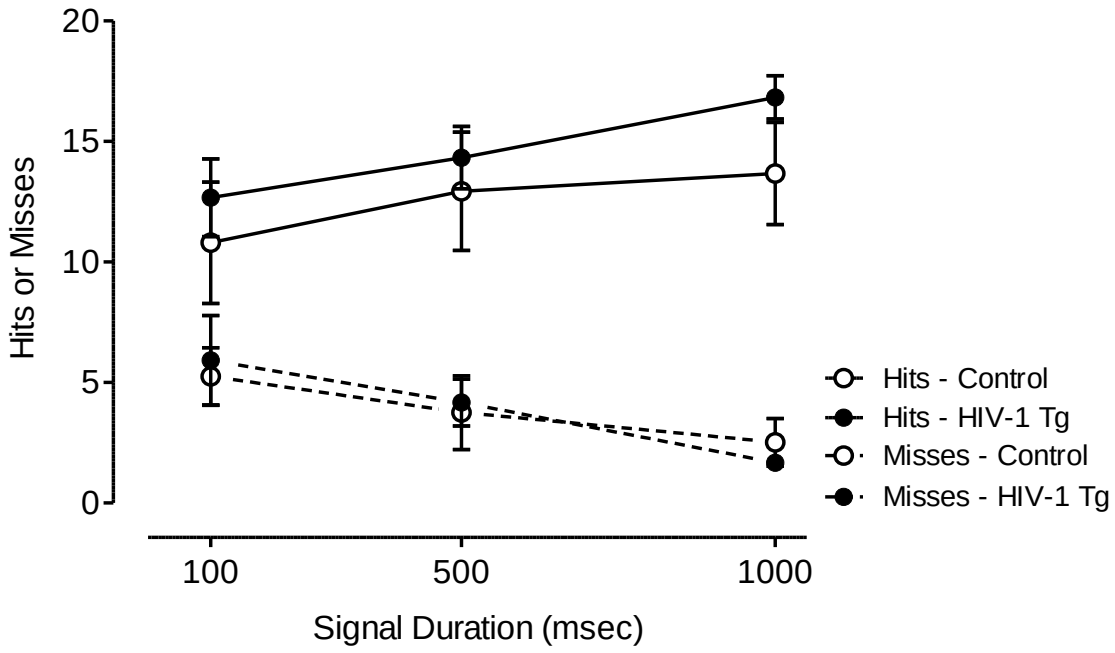

Figure 2.12. Mean (+/- SEM) number of hits and misses across signal duration on the signal detection task after 45 days of S-equol treatment. Control values represent the average of the top third of control animals, across all dose groups including the vehicle group. HIV-1 Tg values represent performance of the top third of HIV-1 Tg animals that received 0.2 mg of S-equol.

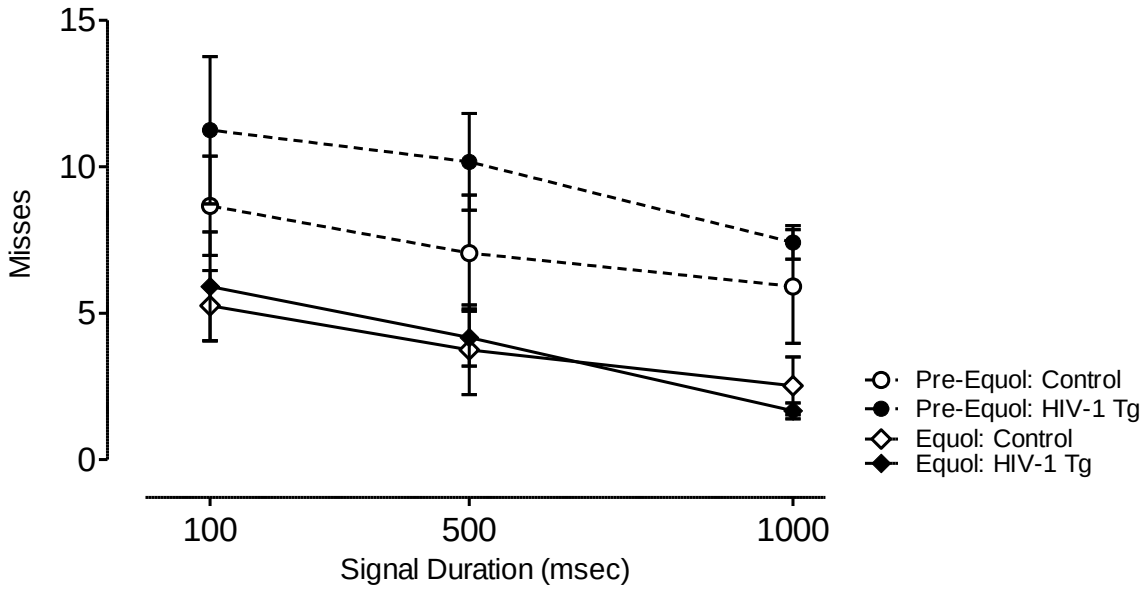

Figure 2.13. Mean (+/-SEM) number of misses during the first 3 days of S-equol treatment and after 45 days of S-equol treatment. Control values represent the average of the top third of control animals, across all dose groups including the vehicle group. The HIV-1 Tg values represent the top third of the HIV-1 Tg animals that received the 0.2 mg dose of S-equol.

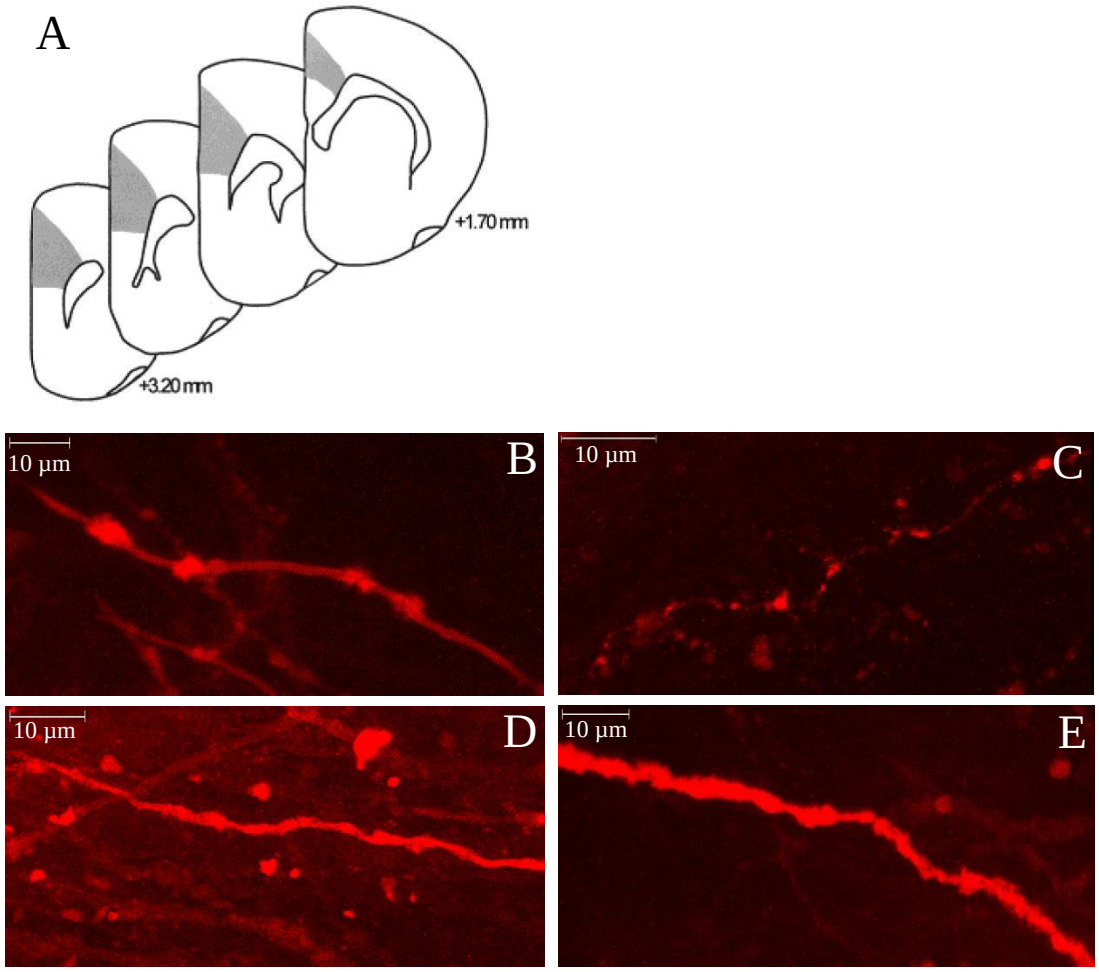

Figure 2.14. (A) Coronal sections through rat PFC. Neurons were sampled from layers II and III of the anterior cingulate cortex (shaded area). Coordinates indicate position relative to bregma. DiOlistically labeled axons with varicosities from pyramidal neurons in the anterior cingulate cortex of a control animal treated with sucrose (B), an HIV-1 Tg animal treated with sucrose (C), a control animal treated with 0.2 mg S-equol (D), and an HIV-1 Tg animal treated with 0.2 mg S-equol (E). Scale bars = 10 µm.

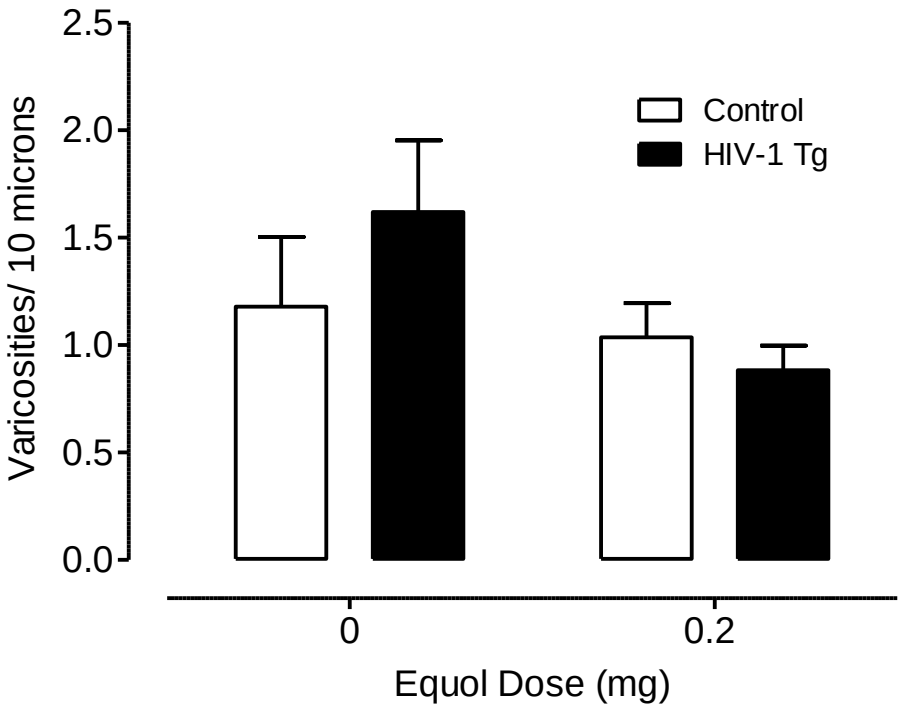

Figure 2.15. Mean (+/- SEM) number of varicosities per 10 micron axon segment derived from pyramidal neurons in the anterior cingulate area.

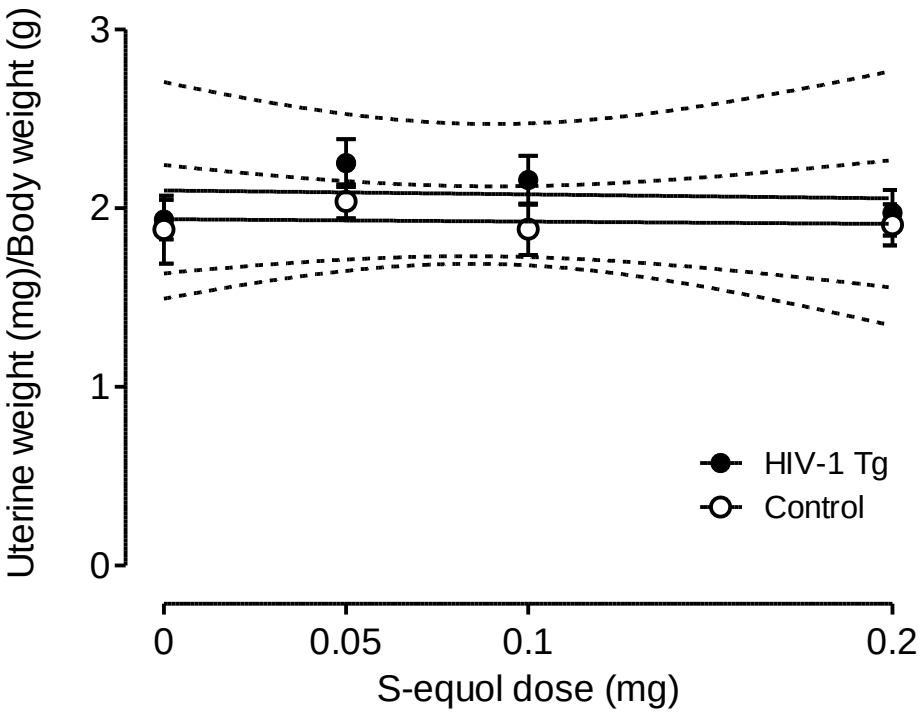

Figure 2.16. Mean relative uterine weight (±95% CI) as a function of S-equol dose.

CHAPTER 3

EXPERIMENT 2

In Experiment 1, assessments of preattentive processing and sustained attention revealed prominent temporal processing deficits in the HIV-1 Tg animals. The results of Experiment 1C suggested that the 0.2 mg dose of S-equol improved temporal processing, as assessed with sustained attention, in the top third of the HIV-1 Tg animals, when administered daily for 45 days during testing between 6-8 months of age. In Experiment 2, preattentive processing and sustained attention, as measures of temporal processing, were again assessed in HIV-1 Tg and control animals. Performance on the sustained attention task was assessed while the animals were administered 0.2 mg of S-equol or sucrose for 60 days, beginning at 2-3 months of age, to determine if the S-equol treatment would be more effective if administered prophylactically. After the conclusion of the S-equol treatment period, the animals were also tested on additional sustained and selective attention tasks to further characterize the attentional deficits of the HIV-1 Tg rat. The following hypotheses were proposed: 1) the HIV-1 Tg animals will exhibit temporal processing deficits as observed in Experiment 1, both in preattentive processing, as measured with PPI of the ASR, and in sustained attention and selective attention tasks; and 2) the initiation of the 0.2 mg dose of S-equol after a shorter duration of constitutive expression of the HIV-1 transgene will delay, or perhaps prevent, the development of sustained attention deficits relative to the controls.

Experiment 2A: Preattentive Processing

Materials and Methods

Animals.

Two-month-old ovariectomized female Fischer (F344/N; Harlan Laboratories) rats (HIV-1 Tg, n=20; control, n=20) were delivered to the facility in two separate batches, one week apart in delivery and age. All animals were group- or pair-housed throughout the experiments. Rodent food (2020X Teklad Global Extruded Rodent Diet (Soy Protein-Free)) and water were available *ad libitum* throughout the first PPI test period. The animals were under food restriction (85% body weight) beginning one week prior to and throughout operant testing, and were free-fed again at the conclusion of operant testing and during PPI testing. The animals were maintained according to the National Institute of Health (NIH) guidelines in AAALAC-accredited facilities. The animal facility was maintained at 21° ± 2°C, 50% ± 10% relative humidity and had a 12-h light:12-h dark cycle with lights on at 0700 h (EST). Rats were handled for one week prior to any behavioral testing procedures. The Institutional Animal Care and Use Committee (IACUC) of the University of South Carolina approved the project protocol.

Procedure.

Preattentive processing was assessed by measuring PPI of the auditory startle response (ASR) with both auditory and visual prepulses. As in Experiment 1, the animals were tested at 2 months of age to assess alterations in PPI that occur early in the expression of the transgene, and subsequently tested at 8 months of age to determine the development of PPI with age and continued expression of the transgene. Based on the results of Experiment 1, the 22 lux light stimulus was used as the visual prepulse, as this

intensity was sufficient to cause robust inhibition of the ASR. It was hypothesized that the HIV-1 Tg animals would exhibit a flatter ISI function which would not sharpen with age, relative to the control animals, as observed in Experiment 1, thereby demonstrating temporal processing deficits.

One week after arrival, at approximately 2 months of age, all animals were administered a 36-trial auditory startle test session to habituate them to the auditory stimulus and test procedures. Each session began with a 5-min acclimation period of 70dB(A) background white noise, followed by 36 trials of a 100dB(A) white noise stimulus with a 20-msec duration and a 10-sec intertrial interval (ITI). All test sessions were conducted in the dark.

One day after ASR habituation, animals were tested for PPI of the ASR with both visual and auditory prepulse stimuli. As in the two later test periods in experiment 1, the visual prepulse was presented as one LED light (22 lux at the level of the test cylinder), as it was determined in experiment 1 that this stimulus was sufficient to cause robust inhibition of the ASR. The animals were tested again at 8 months of age. All other PPI procedures from experiment 1 were followed in experiment 2.

Experiment 2A: Results

Body Weight.

The HIV-1 Tg group weighed significantly less than the control group across the 6-month period during which they were tested, $F(1, 36)=61.0$, $p\leq0.001$. While food restriction was implemented (from week 10 to week 28), both groups increased in weight in a linear function [HIV-1 Tg: $F(1,18)= 562.5$, $p\leq0.001$; Control: $F(1,18)=1005.4$,

$p \leq 0.001$]. There was no significant difference in the slope of these lines, indicating that the groups did not differ in their rates of growth (see Figure 3.1). There was also no effect of S-equol treatment on weight in either group.

ASR Intrasession Habituation.

Linear regression analysis revealed that there was no difference in the rate of habituation across the test session for the HIV-1 Tg and control groups (regression line slopes: HIV-1 Tg, -3.5+/-0.94; Control: -4.2+/-1.2; see Figure 3.2), with no difference in overall ASR between groups.

PPI with an Auditory Prepulse Across Age.

Mean peak ASR amplitude during auditory prepulse trials was assessed at 2 and 8 months of age. The ANOVA conducted on mean peak amplitude across both test periods revealed a significant genotype x age x ISI interaction, $F(5,190)=4.7$, $p \leq 0.001$, as well as a genotype x ISI interaction, $F(5,190)=3.9$, $p \leq 0.005$, an age x ISI interaction, $F(5,190)=7.5$, $p \leq 0.001$, and a genotype x age interaction, $F(1,38)=8.4$, $p \leq 0.01$. Additional analyses were conducted to identify the locus of these interactions.

Separate analyses of each group revealed a main effect of age, $F(1,19)=23.3$, $p \leq 0.001$, and an age x ISI interaction, $F(5,95)=16.1$, $p \leq 0.001$, in the control group. These effects were not observed in the HIV-1 Tg group, suggesting that the expression of the HIV-1 transgene interfered with the age-dependent development of perceptual sharpening (see Figure 3.3).

Complementary results were obtained after separate analyses at each age, which revealed a significant genotype x ISI interaction at 8 months of age, $F(5,190)=5.5$, $p\leq 0.001$, but not at 2 months of age. The genotype x ISI interaction at the later age indicates, as does the age x ISI interaction in the control group but not the HIV-1 Tg group, altered development of the ISI function in the HIV-1 Tg group; they did not exhibit the same sharpening of the ISI function with age that is apparent with the control group.

The overall genotype x ISI interaction reflects not only the relative insensitivity of the HIV-1 Tg group to manipulation of ISI duration, but also the differential peak inhibition of the two groups, observed at the 40 msec ISI at both 2 and 8 months of age for the HIV-1 Tg group, and at the 40 ms ISI at 2 months and at the 80 ms ISI at 8 months for the control group. Both groups showed significant quadratic trends for ISI at each age, characteristic of the fundamental temporal domain of PPI [2 months, Control: $F(1,19)=24.8$, $p\leq 0.001$; HIV-1 Tg: $F(1,19)=26.0$, $p\leq 0.001$; 8 months, Control: $F(1,19)=97.2$, $p\leq 0.001$; HIV: $F(1,19)=25.5$, $p\leq 0.001$].

PPI with a Visual Prepulse Across Age.

The ANOVA conducted on mean peak amplitude with a visual prepulse across both test periods revealed a significant genotype x ISI interaction, $F(5,190)=8.6$, $p\leq 0.001$, a genotype x age interaction, $F(1,38)=10.33$, $p\leq 0.01$, and a significant effect of genotype, $F(1,38)=19.9$, $p\leq 0.001$. Additional analyses were conducted to identify the locus of these interactions.

Separate analyses of each group revealed a main effect of age, $F(1,19)=34.1$, $p\leq0.001$, and an age x ISI interaction, $F(5,95)=4.0$, $p\leq0.01$, in the control group. These effects were not observed in the HIV-1 Tg group, suggesting that the expression of the HIV-1 transgene interfered with the age-dependent development of perceptual sharpening (see Figure 3.4). Both groups exhibited peak inhibition at the 40 ms ISI at both age. At 2 months of age, there was no difference between groups in percent PPI at the 40 ms ISI [Control: *M*=74.4, *SEM*=4.3; HIV-1 Tg: *M*=73.8, *SEM*=3.0], but the HIV-1 Tg group had significantly lower percent PPI than the control group at 8 months of age, $t(38)=2.3$, $p\leq0.05$ [Control: *M*=76.9, *SEM*=3.2; HIV: *M*=53.9, *SEM*=9.3]. Both groups showed significant quadratic trends for ISI at each age, characteristic of the fundamental temporal domain of PPI [2 months, Control: $F(1,19)=70.2$, $p\leq0.001$; HIV-1 Tg: $F(1,19)=68.2$, $p\leq0.001$; 8 months, Control: $F(1,19)=67.9$, $p\leq0.001$; HIV-1 Tg: $F(1,19)=35.9$, $p\leq0.001$].

Experiment 2A: Discussion

An assessment of preattentive processing was conducted with PPI of the ASR at 2 and 8 months of age. As in Experiment 1, alterations in PPI were demonstrated in the HIV-1 Tg rat early in the expression of the HIV-1 transgene and prior to any documented neurological symptoms or signs of wasting. In the absence of any difference between the HIV-1 Tg and control animals in overall ASR or rate of habituation to the startle stimulus, significant differences were observed in PPI. The most apparent difference was that the HIV-1 Tg group exhibited a flatter ISI function, which did not sharpen with age, as it did with control animals. Furthermore, the flatter ISI function was observed in both auditory and visual prepulse conditions, demonstrating the generality of sensorimotor

gating deficits across prepulse modality. Over time, auditory prepulses precipitated a temporal shift in peak inhibition in HIV-1 Tg animals relative to controls, whereas with visual prepulses, both groups displayed peak inhibition at the 40 msec ISI at both 2 and 8 months of age, parallel to the findings in Experiment 1.

Alterations in temporal processing were observed in the HIV-1 Tg rats in Experiment 1, with tests of both PPI and sustained attention. The present results parallel those observed in Experiment 1; the HIV-1 Tg rats demonstrate a lack of perceptual sharpening with age and a relative insensitivity to the temporal dimension of preattentive processing. Thus, the present results further support the conclusion that neurocognitive impairment reported in HIV-1+ individuals early in the disease course can be modeled in the HIV-1 Tg rat.

Experiment 2B: Sustained and Selective Attention, and Prevention of Attentional Deficits with S-equol Treatment

Performance on the sustained attention task was assessed while the animals were administered 0.2 mg of S-equol or sucrose for 60 days, beginning at 2-3 months of age, to determine if the S-equol treatment would be more effective if administered prophylactically. It was hypothesized that the HIV-1 Tg animals would display deficits in sustained attention, tested with the signal detection task with varying signal durations as in Experiment 1, and that the initiation of the 0.2 mg dose of S-equol after a shorter duration of constitutive expression of the HIV-1 transgene will delay, or perhaps prevent, the development of sustained attention deficits relative to the controls. After the conclusion of the S-equol treatment period, animals were additionally tested with

sustained and selective attention tasks to further characterize attentional deficits of the HIV-1 Tg rat.

Materials and Methods

S-equol.

S-equol was obtained from Cayman Chemical Company (Ann Arbor, MI) and incorporated into 90 mg sucrose pellets by Bio-Serv (Frenchtown, NJ), to produce pellets containing 0.05 mg S-equol. Plain 90 mg sucrose pellets were also obtained from Bio-Serv to provide to the vehicle group.

Design.

Animals began daily treatment with S-equol or vehicle at approximately 2-3 months of age, one week before operant training began. Animals were randomly assigned to either the S-equol group or the vehicle group. The S-equol group received four 90 mg sucrose pellets, each containing 0.05 mg of S-equol, for a total daily dose of 0.2 mg of S-equol. The vehicle group received four 90 mg sucrose pellets. Each rat was administered its treatment in a separate cage, at least an hour after behavioral testing, and typically consumed their pellets within seconds.

Sustained Attention: Varying Signal Durations

Animals were trained with autoshaping and the stimulus detection task as described in Experiment 1 (section 2.1.3). Once the animals achieved the criterion of 70% accuracy on three consecutive days for the stimulus detection task, they were tested

with a more difficult version in which the stimulus was presented for one second, followed by the presentation of the levers. This version of the task had been employed in experiment 1 after the initial lever-press training, but was modified with the lever press/ stimulus-off contingency after the majority of the animals failed to achieve 70% or greater accuracy after a month of training. It was thus determined that for experiment 2, the animals would need to be trained with the lever-press /stimulus-off contingency before training with the one-second stimulus.

After each animal achieved the criterion of 70% or greater accuracy on three consecutive sessions of the stimulus detection task with the one second stimulus, it was again tested with the stimulus detection task, now with three different light stimulus lengths (100, 500, and 1000 ms), presented an equal number of times randomly throughout the session. Animals were tested daily with the varying stimulus lengths until all animals acquired the task with at least 70% accuracy. Three consecutive days at 70% accuracy was the criterion used for inclusion in the data analyses.

Sustained Attention: Highly Variable ITI

After acquiring the stimulus detection task with varying stimulus lengths, all animals were tested again with varying stimulus lengths as well as with a greater range of ITIs (9+/-6 seconds), such that over a 160-trial session, ITIs of 3, 5, 7, 9, and 11 were used equally and presented randomly. Increased ITI variability degrades performance efficiency when compared with regular event schedules (Scerbo et al., 1987; McGaughy and Sarter, 1995). Because the variability of the ITI was kept relatively small (9+/-3 s), the possibility that animals were able to approximate the onset of the signal or nonsignal

event could not be completely excluded. Therefore, the effects of an increase in the variability of the ITI to 9+/-6 s were examined. Animals were tested for three sessions in the task using the highly variable ITI. These sessions allowed the assessment of increased sustained attention demands.

Selective Attention

To test selective attention, the stimulus detection task was modified to include a 1.5 s distractor stimulus at the beginning of each trial, for the second block of 54 trials within each session. The distractor stimulus onset and offset was 1 s prior to and 0.5 s after the signal onset (center panel light), respectively, for a total 1.5 s duration. Animals were tested a visual distractor for three consecutive daily sessions, and then with an auditory distractor for an additional three consecutive sessions. The house light was used as the visual distractor, with a light intensity of 5.5 lux measured from the center of the chamber at the level of the animal's height. A sonalert (Mallory model SC24) provided a 71 dB(A) (~5 dB(A) above background noise) auditory distractor stimulus, measured from the center of the chamber at the level of the animal's height.

Statistics

All data were analyzed using SPSS Statistics 20 (IBM Corp., Somers, NY). Trials and errors to criterion were analyzed with independent T-tests and sessions to criterion were analyzed with linear regression to assess the temporal process of acquisition of the initial stimulus detection task, as well as the following stimulus detection task with varying signal durations. For the initial stimulus detection task, a

three-way mixed-factor ANOVA was performed on percent accuracy and the number of hits, misses, false alarms, and correct rejections, with genotype (HIV-1 Tg vs. control) and S-equol treatment as the between-subjects factors, and time (first three days of testing vs. performance at criterion) as the within-subjects factor.

For the stimulus detection task with varying signal durations, the same analyses were conducted, with the addition of signal duration (100, 500, or 1000 ms) as a within-subjects factor for the dependent measures of hits and misses. Separate ANOVAs were also conducted to compare performance on the first three days of testing to performance one month after S-equol treatment ended. Each animal's performance on each measure was averaged across three consecutive days at the respective time points to provide the data for the analyses.

A three-way mixed-factor ANOVA was also conducted on the same dependent measures to compare the effects of different ITI ranges, with genotype and S-equol treatment as the between-subjects factors, and ITI variability (9+/-3 s vs. 9+/-6 s) as the within-subjects factor. Signal duration was also included as a within-subjects factor for the dependent measures of hits and misses.

For analysis of performance during the selective attention tasks, test sessions were divided in three blocks of 54 trials (distractor stimuli were presented during trials in the second block). A three-way mixed-factor ANOVA was conducted on the same previously mentioned dependent measures with genotype and S-equol treatment as the between-subjects factors, and block as the within-subjects factor.

An alpha level of $p \leq 0.05$ was considered significant for all statistical tests. Sample sizes were chosen with the goal of sufficient statistical power (> 0.80) to

maximize the likelihood of detecting subtle early alterations of expression of the HIV-1 transgene.

Experiment 2B: Results

Stimulus Detection-1000 ms signal duration.

All control and HIV-1 Tg animals acquired the criterion for the stimulus detection task with the 1000 ms signal duration within approximately 2 months of daily testing. There was no significant difference in the number of trials, errors, or sessions to criterion between the HIV-1 Tg and control animals, nor was there a significant effect of S-equol on these measures. However, linear regression on sessions to criterion revealed that the groups differed in the rate of acquisition, illustrated in Figure 3.5 as the cumulative proportion of subjects that attained the criterion over sessions. The HIV-1 Tg animals that received S-equol acquired the task significantly faster than the HIV-1 Tg animals that received sucrose, $F(1,101)=21.8$, $p\leq0.0001$. The HIV-1 Tg animals that received S-equol also achieved the criterion faster than the control animals, whereas the HIV-1 Tg animals that received sucrose took the longest amount of time to acquire the criterion compared to all animals. The control animals that received S-equol also showed a significantly faster overall rate of acquisition compared to the control animals that received sucrose, $F(1,105)=49.6$, $p\leq0.0001$, although they had a slower rate of acquistion than the other animals during the first month, which may indicate an initial detrimental effect of the S-equol treatment.

During the first 3 days on the signal detection task, there was no significant difference between the HIV-1 Tg and control animals in percent accuracy (see Figure 3.5), nor in the number of hits, misses, correct rejections, or false alarms (see Figure 3.6).

There was also no significant effect of S-equol on these measures during the first 3 days of the task. Once the animals met the criterion of 70% accuracy on 3 consecutive days, there was still no significant difference in percent accuracy between the HIV-1 Tg and control groups (see Figure 3.5), nor a difference in the number of hits, misses, or correct rejections (see Figure 3.6). However, the control animals had significantly more false alarms, $F(1,36)=7.4$, $p\leq0.01$. There was no significantly overall difference in the number of responses between groups, and there was also no effect of S-equol treatment on these measures.

A comparison of performance during the first 3 days that the animals were on the duration task and the last 3 days (at which point they attained the criterion) was conducted to determine changes in performance over time. Performance on most measures improved over time, as expected. The number of correct rejections increased over time for both the HIV-1 Tg group, $F(1,18)=7.9$, $p\leq0.05$, and the control group, $F(1,18)=15.3$, $p\leq0.001$. A significant genotype x time interaction was observed for the number of hits, $F(1,36)=5.9$, $p\leq0.05$, by which the control group had a significantly greater number of hits over time, $F(1,18)=20.0$, $p\leq0.001$, but the HIV-1 Tg group did not change over time. There was no overall difference between groups in the number of hits. A significant genotype x time interaction was observed for false alarms, $F(1,36)=7.7$, $p\leq0.01$, by which the HIV-1 Tg group had a significant reduction in false alarms over time, $F(1,18)=17.0$, $p\leq0.001$, but the control group did not change in this measure. There was no change in the number of misses over time or between groups. Percent accuracy improved over time for both the HIV-1 Tg group, $F(1,18)=59.2$, $p\leq0.001$, and the control

group, $F(1,18)=57.5$, $p\leq0.001$, with no overall difference between groups. There was also no effect of S-equol on any measure.

Stimulus Detection with Varying Stimulus Lengths.

All but three animals performed at 70% or greater accuracy across 3 consecutive days within two weeks of training. Among these subjects, a significant genotype x S-equol treatment effect was observed on trials to criterion, $F(1,33)=6.4$, $p\leq0.05$, errors to criterion, $F(1,33)=4.2$, $p\leq0.05$, and sessions to criterion, $F(1,33)=6.5$, $p\leq0.05$. Linear regression also revealed a significant difference among genotype/S-equol treatment groups in the rate of acquisition, $F(3,23)=4.45$, $p\leq0.05$, illustrated in Figure 3.7 as the cumulative proportion of subjects meeting the criterion over sessions. HIV-1 Tg animals that received sucrose for two months displayed the slowest rate of acquisition, with one animal requiring one month of daily training to attain the criterion. Control animals that received sucrose showed a faster rate of acquisition than the HIV-1 animals that received sucrose. Control animals that received equol exhibited an initial lag in acquisition as did the HIV-1 Tg animals on sucrose, with only 2 animals attaining the criterion during the first week. After the first week, however, the control animals on equol increased in their rate of acquisition, matching that of the control animals that received sucrose. As in the initial signal detection task, the HIV-1 Tg animals that received equol showed the fastest rate of acquisition, with all but one animal attaining the criterion within one week of training.

Once the animals met the criterion, there was no significant difference between the HIV-1 Tg and control animals in percent accuracy (see Figure 3.7), nor was there any difference between groups in the number of hits, misses, false alarms, or correct rejections. The HIV-1 Tg animals that received S-equol had more misses than HIV-1 Tg animals that received sucrose, $F(1,15)=4.6$, $p\leq0.05$. A linear trend for signal duration was observed in both groups for hits [HIV-1 Tg: $F(1,15)=49.7$, $p\leq0.001$; Control: $F(1,18)=81.4$, $p\leq0.001$] and misses [HIV-1 Tg: $F(1,15)=49.6$, $p\leq0.001$; Control: $F(1,18)=71.8$, $p\leq0.001$].

A comparison of performance during the first 3 days that the animals were tested and the last 3 days (at which point they attained the criterion) was conducted to determine changes in performance over time. Performance on most measures improved over time, as expected. The number of misses decreased over time for both the HIV-1 Tg animals, $F(1,15)=16.7$, $p\leq0.001$, and the control animals, $F(1,18)=9.6$, $p\leq0.01$. The number of false alarms also decreased by the time the animals met the criterion [HIV-1 Tg: $F(1,15)=14.8$, $p\leq0.005$; Control: $F(1,18)=40.9$, $p\leq0.001$]. A significant genotype x condition interaction was observed on correct rejections, $F(1,33)=4.4$, $p\leq0.05$, by which the control group showed an increase in the number of correct rejections over time, $F(1,18)=5.4$, $p\leq0.05$, but the HIV-1 animals did not change in this measure. There was no difference between groups on any of these measures, nor was there an effect of S-equol on performance.

To assess long-term effects of S-equol, additional analyses were conducted on performance on signal detection with varying lengths during the first 3 days on the task and 1 month after S-equol/sucrose treatment ended. Performance of only the top 50% of

animals at 1 month post-treatment was included in the analysis, as it was expected that only the higher performing animals would benefit from the S-equol treatment, as observed in experiment 1.

A significant genotype x time x S-equol treatment interaction was observed on the number of hits, $F(1,16)=7.9$, $p\leq0.05$. For animals that received S-equol, there was a significant genotype x time interaction, $F(1,8)=12.3$, $p\leq0.01$, whereby the control animals that received S-equol did not change in the number of hits over time, and the HIV-1 Tg animals that received S-equol showed a trend for an increase in the number of hits over time, $F(1,4)=7.5$, $p=0.52$ (see Figure 3.9). There was no difference in the number of hits during the first 3 days of training between the HIV-1 Tg and control animals that received S-equol (or sucrose), but the HIV-1 Tg animals that received S-equol had a significantly greater number of hits than the control animals one month after the treatment ended, $F(1,8)=7.3$, $p\leq0.05$.

For the number of misses, there was a significant effect of time for both the HIV-1 Tg group, $F(1,8)=65.9$, $p\leq0.001$, and the control group, $F(1,8)=13.6$, $p\leq0.01$, with fewer misses occurring with training. For the animals on the S-equol treatment, there was a significant effect of time, $F(1,8)=44.7$, $p\leq0.001$, as well as a significant genotype x time interaction, $F(1,8)=6.7$, $p\leq0.05$. The HIV-1 Tg animals treated with S-equol showed a significant decrease in misses over time, $F(1,4)=50.3$, $p\leq0.005$, but this effect was not observed with the control animals (see Figure 3.9). The animals that were treated with sucrose also showed a significant decrease in misses over time, $F(1,8)=21.9$, $p\leq0.005$, but there was no differential effect of genotype. There was no difference between the HIV-1 Tg and control groups in the number of misses at either time point.

There was a significant genotype x S-equol treatment interaction for percent accuracy, $F(1,16)=8.1$, $p\leq0.05$ (see Figure 3.8). The HIV-1 Tg group did not show a significant effect of S-equol treatment, but in the control group, those that received S-equol had significantly lower percent accuracy, $F(1,8)=8.4$, $p\leq0.05$. However, there was a significant overall increase in percent accuracy over time for both the HIV-1 Tg group, $F(1,8)=83.2$, $p\leq0.001$, and the control group, $F(1,8)=255.7$, $p\leq0.001$, regardless of treatment.

The general improvement in performance over time was also observed with false alarms, which decreased significantly for both the HIV-1 Tg group, $F(1,8)=63.2$, $p\leq0.001$, and the control group, $F(1,8)=57.4$, $p\leq0.001$ (see Figure 3.8). Neither group showed an effect of S-equol treatment on the number of false alarms.

The HIV-1 Tg animals generally had more correct rejections than the control group, $F(1,16)=10.1$, $p\leq0.01$, but there was no effect of S-equol treatment or time on the number of correct rejections (see Figure 3.8).

Stimulus Detection with Higher ITI Variability.

During sessions with higher ITI variability (9+/-6 s), a significant linear trend for signal duration was observed for hits and misses for both the HIV-1 Tg animals [Hits: $F(1,18)=19.8$, $p\leq0.001$; Misses, $F(18)=20.4$, $p\leq0.001$] and the control animals [Hits: $F(1,18)=25.9$, $p\leq0.001$; Misses: $F(1,18)=22.8$, $p\leq0.001$], as is seen during sessions with standard ITI variability (see previous section).

The control animals exhibited a greater number of hits, $F(1,18)=5.1$, $p\leq0.05$, false alarms, $F(1,18)=10.1$, $p\leq0.01$, and correct rejections, $F(1,18)=8.3$, $p\leq0.05$, as well as

reduced percent accuracy, $F(1,18)=8.6$, $p\leq0.01$, in sessions with higher ITI variability compared to sessions with standard ITI variability. The performance of the HIV-1 Tg animals, however, was not affected by the higher ITI variability (see Figure 3.10). For both the HIV-1 Tg and control groups, performance was also not differentially altered by the animals' previous treatments with S-equol or sucrose.

The HIV-1 Tg animals generally had more misses than the control animals, regardless of the ITI range, $F(1,38)=5.2$, $p\leq0.05$. Collapsing across ITI ranges, the groups did not differ in the number of hits, false alarms, or correct rejections, or in percent accuracy.

Stimulus Detection with Distractors: Houselight Distractor.

For both HIV-1 Tg and control animals, a significant effect of trial block [HIV-1 Tg: $F(2,36)=38.9$, $p\leq0.001$; Control: $F(2,36)=27.6$, $p\leq0.001$] and signal duration [HIV-1 Tg: $F(2,36)=17.0$, $p\leq0.001$; Control: $F(2,36)=8.9$, $p\leq0.005$], as well as a significant trial block x signal duration interaction [HIV-1 Tg: $F(4,72)=3.8$, $p\leq0.05$; Control: $F(4,72)=6.9$, $p\leq0.005$], were found on the number of hits during signal detection sessions with houselight distractor trials (see Figure 3.11). The number of hits decreased according to a linear trend across trial blocks for both the HIV-1 Tg group, $F(1,18)=82.7$, $p\leq0.001$, and the control group, $F(1,18)=50.4$, $p\leq0.001$. The number of hits also decreased with decreasing signal duration in a linear trend for the control group during the first block, $F(1,19)=18.9$, $p\leq0.001$, but there were no differences in the number of hits across signal duration during the second or third blocks for the control group, reflecting the trial block x signal duration interaction. The HIV-1 Tg group, in contrast, showed the

linear trend for signal duration during all trial blocks [Block 1: $F(1,19)=24.1$, $p\leq0.001$; Block 2: $F(1,19)=8.3$, $p\leq0.05$; Block 3: $F(1,19)=8.7$, $p\leq0.01$]. There was no difference in the total number of hits between groups.

Similar effects were observed on the number of misses (see Figure 3.11). For both groups, there was a significant effect of trial block [HIV-1 Tg: $F(2,36)=5.3$, $p\leq0.05$; Control: $F(2,36)=4.1$, $p\leq0.05$] and signal duration [HIV-1 Tg: $F(2,36)=19.8$, $p\leq0.001$; Control: $F(2,36)=9.6$, $p\leq0.001$], as well as a significant trial block x signal duration interaction [HIV-1 Tg: $F(4,72)=5.4$, $p\leq0.005$; Control: $F(4,72)=7.7$, $p\leq0.001$]. The number of misses decreased with a linear trend across block for the HIV-1 Tg animals, $F(1,18)=6.6$, $p\leq0.05$, but not for the control animals. The control group showed a significant decrease in misses in the second block compared to the first, $F(1,18)=6.6$, $p\leq0.05$, but no subsequent decrease in misses in the third block. The number of misses increased with decreasing signal duration in a linear trend for both groups during block 1 [HIV-1 Tg, $F(1,19)=23.5$, $p\leq0.001$; $F(1,19)=20.4$, $p\leq0.001$], but not during block 2. A linear trend for misses was observed with the HIV-1 Tg group during block 3, $F(1,19)=12.1$, $p\leq0.005$, but not for the control group.

For the number of false alarms, both groups showed a quadratic trend across trial block [HIV-1 Tg: $F(1,18)=95.1$, $p\leq0.001$; Control: $F(1,18)=134.4$, $p\leq0.001$], reflecting the increase in false alarms during the house light distractor trials in the second block, compared to the low number of false alarms during no-distractor trials in the first and third blocks (see Figure 3.12). There was no difference in the number of false alarms between groups.

For the number of correct rejections, both groups showed a quadratic trend across trial blocks [HIV-1 Tg: $F(1,18)=49.5$, $p\leq0.001$; Control: $F(1,18)=180.5$, $p\leq0.001$], reflecting a decrease in correct rejections during the distractor trials in the second block compared to the no-distractor trials in the first and third blocks (see Figure 3.12). However, the HIV-1 Tg animals had more correct rejections during the distractor trials in the second block compared to the control animals, $t(38)=-2.1$, $p\leq0.05$. There was no difference in the number of correct rejections between groups during the first or third trial blocks.

For percent accuracy, both groups showed a quadratic trend across trial blocks [HIV-1 Tg: $F(1,18)=60.3$, $p\leq0.001$; Control: $F(1,18)=296.3$, $p\leq0.001$], reflecting the decrease in percent accuracy during the distractor trials in the second block compared to the no-distractor trials in the first and third blocks (see Figure 3.12). However, the HIV-1 Tg animals had greater percent accuracy during the distractor trials in the second block compared to the control animals, $t(38)=-2.6$, $p\leq0.05$.

For the total number of responses, including both correct and incorrect responses, both groups showed a linear trend across trial blocks [HIV-1 Tg: $F(1,18)=60.7$, $p\leq0.001$; Control: $F(1,18)=56.1$, $p\leq0.001$], reflecting a decrease in responding across the session (see Figure 3.12). The HIV-1 Tg animals had a greater number of total responses in the first [$t(38)=-2.5$, $p\leq0.05$] and second blocks [$t(38)=-2.1$, $p\leq0.05$] compared to the control animals. However, there was no difference between groups in the third block.

There was no effect of previous S-equol treatment on any of the measures.

Stimulus Detection with Distractors: Tone Distractor.

For both HIV-1 Tg and control animals, a significant effect of trial block [HIV-1 Tg: $F(2,36)=31.1$, $p\leq0.001$; Control: $F(2,36)=92.6$, $p\leq0.001$] and signal duration [HIV-1 Tg: $F(2,36)=9.9$, $p\leq0.001$; Control: $F(2,36)=12.3$, $p\leq0.001$], as well as a significant trial block x signal duration interaction [HIV-1 Tg: $F(4,72)=5.2$, $p\leq0.005$; Control: $F(4,72)=6.9$, $p\leq0.001$], were found on the number of hits during signal detection sessions with tone distractor trials (see Figure 3.13). The number of hits decreased according to a linear trend across trial blocks for both the HIV-1 Tg group, $F(1,18)=46.8$, $p\leq0.001$, and the control group, $F(1,18)=245.0$, $p\leq0.001$. The number of hits also decreased with decreasing signal duration in a linear trend for both groups during the first trial block [HIV-1 Tg: $F(1,18)=31.1$, $p\leq0.001$; Control: $F(1,18)=17.3$, $p\leq0.005$], but there were no differences in the number of hits across signal duration during the second or third blocks for either group, reflecting the trial block x signal duration interaction. The HIV-1 Tg group had significantly more hits than the control group during the third trial block, $F(1,36)=7.7$, $p\leq0.01$, but there were no differences between groups during the first two trial blocks.

Similar effects were found on the number of misses (see Figure 3.13). For both HIV-1 Tg and control animals, there was a significant effect of trial block [HIV-1 Tg: $F(2,36)=11.2$, $p\leq0.001$; Control: $F(2,36)=20.6$, $p\leq0.001$] and signal duration [HIV-1 Tg: $F(2,36)=7.7$, $p\leq0.005$; Control: $F(2,36)=17.4$, $p\leq0.001$], as well as a significant trial block x signal duration interaction [HIV-1 Tg: $F(4,72)=11.7$, $p\leq0.001$; Control: $F(4,72)=14.3$, $p\leq0.001$]. The number of misses decreased according to a linear trend across trial blocks for both the HIV-1 Tg group, $F(1,18)=26.0$, $p\leq0.001$, and the control

group, $F(1,18)=27.0$, $p\leq0.001$. The number of misses increased with decreasing signal duration in a linear trend for both groups during the first trial block [HIV-1 Tg: $F(1,18)=27.9$, $p\leq0.001$; Control: $F(1,18)=24.1$, $p\leq0.001$], but there were no differences in the number of misses across signal duration during the second or third blocks for either group, reflecting the trial block x signal duration interaction. HIV-1 Tg animals had more misses overall than the control animals during the third trial block, $F(1,36)=6.2$, $p\leq0.05$, but there were no differences between groups during the first two trial blocks.

The HIV-1 Tg animals had more false alarms overall than the control animals, $F(1,36)=5.3$, $p\leq0.05$, but there were no differences between groups in any particular trial block (see Figure 3.14). The HIV-1 Tg group showed a significant decrease in false alarms in the third trial block compared to the average of the first and second trial blocks, $F(1,19)=8.8$, $p\leq0.01$. There were no changes in the number of false alarms across trial blocks for the control animals.

The HIV-1 Tg animals also had more correct rejections overall than the control animals, $F(1,36)=5.8$, $p\leq0.05$, and more correct rejections than the control animals during the third block specifically, $t(38)=-2.5$, $p\leq0.05$ (see Figure 3.14). The number of correct rejections decreased in a linear trend for both the HIV-1 Tg group, $F(1,18)=67.7$, $p\leq0.001$, and the control group, $F(1,18)=399.4$, $p\leq0.001$, with the largest decrease in correct rejections observed during the second trial block, when the tone distractor was presented on each trial.

A significant group x trial block interaction was observed for percent accuracy, $F(2,72)=3.5$, $p\leq0.05$. The HIV-1 Tg group showed significantly lower percent accuracy

than the control group during the first block, $t(38)=2.0$, $p\leq0.05$, but there was no difference between the groups during the second and third blocks, as illustrated in Figure 3.14. The control group had a significant decrease in percent accuracy from the first to second block, $F(1,18)=18.0$, $p\leq0.001$. Percent accuracy in the third block was also significantly lower than that in the first block for the control group, $F(1,18)=16.9$, $p\leq0.005$, but there was no difference between the second and third blocks. The HIV-1 Tg group also had a significant decrease in percent accuracy from the first to second block, $F(1,18)=6.0$, $p\leq0.05$. Percent accuracy during the third block was also significantly less than that of the first block, $F(1,18)=5.2$, $p\leq0.05$, but not different from that of the second block.

The HIV-1 Tg animals had a greater overall number of responses (correct and incorrect) than the control animals, $F(1,36)=9.3$, $p\leq0.005$ (see Figure 3.14). The control animals showed a significant decrease in their total number of responses from the first block to the second block, $F(1,18)=154.6$, $p\leq0.001$, as well as significantly less responding during the third block compared to the first block, $F(1,18)=338.8$, $p\leq0.001$. There was no difference between responding in the second and third blocks. The HIV-1 Tg group also showed a significant decrease in responding in the second block compared to the first block, $F(1,18)=54.5$, $p\leq0.001$, and in the third block compared to the first block, $F(1,18)=76.7$, $p\leq0.001$, with no difference between the second and third blocks.

There was no effect of previous S-equol treatment on any of the measures.

Experiment 2B: Discussion

S-equol (0.2 mg) was administered to the animals beginning at 2-3 months of age to determine if the temporal processing deficits of the HIV-1 Tg rats, observed with the sustained attention task in Experiment 1, could be delayed or prevented with an early course of treatment. The most prominent effect of the S-equol treatment was on the rate of acquisition of the sustained attention tasks, with both the 1-second signal and with varying signal durations. The HIV-1 Tg animals that received S-equol achieved the criterion for acquisition faster than the animals that received sucrose, and also achieved it faster than the control animals that received S-equol. The HIV-1 Tg animals that received sucrose showed the slowest rate of acquisition, consistent with the results of Experiment 1, in which the HIV-1 Tg animals acquired most of the tasks at a significantly slower rate relative to controls.

On the signal detection task, with either the 1 second signal or with varying signal duration, individual measures of performance, including percent accuracy and the number of hits, misses, correct rejections, and false alarms, generally did not differ significantly between the HIV-1 Tg and control groups, or between the sucrose or S-equol treated animals, either at the beginning of training or by the point at which the animals achieved the criterion. Long-term effects of S-equol were observed, however, one month after the treatment ended. The HIV-1 Tg animals that received S-equol showed a trend for an increase in the number of hits a month after treatment ended compared to the beginning of training, whereas the control animals and the HIV-1 Tg animals that received sucrose did not show any change in the number of hits. In addition, the HIV-1 Tg animals, but not control animals, that received S-equol had a significant decrease in misses from the first

week of training to a month post-treatment. Interestingly, the control animals that received S-equol had lower overall percent accuracy compared to the controls that received sucrose, although all animals improved in percent accuracy over time, as expected.

In order to further tax sustained attention, the ITI range was increased to 9+/-6 seconds. With these conditions, the control animals showed an increased overall amount of responding along with decreased percent accuracy. The HIV-1 Tg animals were not affected by the change in ITI range, however. There was also no effect of previous S-equol treatment on either group.

To assess selective attention, a distractor stimulus was introduced in the second block of trials of the signal detection task with varying signal durations. The HIV-1 Tg and control animals both showed reductions in accuracy and overall responsiveness during the house light distractor trials. Responsiveness remained low during the third and last block of trials, with no distractor stimulus, suggesting a residual effect of the distractors on attention. The HIV-1 Tg group was less sensitive to the house light distractor than the control group, however, as indicated by higher percent accuracy than the control animals during the distractor trials. Also, during distractor trials, the HIV-1 Tg group maintained the linear function for the effect of signal duration on the number of hits, whereas the control group did not respond differentially to signal duration when the distractor was presented. The control group maintained a low level of responding across all signal durations, indicating that the distractor stimulus reduced their ability to detect even the longest signal. The lack of this effect on the HIV-1 Tg group may reflect a reduced sensitivity to brightness due to their cataracts. It is possible that the house light

distractor did not appear as bright to the HIV-1 Tg animals as it did to the control animals, and therefore it did not affect their ability to detect the signal to the same extent as that observed with the control animals. For both groups, though, the house light distractor significantly increased the number of false alarms, which was expected, given that the distractor was of the same modality as the signal. Further, the animals' sensitivity to brightness was confirmed in the PPI procedure in Experiments 1A and 2A.

The tone distractor was also effective in testing selective attention. The HIV-1 Tg and control animals both showed reductions in accuracy and overall responsiveness during the tone distractor trials. Responsiveness remained low during the third and last block of trials, with no distractor stimulus, suggesting a residual effect of the distractors on attention. Both the HIV-1 Tg and control groups also exhibited non-differential responding across signal duration during the tone distractor trials. A low level of hits and misses was maintained across all signal durations for both groups, indicating the effect of increased attentional load provided by the presence of the distractor. The HIV-1 Tg animals were generally more responsive than the control animals, regardless of whether the response was correct or incorrect.

There was no effect of previous S-equol treatment on performance on signal detection with higher ITI variability, or on the selective attention tasks. As these tests were conducted approximately two months after the treatment ended, it was not predicted that there would be any effect. The HIV-1 Tg rats generally did not exhibit any deficits in performance relative to the control animals on these tasks. Their cataracts may have contributed to their attenuated response to the visual distractor stimulus.

In summary, the initiation of the 0.2 mg dose of S-equol after a shorter duration of constitutive expression of the HIV-1 transgene prevented the delay in acquisition of the signal detection task that was observed in the HIV-1 Tg rats in Experiment 1. In fact, HIV-1 Tg animals that received S-equol acquired the task significantly more rapidly than the other groups. Long-term improvements in performance after S-equol treatment had ended were also demonstrated in the HIV-1 Tg animals. Thus, the present results suggest that S-equol can effectively prevent, or at least delay, neurocognitive impairment of HAND when administered early in the progression of HIV-1.

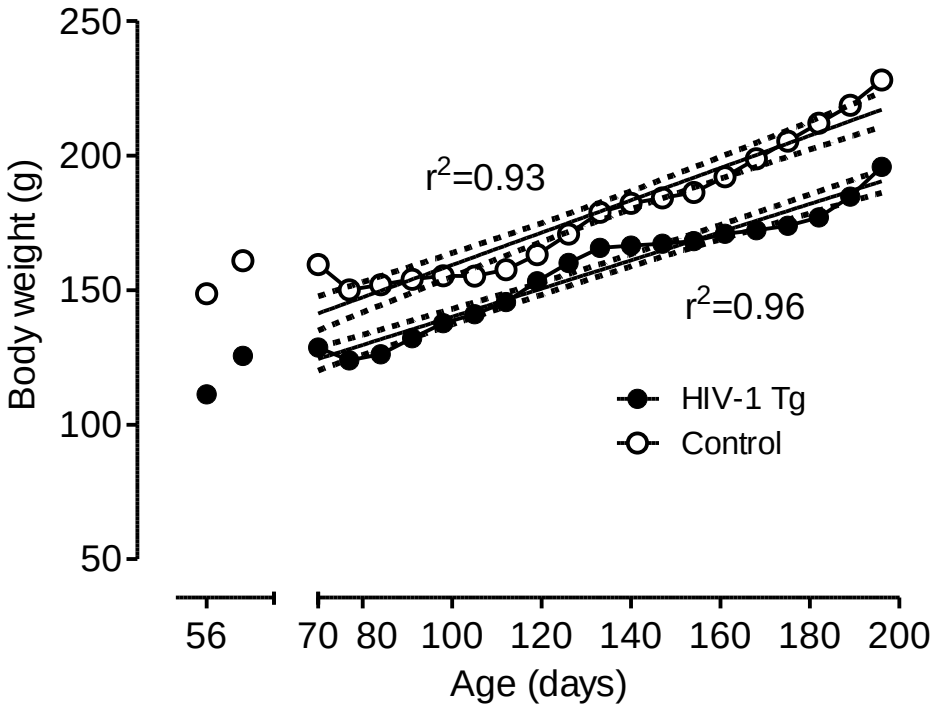

Figure 3.1. Mean body weight of the HIV-1 Tg animals and control animals across age with the best fit linear regression for each group (±95% CI). The HIV-1 Tg group weighed significantly less than the control group across the 6-month period during which they were tested. Both groups increased significantly in body weight across this period and did not differ in their rates of growth. The x-axis break at 65 days indicates the point at which animals began food restriction, prior to testing.

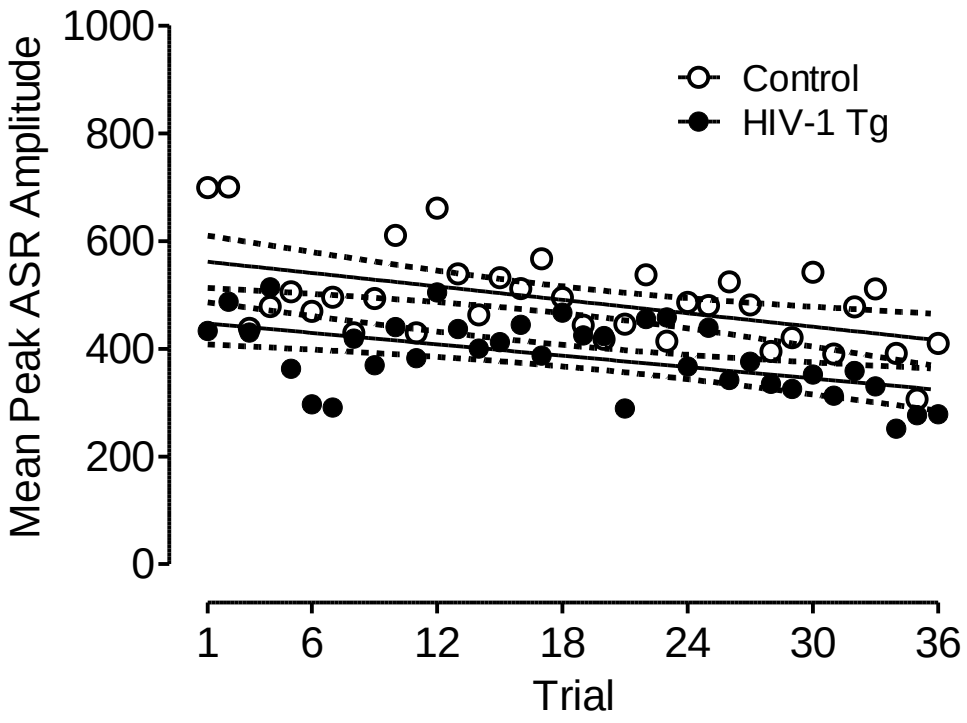

Figure 3.2. Mean peak ASR amplitude data from the habituation session (±95% CI). The HIV-1 Tg and control groups showed no difference in overall ASR, and did not differ in rate of habituation to the auditory startle stimulus. Regression line slopes: HIV-1 Tg, -3.5+/-0.94; Control: -4.2+/-1.2.

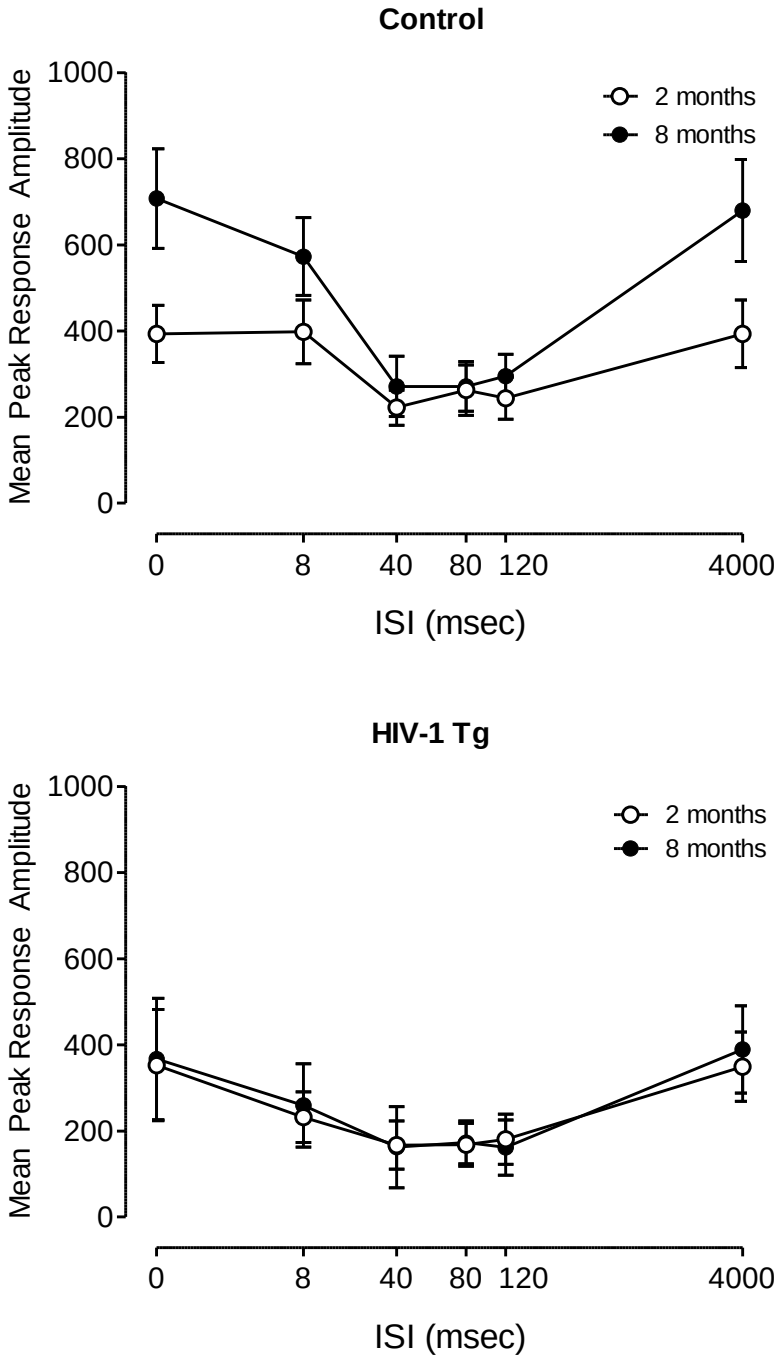

Figure 3.3 Mean (+/- SEM) peak response amplitude during PPI trials with a prepulse at 2 and 8 months of age for the control animals (top panel) and HIV-1 Tg animals (bottom panel).

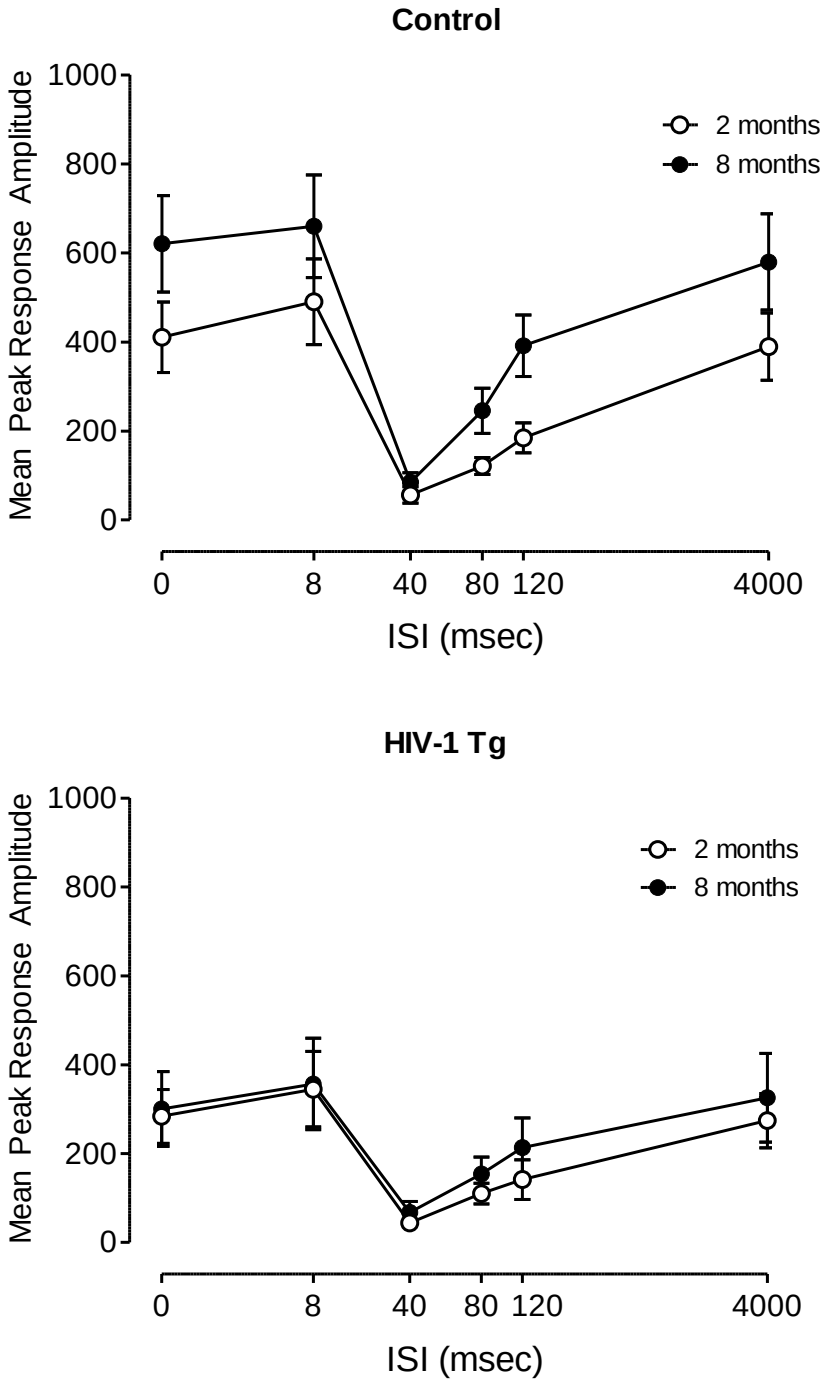

Figure 3.4. Mean (+/- SEM) peak response amplitude during PPI trials with a visual prepulse at 2 and 8 months of age for the control animals (top panel) and HIV-1 Tg animals (bottom panel).

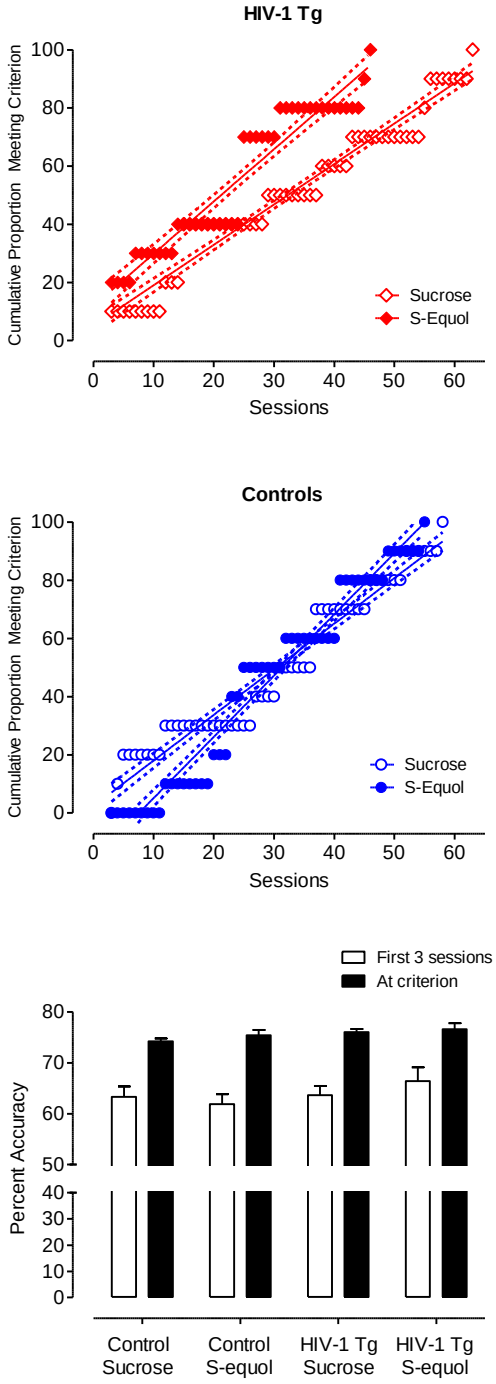

Figure 3.5. Cumulative proportion of HIV-1 Tg animals (top panel) and control animals (middle panel) achieving the signal detection task criterion of 70% accuracy on 3 consecutive sessions (±95% CI). Regression line slopes: HIV-1 Tg sucrose, 1.4+/-0.04; HIV-1 Tg S-equol, 1.8+/-0.08; Control sucrose, 1.6+/-0.05; Control S-equol, 2.1+/-0.06. Bottom panel: Mean (+/-SEM) percent accuracy at the beginning of training and at the point of achieving the criterion.

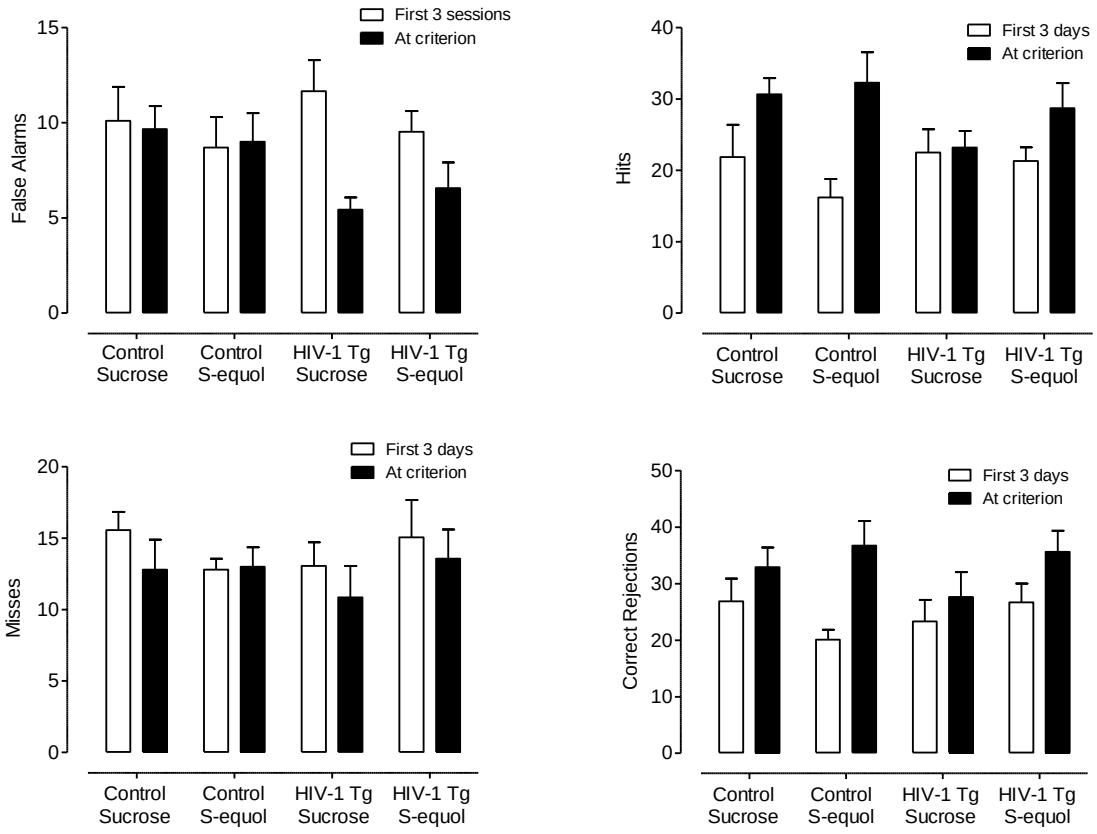

Figure 3.6. Mean (+/- SEM) number of false alarms, hits, misses, and correct rejections during the first 3 days of training and at the point of achieving the criterion for the signal detection task.

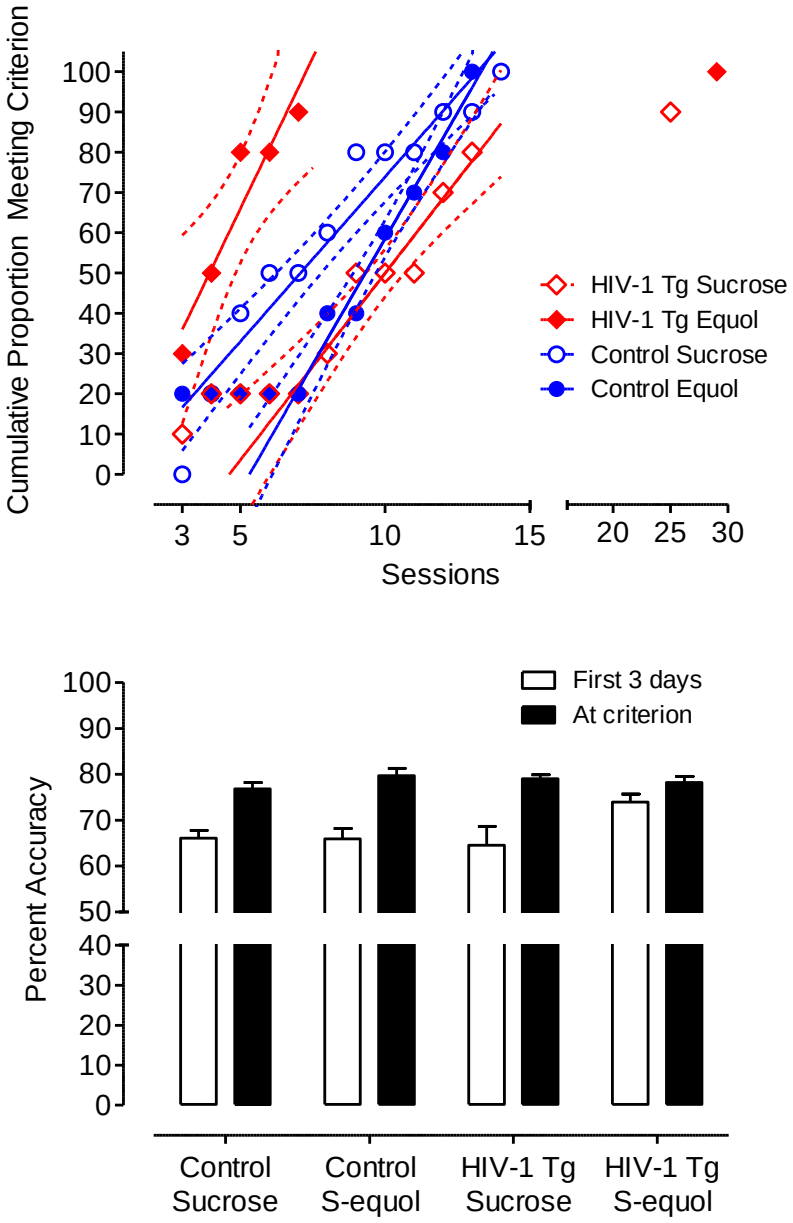

Figure 3.7. Top panel: Cumulative proportion of animals achieving the criterion for the stimulus detection task with varying signal durations (70% accuracy on 3 consecutive days) (±95% CI). Regression line slopes: HIV-1 Tg Sucrose, 9.3+/-1.2; HIV-1 Tg S-equol, 15+/-3; Control sucrose, 8.2+/-0.7; Control S-equol, 12.5+/-0.9. Bottom panel: Mean (+/- SEM) percent accuracy at the beginning of training and at the point of achieving the criterion for the signal detection task with varying signal durations.

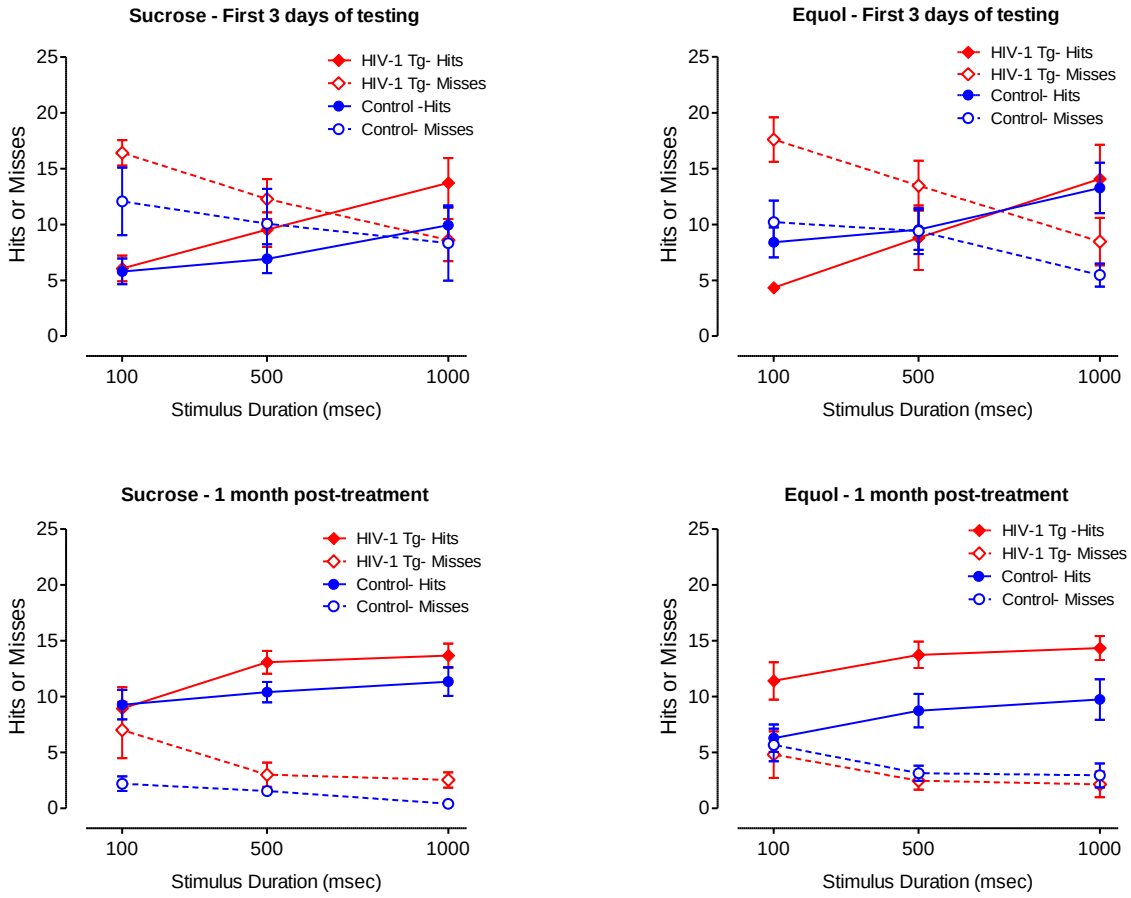

Figure 3.8. Mean (+/- SEM) number of hits and misses across signal duration during the first 3 days of training and one month after the end of the S-equol treatment period for the top 50% of subjects (based on percent accuracy at the beginning of testing).

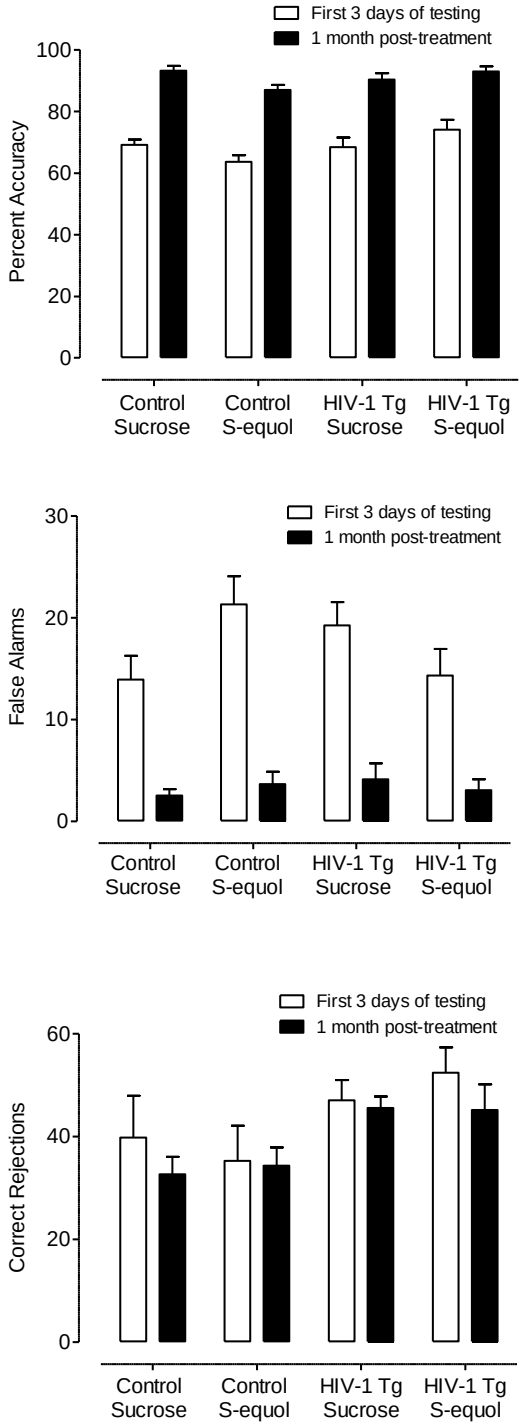

Figure 3.9. Mean (+/-SEM) percent accuracy, and mean (+/-SEM) number of false alarms and correct rejections for the top 50% of subjects (based on percent accuracy at the beginning of testing) during the first 3 days of training and 1 month post-treatment for the signal detection task with varying signal durations top 50%.

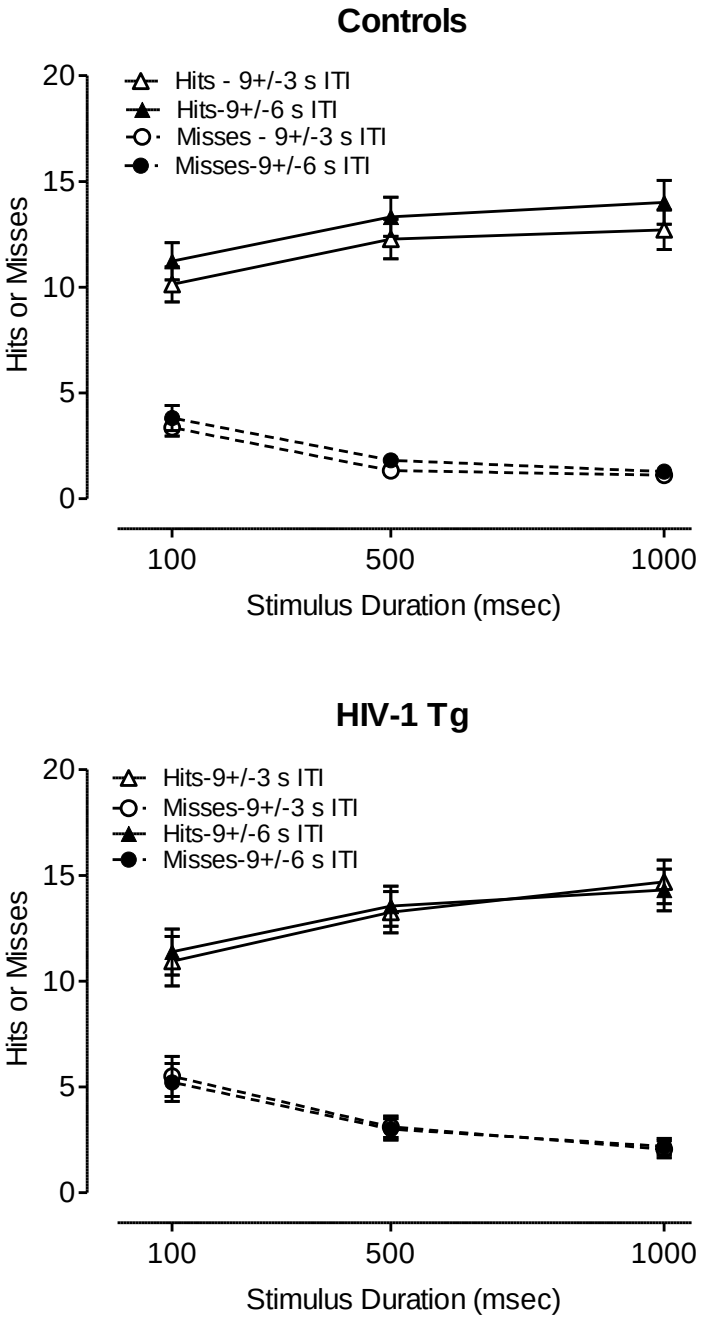

Figure 3.10. Mean (+/-SEM) number of hits and misses across signal duration with standard ITI variability (9+/-3 s) and with higher ITI variability (9+/-6 s).

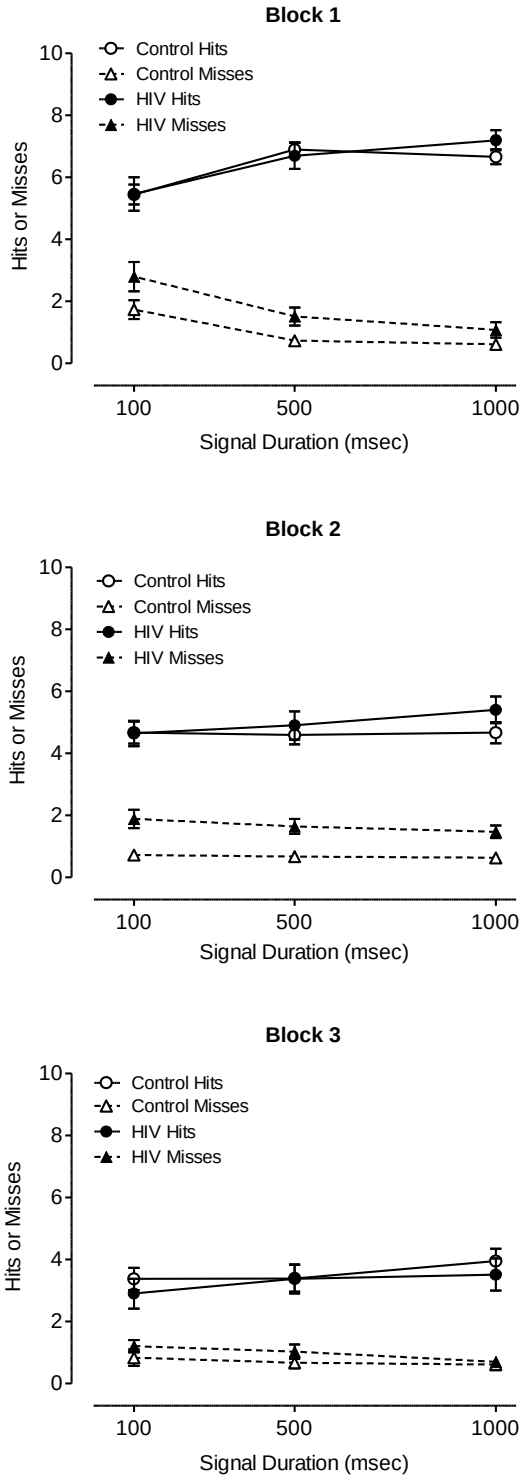

Figure 3.11. Mean (+/- SEM) number of hits and misses across signal duration during sessions with a visual stimulus distractor. The distractor was presented at the beginning of each trial of block 2.

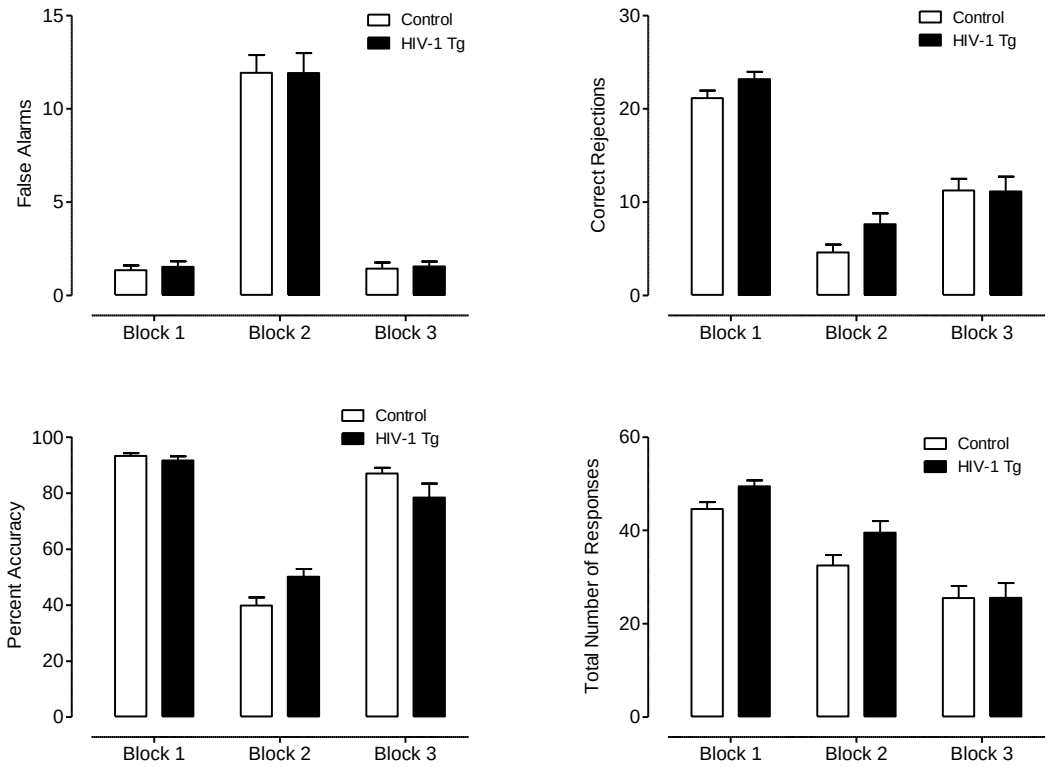

Figure 3.12. Mean (+/-SEM) number of false alarms, correct rejections, total number of responses, and percent accuracy during signal detection sessions with a visual stimulus distractor. The distractor was presented at the beginning of each trial of block 2.

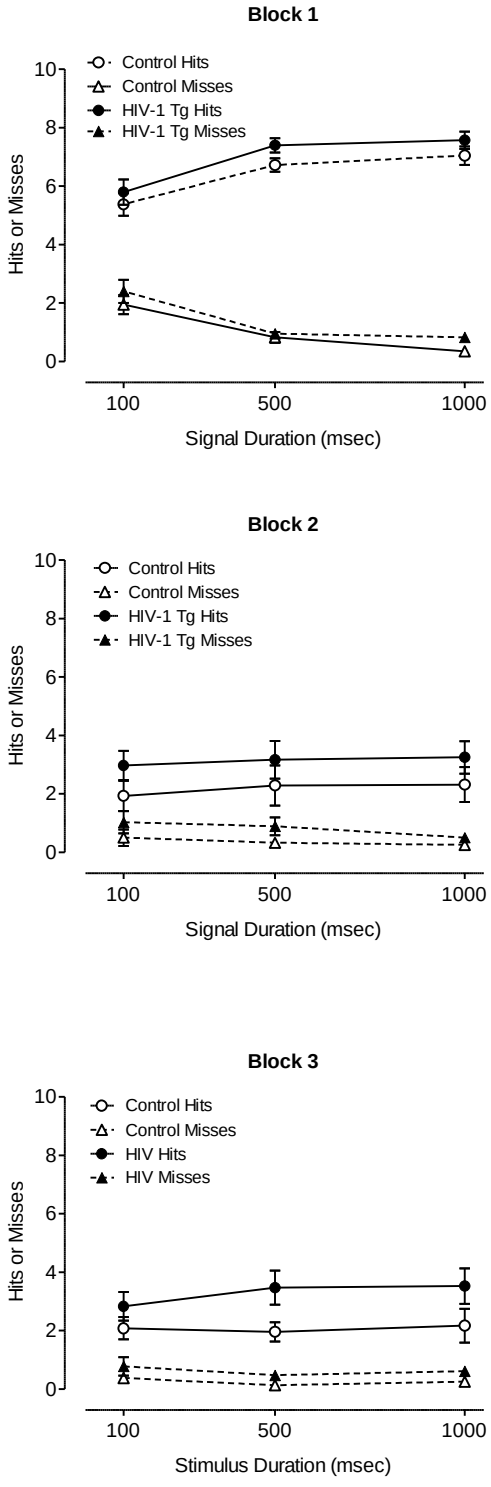

Figure 3.13. Mean (+/-SEM) number of hits and misses across signal duration during sessions with an auditory stimulus distractor. The distractor was presented at the beginning of each trial of block 2.

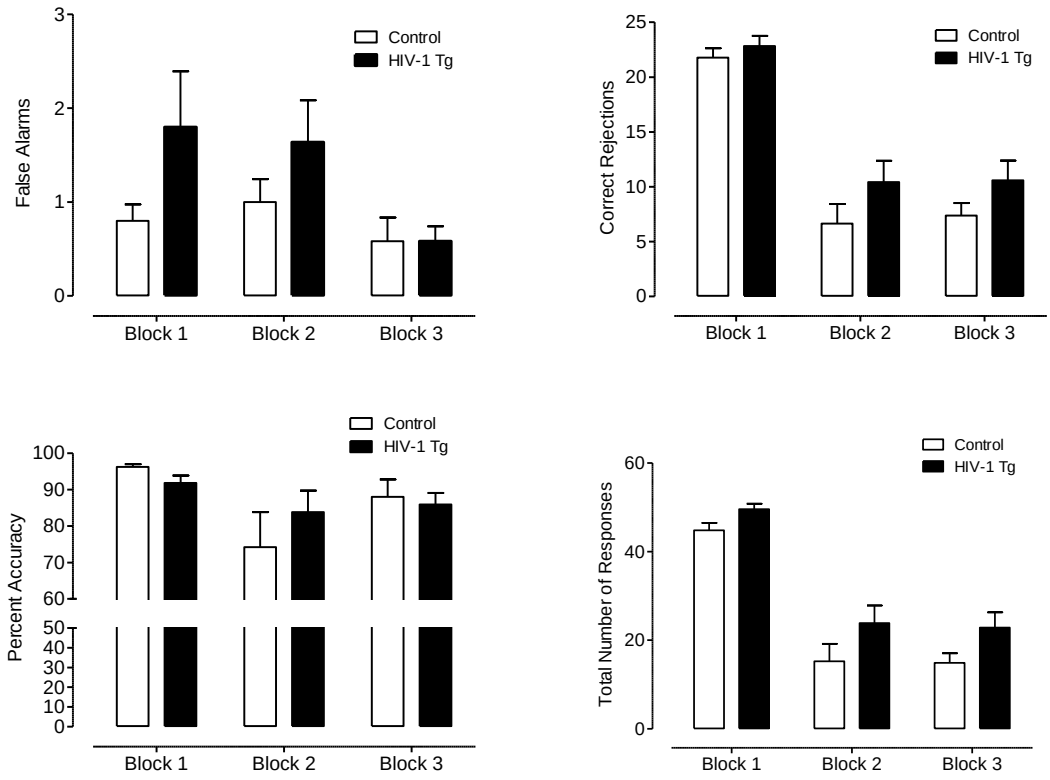

Figure 3.14. Mean (+/- SEM) number of false alarms, correct rejections, total number of responses, and percent accuracy during signal detection sessions with an auditory stimulus distractor. The distractor was presented at the beginning of each trial of block 2.

CHAPTER 4

GENERAL DISCUSSION

The neurocognitive impairment that defines HAND in the cART era can be modeled and demonstrated in the HIV-1 Tg rat, providing opportunities to develop therapeutics for HAND. The HIV-1 Tg rat demonstrates deficits in executive function, attention, and preattentive processing, as well as alterations in neuronal networks in the medial PFC. Assessment of attention and preattentive processing also revealed temporal processing deficits as a common impairment across behavioral tests in the HIV-1 Tg rat. In experiment 1, daily S-equol treatment for 45 days at 6-8 months of age improved sustained attention in the top-performing HIV-1 Tg and control animals, and the HIV-1 Tg animals exhibited an improvement that was twofold that of the control group. HIV-1 Tg animals that received S-equol also displayed a neuronal network profile that more closely resembled that of the control animals than that of the HIV-1 Tg animals that received sucrose. In experiment 2, daily S-equol treatment began at 2-3 months of age and continued for two months to determine if a prophylactic treatment would delay, or perhaps prevent, the development of sustained attention deficits relative to the controls. The HIV-1 Tg animals that received S-equol acquired the sustained attention tasks faster than control animals and HIV-1 Tg animals that received sucrose. Also, for both groups, the animals that were treated with S-equol improved in performance on the sustained attention task from the beginning of testing to a month post-treatment. Thus, S-equol is

able to both improve as well as delay, if not prevent, attentional deficits observed in the HIV-1 Tg rat, suggesting that S-equol may be a suitable therapeutic for neurocognitive impairment in HAND.

In the cART era, individuals with HAND typically exhibit deficits in executive function, affecting the core cognitive components of flexibility, inhibition, and set-shifting. Attention provides an important foundation to executive function and is also impaired in HAND, as is preattentive processing. Therefore, assessments of these areas of cognition are necessary in modeling HAND in preclinical studies. Homology of executive function and attention have been demonstrated across species (Kesner and Churchwill 2011), supporting the use of the HIV-1 Tg rat to study these components of cognition.

The selection of operant tasks in the present study was based on several factors. Each task tapped into basic, lower level components of executive function, which can be operationally defined in a fairly precise manner. In addition, there are many well-established and relatively simple methods available to study each function. The preclinical assessment of sustained attention, and inhibition, flexibility, and set-shifting, as fundamental components of executive function, is important in understanding deficits observed in more complex components of executive function, such as decision-making and planning. In the present experiments, assessments of specific aspects of executive function provided the opportunity to characterize conditions under which the various components are deficient or intact in the HIV-1 Tg rat.

Sustained attention is the process by which one detects infrequent target stimuli over long periods of time. It has been characterized in both humans and rats, with analogous changes in performance related to varying task parameters, such as signal duration, intensity, and frequency (McGaughy and Sarter, 1995; Bushnell, 1999; Parasuraman and Davies, 1977). In the present experiments, a range of signal durations and higher ITI variability were implemented to increase demands on the temporal domains of sustained attention. Although the HIV-1 Tg and control groups both showed decreases in correct responses and increases in incorrect responses with the shorter signal durations, the performance of the HIV-1 Tg group was clearly more impaired. Interestingly, the HIV-1 Tg animals did not perform worse with the higher ITI variability, although the control group had significantly lower percent accuracy. Selective attention, the ability to attend to a target stimulus while ignoring irrelevant stimuli, was also assessed by presenting distractor stimuli during signal detection sessions, which reliably reduces performance accuracy (McGaughy and Sarter 1995). In the present experiments, both groups showed decrements in performance accuracy and overall responsiveness during distractor trials.

The core components of executive function that were targeted for assessment in this study – flexibility, inhibition, and set-shifting – are both interrelated and dissociable, given the use of specific measures. Flexibility, as the ability to modify behavior to changing environmental contingencies, requires the formation of a new representation of a learned stimulus-response rule. In the reversal task, the animal learns to respond to a stimulus that was previously not reinforced, and vice versa. The extradimensional set-shifting task, in contrast, requires one to learn stimulus-response contingencies within a

novel stimulus set. Inhibition is another component of executive function which is necessarily involved in learning new stimulus-response contingencies. In the reversal and set-shifting tasks, the animal must deliberately inhibit responses that were previously reinforced in order to respond correctly to the present contingency.

Set-shifting, or attention-switching, has also been defined as a basic component of executive function, in which one disengages from a learned stimulus set and actively engages in another. It is a composite of flexibility and inhibition, but it can be more specifically applied to circumstances in which a novel stimulus set is associated with the appropriate response, referred to as set-shifting tasks, as described above. Thus, "set-shifting" is both a construct and an operationally defined task used to measure the construct. In contrast, the reversal task does not involve shifting to a new stimulus set; it requires learning the opposite stimulus-response contingencies with the same stimulus set.

Different brain regions have been associated with performance on each task; the lateral prefrontal cortex of humans and nonhuman primates (Dias et al., 1996; Manes et al., 2002) and the medial prefrontal cortex of the rat (Birrell and Brown 2000) support extradimensional set-shifting, whereas the orbitofrontal cortex is associated with the reversal learning in nonhuman primates (Dias et al., 1996) and rats (McAlonan and Brown, 2003). The double dissociation of brain region and cognitive function may explain the difference in the HIV-1 Tg group's performance on the reversal and extradimensional tasks. Although both tasks place demands on executive function, each requires the use of a different cognitive process and thus, presumably, a different brain region.

Executive function in individuals with HIV-1 is commonly assessed with the Wisconsin Card Sorting Test, as a measure of flexibility, set-shifting, and inhibition of prepotent responses (Tozzi et al. 1993; Basso and Bornstein, 2003; Heaton et al., 2011). The Stroop Color Word Test (Chang et al., 2002; Maki et al., 2009) and category switching tests (Iudicello et al. 2008) have also been used to detect impairments in flexibility and inhibition as components of executive function in HIV-1+ individuals.

Most studies on attention in HIV-1+ individuals have only included measures of reaction time (for review, see Hardy and Hinkin, 2002), which is related more to psychomotor function than attention per se. However, a few studies have reported reduced accuracy on sustained attention tasks by individuals with HIV-1 (Fein et al., 1995; Watkins et al., 2000), which indicate specifically attentional deficits, rather than psychomotor impairment.

Alterations in preattentive processes, as measured with auditory evoked potentials (AEPs), have long been identified as an early marker of the neurological effects of HIV-1, both without (Castello et al. 1998; Fein et al. 1995; Gil et al. 1992; Koralnik et al. 1990; Pagano et al. 1992; Schroeder et al. 1996; Vigliano et al. 2000), and after, cART initiation (Chao et al. 2004; Matas et al. 2010). Individuals with HAND are also more likely to demonstrate reduced prepulse inhibition (Minassian et al. 2013). For the present experiments, the use of prepulse inhibition as a measure of preattentive processing in the HIV-1 Tg rat allowed for a characterization of deficits that occur early in the expression of HIV-1, as well as the progression of those deficits over time.

Previous studies have shown that HIV-1 Tg animals exhibit a peak shift in inhibition relative to controls (Moran et al. 2013). Shifts in peak inhibition have also been demonstrated in rats administered stereotaxic HIV-1 viral protein injections. Leftward shifts were observed in 30- and 60-day old male Sprague-Dawley rats following neonatal Tat injection (Fitting et al. 2006a) and in 9-month old male and female Sprague-Dawley rats given neonatal gp120 injections (Fitting et al. 2006b). In the present experiments, peak shifts in inhibition during auditory PPI trials were also displayed by the HIV-1 Tg animals, but the most striking effect was the relative insensitivity to the temporal dimension of preattentive processing and the lack of development of perceptual sharpening of the HIV-1 Tg group. Furthermore, these effects were observed with both auditory and visual prepulses, demonstrating the generality of the impairment across prepulse modality.

As discussed in the introduction, the brain DA system has been implicated as a major target of HIV-1 infection. Reports of HIV-1 positive individuals with lower cerebrospinal fluid DA (Berger et al., 1994), Parkinsonian symptoms, sensitivity to DA receptor antagonists, or abnormalities in basal ganglia structure and function (Berger & Nath, 1997; Koutsilieri et al., 2002) provided some of the earliest lines of evidence that HIV-1 infection disrupts the DA system. *In vitro* studies have shown that DAT is targeted by HIV-1 proteins Tat and gp120, resulting in transporter impairment (Aksenov et al. 2008; Ferris et al. 2009; Midde et al. 2013; Zhu et al. 2009; Zhu et al. 2011), due to direct protein-protein interactions (Zhu et al. 2009) involving an allosteric modulation of DAT by the Tat protein (Zhu et al. 2011). In addition, DA-dependent signaling has been

identified as a mechanism of HIV-1 protein neurotoxicity (Aksenova et al. 2006; Silvers et al. 2007; Wallace et al. 2006).

Symptoms of HAND have also been associated with DA system dysfunction. Reduced DA transporter (DAT) levels in the putamen and ventral striatum are found in HIV-1+ individuals with cognitive impairment (Wang et al. 2004; Chang et al., 2008). In HIV-1+ individuals, significant reductions in DA levels in the substantia nigra are correlated with poor performance on learning and memory tasks (Kumar et al., 2011), and decreases in homovanillic acid has been associated with deficits in executive function (di Rocco et al., 2000).

Indeed, DA systems play a critical role in the processes of attention and executive function. Sustained attention depends on DA systems in the PFC, as evidenced by the differential effects of PFC-infused DA agonists and antagonists on sustained attention (Granon et al. 2000; Chudasama and Robbins, 2004), and DA receptor activity regulates cortical ACh efflux (Moore et al., 1999; Zmarowski et al. 2005), which is essential in the mediation of attentional processes, as demonstrated with the stimulus detection task which taps sustained attention (McGaughy et al. 1996; Himmelheber et al. 2000). Executive function processes also rely on the integrity of DA systems; performance on the reversal task is disrupted by D2 receptor antagonists (Lee et al., 2007; Ridley et al., 1981) and DA depletion in the striatum (Clarke et al. 2011; O'Neill and Brown 2007), and is also correlated with D2 receptor activity in the striatum (Clatworthy et al, 2009; Kellendonk et al, 2006; Groman et al., 2011). Increases in DA in the rat PFC improve performance on the extradimensional set-shifting task (Tunbridge et al. 2004), whereas depletion of PFC DA impaired performance on the task in monkeys (Crofts et al., 2001).

Rodent models of schizophrenia often incorporate the extradimensional set-shifting task to demonstrate deficits in executive function, which are attenuated by D2 antagonist antipsychotics (McLean et al. 2008; Rodefer et al., 2008; Tait et al., 2009).

In addition to the role of DA systems in set-shifting, lesions of the dorsal noradrenergic ascending bundle (Tait et al. 2007) and noradrenergic deafferentiation of rat medial PFC (McGaughy et al. 2008) result in impaired performance on the extradimensional set-shifting task. Noradrenergic systems are also implicated in sustained attention, especially under conditions of increased attentional load (Aston-Jones and Cohen 2005; Carli et al. 1983; Milstein et al. 2007). NE plays a significant role in mediating PFC function, via stimulation of α_{2A}-receptors on dendritic spines of pyramidal neurons in the PFC, which strengthens synaptic connections by closing the ion channels on the spines, increasing the efficacy of synaptic inputs (Wang et al. 2007). Further study of neural network structures implicating NE as well as DA in the PFC of the HIV-1 Tg rat will be important in clarifying alterations underlying neurocognitive impairment of HAND.

The alterations in preattentive processing observed in HIV-1 Tg rats may also be explained by the disruptions in the DA system that are consequent to HIV-1 infection. Pharmacological studies have shown reductions in PPI after administration of direct and indirect DA agonists, such as apomorphine and amphetamine (Geyer et al. 2001). Apomorphine-induced PPI deficits have been used as a preclinical model of schizophrenia, capturing both the dysfunction of the DA system and preattentive sensory gating deficits as measured with event-evoked potentials (Adler et al. 1982) and the eyeblink response (Braff et al. 1978) in individuals with schizophrenia. The

aforementioned early studies on sensory gating in schizophrenic patients revealed that they have flatter ISI functions than the healthy controls, indicating an insensitivity to manipulation of the duration of the ISI. A "flattening" of the ISI function in rats administered apomorphine has previously been observed (Moran et al., 2009), comparable to the ISI functions exhibited by the HIV-1 Tg rats in the present study. Although other neural systems may be involved, central DA system dysfunction often results from HIV-1 infection and is associated with subsequent cognitive deficits (Kumar et al. 2011; Chang et al. 2008; diRocco et al. 2000; Kumar et al. 2011; Purohit et al. 2011; Wang et al. 2004). The use of behavioral measures such as the ASR and PPI that can detect early neurological alterations, especially those of the DA system, may be instrumental in predicting the development of HAND and thus determining an appropriate course of treatment.

The therapeutic effects of S-equol on cognitive deficits in the HIV-1 Tg rat were clearly demonstrated in the present experiments. S-equol both ameliorated deficits that had already developed with expression of the transgene, and prevented, or at least delayed, those deficits when administered early in the expression of the transgene. There is also preliminary evidence that S-equol ameliorated alterations in varicosity density that were observed in the HIV-1 Tg animals. Other estrogenic compounds have previously been demonstrated to reduce synaptodendritic damage as well as restore neuronal networks affected by Tat (Bertrand et al. 2013). Phytoestrogens have also been shown to attenuate damage to DA neurons in other disease models (Chen et al. 2007; Wang et al., 2005). Some benefits and potential drawbacks of the use of estrogenic compounds in treating neurocognitive impairment have been described, but there is still more to learn

about the potential uses of various compounds. S-equol, as a metabolite of the isoflavone daidzein, appears to be a particularly promising potential treatment for attention and executive function deficits, with selective affinity for ERβ, which is highly expressed in the PFC. Only individuals who can produce S-equol can benefit from its precursor, daidzein; thus, S-equol, rather than daidzein, may be a more efficient and effective potential therapeutic for neurocognitive impairment of HAND.

In conclusion, the present experiments demonstrate that HIV-1 Tg rats exhibit neurological and cognitive deficits early in the expression of the HIV-1 transgene, prior to clinical signs of wasting, bearing a marked resemblance to neurological and neurocognitive impairment observed in individuals with HAND. The functional consequences of chronic low level of exposure to the HIV-1 proteins are apparent under conditions which resemble the suppression of infection in HIV-1+ individuals under cART (Peng et al., 2010). The phytoestrogen metabolite S-equol both ameliorated and prevented cognitive deficits in the HIV-1 Tg rat, providing strong evidence that S-equol may represent a promising new therapeutic for neurocognitive impairment in HAND.

REFERENCES

Adams, S. M., Aksenova, M. V., Aksenov, M. Y., Mactutus, C. F., & Booze, R. M. (2010). ER-beta Mediates 17 beta-Estradiol Attenuation of HIV-1 Tat-Induced Apoptotic Signaling. *Synapse, 64,* 829-838.

Adler, L. E., Pachtman, E., Franks, R. D., Pecevich, M., Waldo, M. C., & Freedman, R. (1982). Neurophysiological Evidence for A Defect in Neuronal Mechanisms Involved in Sensory Gating in Schizophrenia. *Biological Psychiatry, 17,* 639-654.

Agster, K. L., Mejias-Aponte, C. A., Clark, B. D., & Waterhouse, B. D. (2013). Evidence for a regional specificity in the density and distribution of noradrenergic varicosities in rat cortex. *Journal of Comparative Neurology, 521,* 2195-2207.

Aksenov, M. Y., Aksenova, M. V., Silvers, J. M., Mactutus, C. F., & Booze, R. M. (2008). Different effects of selective dopamine uptake inhibitors, GBR 12909 and WIN 35428, on HIV-1 Tat toxicity in rat fetal midbrain neurons. *Neurotoxicology, 29,* 971-977.

Aksenova, M. V., Silvers, J. M., Aksenov, M. Y., Nath, A., Ray, P. D., Mactutus, C. F. et al. (2006). HIV-1 Tat neurotoxicity in primary cultures of rat midbrain fetal neurons: Changes in dopamine transporter binding and immunoreactivity. *Neuroscience Letters, 395,* 235-239.

Alfahad, T.B., & Nath, A. (2013). Update on HIV-associated neurocognitive disorders. *Current Neurology and Neuroscience Reports, 13,* 387.

An, S. F., Groves, M., Gray, F., & Scaravilli, F. (1999). Early entry and widespread cellular involvement of HIV-1 DNA in brains of HIV-1 positive asymptomatic individuals. *Journal of Neuropathology and Experimental Neurology, 58,* 1156-1162.

Ances, B. M. & Ellis, R. J. (2007). Dementia and neurocognitive disorders due to HIV-1 infection. *Seminars in Neurology, 27,* 86-92.

Antinori, A., Arendt, G., Becker, J.T., Brew, B.J., Byrd, D.A., Cherner, M., et al. (2007). Updated research nosology for HIV-associated neurocognitive disorders. *Neurology, 69,* 1789-1799.

Aston-Jones, G., & Cohen, J.D. (2005). An integrative theory of locus coeruleus-norepinephrine function: adaptive gain and optimal performance. *Annual Review of Neuroscience, 28,* 403-450.

Basso, M.R., & Bornstein, R.A. (2003). Effects of past noninjection drug abuse upon executive function and working memory in HIV infection. *Journal of Clinical and Experimental Neuropsychology, 25,* 893-903.

Berger, J.R., Kumar, M., Kumar, A., Fernandez, J.B., & Levin, B. (1994). Cerebrospinal fluid dopamine in HIV-1 infection. *AIDS, 8,* 67-71.

Berger, J.R., & Nath, A. (1997). HIV dementia and the basal ganglia. *Intervirology, 40,* 122-131.

Bertrand, S.J., Mactutus, C.F., Aksenova, M.V., Espensen-Sturges, T.D., & Booze, R.M. (in press). Synaptodendritic recovery following HIV Tat exposure: Neurorestoration by phytoestrogens. *Journal of Neurochemistry,* Epub ahead of print 2013 Jul 22. doi: 10.1111/jnc.12375.

Birrell, J. M. & Brown, V. J. (2000). Medial frontal cortex mediates perceptual attentional set shifting in the rat. *Journal of Neuroscience, 20,* 4320-4324.

Braff, D., Stone, C., Callaway, E., Geyer, M., Glick, I., & Bali, L. (1978). Prestimulus effects on human startle reflex in normals and schizophrenics. *Psychophysiology, 15,* 339-343.

Bushnell, P.J. (1998). Behavioral approaches to the assessment of attention in animals. *Psychopharmacology, 138,* 231-259.

Campbell, B. A. & Haroutunian, V. (1983). Perceptual sharpening in the developing rat. *Journal of Comparative Psychology, 97,* 3-11.

Carlson, L. E. & Sherwin, B. B. (1998). Steroid hormones, memory and mood in a healthy elderly population. *Psychoneuroendocrinology, 23,* 583-603.

Casini, M.L., Marelli, G., Papaleo, E., Ferrari, A., D'Ambrosio, F., & Unfer, V. (2006). Psychological assessment of the effects of treatment with phytoestrogens on menopausal women: a randomized, double-blind, crossover, placebo-controlled study. *Fertility and Sterility, 85*, 972-978.

Castello, E., Baroni, N., & Pallestrini, E. (1998). Neurotological and auditory brain stem response findings in human immunodeficiency virus-positive patients without neurologic manifestations. *Annals of Otology Rhinology and Laryngology, 107,* 1054-1060.

Cattie, J. E., Doyle, K., Weber, E., Grant, I., & Woods, S. P. (2012). Planning deficits in HIV-associated neurocognitive disorders: Component processes, cognitive correlates, and implications for everyday functioning. *Journal of Clinical and Experimental Neuropsychology, 34,* 906-918.

Chang, L., Ernst, T., Witt, M.D., Ames, N., Gaiefsky, M., & Miller, E. (2002). Relationships among brain metabolites, cognitive function, and viral loads in antiretroviral-naïve HIV patients. *Neuroimage, 17*, 1638-1648.

Chang, L., Speck, O., Miller, E. N., Braun, J., Jovicich, J., Koch, C. et al. (2001). Neural correlates of attention and working memory deficits in HIV patients. Neurology, 57, 1001-1007.

Chang, L., Wang, G. J., Volkow, N. D., Ernst, T., Telang, F., Logan, J. et al. (2008). Decreased brain dopamine transporters are related to cognitive deficits in HIV patients with or without cocaine abuse. *Neuroimage, 42,* 869-878.

Chao, L. L., Lindgren, J. A., Flenniken, D. L., & Weiner, M. W. (2004). ERP evidence of impaired central nervous system function in virally suppressed HIV patients on antiretroviral therapy. *Clinical Neurophysiology, 115,* 1583-1591.

Chen, H. Q., Jin, Z. Y., & Li, G. H. (2007). Biochanin A protects dopaminergic neurons against lipopolysaccharide-induced damage through inhibition of microglia activation and proinflammatory factors generation
1. *Neuroscience Letters, 417,* 112-117.

Chen, J. R., Yan, Y. T., Wang, T. J., Chen, L. J., Wang, Y. J., & Tseng, G. F. (2009). Gonadal Hormones Modulate the Dendritic Spine Densities of Primary Cortical Pyramidal Neurons in Adult Female Rat. *Cerebral Cortex, 19,* 2719-2727.

Chen, S.L., Zhao, H.R., Li, X.M., Zhang, Y.Q., Jin, G.S., Zhao, C.Y., et al. (2009). [Retrospective cohort study on the rate of mother-to-child transmission among mothers infected with human immunodeficiency virus type 1 through blood transfusion]. *Zhonghua Liu Xing Bing Xue Za Zhi, 30*, 564-566. (Chinese).

Chudasama, Y. & Robbins, T. W. (2003). Dissociable contributions of the orbitofrontal and infralimbic cortex to Pavlovian autoshaping and discrimination reversal learning: Further evidence for the functional heterogeneity of the rodent frontal cortex. *Journal of Neuroscience, 23*, 8771-8780.

Chung, R. S., Penkowa, M., Dittmann, J., King, C. E., Bartlett, C., Asmussen, J. W. et al. (2008). Redefining the role of metallothionein within the injured brain - Extracellular metallothioneins play an important role in the astrocyte-neuron response to injury
1. *Journal of Biological Chemistry, 283*, 15349-15358.

Clapham, P. R. & McKnight, A. (2001). HIV-1 receptors and cell tropism. *British Medical Bulletin, 58*, 43-59.

Clarke, H.F., Hill, G.J., Robbins, T.W., Roberts, A.C. (2011). Dopamine, but not serotonin, regulates reversal learning in the marmoset caudate nucleus. *Journal of Neuroscience, 16*, 4290-4297.

Clatworthy, P.L., Lewis, S.J., Brichard, L., Hong, Y.T., Izquierdo, D., Clark, L., et al. (2009). Dopamine release in dissociable striatal subregions predicts the different effects of oral methylphenidate on reversal learning and spatial working memory. *Journal of Neuroscience, 29*, 4690-4696.

Connor, E. M., Sperling, R. S., Gelber, R., Kiselev, P., Scott, G., Osullivan, M. J. et al. (1994). Reduction of Maternal-Infant Transmission of Human-Immunodeficiency-Virus Type-1 with Zidovudine Treatment
3. *New England Journal of Medicine, 331*, 1173-1180.

Crofts, H.S., Dalley, J.W., Collins, P., Van Denderen, J.C., Everitt, B.J., Robbins, T.W., et al. (2001). Differential effects of 6-OHDA lesions of the frontal cortex and caudate nucleus on the ability to acquire an attentional set. *Cerebral Cortex, 11*, 1015-1026.

Cysique, L. A., Maruff, P., & Brew, B. J. (2004). Prevalence and pattern of neuropsychological impairment in human immunodeficiency virus-infected/acquired immunodeficiency syndrome (HIV/AIDS) patients across pre-

and post-highly active antiretroviral therapy eras: A combined study of two cohorts. *Journal of Neurovirology, 10,* 350-357.

Dewhurst, S., Maggirwar, S. B., Schifitto, G., Gendelman, H. E., & Gelbard, H. A. (2007). Glycogen synthase kinase 3 beta (GSK-3 beta) as a therapeutic target in neuroAIDS. *Journal of Neuroimmune Pharmacology, 2,* 93-96.

Dias, R., Robbins, T.W., & Roberts, A.C. (1996). Primate analogue of the Wisconsin Card Sorting Test: effects of excitotoxic lesions of the prefrontal cortex in the marmoset. *Behavioral Neuroscience, 110,* 872-886.

Di Rocco, A., Bottiglieri, T., Dorfman, D., Werner, P., Morrison, C., & Simpson, D. (2000). Decreased homovanilic acid in cerebrospinal fluid correlates with impaired neuropsychologic function in HIV-1-infected patients. *Clinical Neuropharmacology, 23,* 190-194.

Duff, S. J. & Hampson, E. (2000). A beneficial effect of estrogen on working memory in postmenopausal women taking hormone replacement therapy. *Hormones and Behavior, 38,* 262-276.

Duffy, R., Wiseman, H., & File, S. E. (2003). Improved cognitive function in postmenopausal women after 12 weeks of consumption of a soya extract containing isoflavones. *Pharmacology Biochemistry and Behavior, 75,* 721-729.

Eilers, M., Roy, U., & Mondal, D. (2008). MRP (ABCC) transporters-mediated efflux of anti-HIV drugs, saquinavir and zidovudine, from human endothelial cells 1. *Experimental Biology and Medicine, 233,* 1149-1160.

Ellis, R. J., Deutsch, R., Heaton, R. K., Marcotte, T. D., McCutchan, J. A., Nelson, J. A. et al. (1997). Neurocognitive impairment is an independent risk factor for death in HIV infection. *Archives of Neurology, 54,* 416-424.

Ensoli, B., Buonaguro, L., Barillari, G., Fiorelli, V., Gendelman, R., Morgan, R. A. et al. (1993). Release, Uptake, and Effects of Extracellular Human-Immunodeficiency-Virus Type-1 Tat Protein on Cell-Growth and Viral Transactivation. *Journal of Virology, 67,* 277-287.

Everall, I. P., Heaton, R. K., Marcotte, T. D., Ellis, R. J., McCutchan, J. A., Atkinson, J. H. et al. (1999). Cortical synaptic density is reduced in mild to moderate human immunodeficiency virus neurocognitive disorder. *Brain Pathology, 9,* 209-217.

Fein, G., Biggins, C. A., & Mackay, S. (1995). Delayed Latency of the Event-Related Brain Potential P3A Component in Hiv Disease - Progressive Effects with Increasing Cognitive Impairment. *Archives of Neurology, 52,* 1109-1118.

Ferris, M. J., Frederick-Duus, D., Fadel, J., Mactutus, C. F., & Booze, R. M. (2009). The Human Immunodeficiency Virus-1-Associated Protein, Tat(1-86), Impairs Dopamine Transporters and Interacts with Cocaine to Reduce Nerve Terminal Function: A No-Net-Flux Microdialysis Study. *Neuroscience, 159,* 1292-1299.

File, S. E., Hartley, D. E., Elsabagh, S., Duffy, R., & Wiseman, H. (2005). Cognitive improvement after 6 weeks of soy supplements in postmenopausal women is limited to frontal lobe function. *Menopause-the Journal of the North American Menopause Society, 12,* 193-201.

Fitting, S., Booze, R. M., & Mactutus, C. F. (2006a). Neonatal hippocampal Tat injections: developmental effects on prepulse inhibition (PPI) of the auditory startle response. *International Journal of Developmental Neuroscience, 24,* 275-283.

Fitting, S., Booze, R. M., & Mactutus, C. F. (2006b). Neonatal intrahippocampal glycoprotein 120 injection: The role of dopaminergic alterations in prepulse inhibition in adult rats. *Journal of Pharmacology and Experimental Therapeutics, 318,* 1352-1358.

Fitting, S., Xu, R. Q., Bull, C., Buch, S. K., El Hage, N., Nath, A. et al. (2010). Interactive comorbidity between opioid drug abuse and HIV-1 Tat chronic exposure augments spine loss and sublethal dendritic pathology in striatal neurons. *American Journal of Pathology, 177,* 1397-1410.

Folman, Y. & Pope, G. S. (1966). Interaction in immature mouse of potent oestrogens with coumestrol genistein and other utero-vaginotrophic compounds of low potency. *Journal of Endocrinology, 34,* 215-225.

Folman, Y. & Pope, G. S. (1969). Effect of norethisterone acetate, dimethylstilboestrol, genistein and coumestrol on uptake of [3H]Oestradiol by uterus, vagina and skeletal muscle of immature mice. *Journal of Endocrinology, 44,* 213-218.

Fuxe, K., Hamberger, B., Hokfelt, T. (1968). Distribution of noradrenaline nerve terminals in cortical areas of the rat. *Brain Research, 8,* 125-131.

Ganz, L. (1968). An analysis of generalization behavior in the stimulus deprived organism. In Newton, G. (Ed.), *Early experience and behavior: The psychobiology of development* (pp. 365-411). Springfield, IL: Charles C. Thomas.

Garvey, L. J., Yerrakalva, D., & Winston, A. (2009). Correlations between Computerized Battery Testing and a Memory Questionnaire for Identification of Neurocognitive Impairment in HIV Type 1-Infected Subjects on Stable Antiretroviral Therapy. *Aids Research and Human Retroviruses, 25,* 765-769.

Geyer, M. A., Krebs-Thomson, K., Braff, D. L., & Swerdlow, N. R. (2001). Pharmacological studies of prepulse inhibition models of sensorimotor gating deficits in schizophrenia: a decade in review. *Psychopharmacology, 156,* 117-154.

Gibson, E. (1969). *Principles of perceptual learning and development*. New York: Appleton-Century-Crofts.

Gil, R., Breux, J.P., Neau, J.P., & Becq-Giraudon, B. (1992). [Cognitive evoked potentials and HIV infection]. *Neurophysiologie Clinique, 22,* 385-391. (French).

Golob, E. J., Irimajiri, R., & Starr, A. (2007). Auditory cortical activity in amnestic mild cognitive impairment: relationship to subtype and conversion to dementia. *Brain, 130,* 740-752.

Goodwin, G. M., Pretsell, D. O., Chiswick, A., Egan, V., & Brettle, R. P. (1996). The Edinburgh cohort of HIV-positive injecting drug users at 10 years after infection: A case-control study of the evolution of dementia. *Aids, 10,* 431-440.

Gottlieb, G. (1971). Ontogenesis of sensory function in birds and mammals. In Tobach, E. (Ed.), *The Biopsychology of Development.* (pp. 67-128). New York: Academic Press.

Granon, S., Passetti, F., Thomas, K.L., Dalley, J.W., Everitt, B.J., & Robbins, T.W. (2000). Enhanced and impaired attentional performance after infusion of D1 dopaminergic receptor agents into rat prefrontal cortex. *Journal of Neuroscience, 20,* 1208–1215.

Groman, S.M., Lee, B., London, E.D., Mandelkern, M.A., James, A.S., Feiler, K., et al. (2011). Dorsal striatal D2-like receptor availability covaries with sensitivity to positive reinforcement during discrimination learning. *Journal of Neuroscience, 31*, 7291-7299.

Hao, J. D., Rapp, P. R., Leffler, A. E., Leffler, S. R., Janssen, W. G. M., Lou, W. et al. (2006). Estrogen alters spine number and morphology in prefrontal cortex of aged female rhesus monkeys. *Journal of Neuroscience, 26*, 2571-2578.

Hardy, D.J., & Hinkin, C.H. (2002). Reaction time performance in adults with HIV/AIDS. *Journal of Clinical and Experimental Neuropsychology, 24*, 912-929.

Heaton, R. K., Clifford, D. B., Franklin, D. R., Woods, S. P., Ake, C., Vaida, F. et al. (2010). HIV-associated neurocognitive disorders persist in the era of potent antiretroviral therapy CHARTER Study. *Neurology, 75*, 2087-2096.

Heaton, R. K., Franklin, D. R., Ellis, R. J., McCutchan, J. A., Letendre, S. L., LeBlanc, S. et al. (2011). HIV-associated neurocognitive disorders before and during the era of combination antiretroviral therapy: differences in rates, nature, and predictors. *Journal of Neurovirology, 17*, 3-16.

Heaton, R.K., Grant, I., Butters, N., White, D.A., Kirson, D., Atkinson, J.H., et al. The HNRC 500—neuropsychology of HIV infection at different disease stages. HIV Neurobehavioral Research Center. *Journal of the International Neuropsychological Society, 1*, 231-51.

Himmelheber, A.M., Sarter, M., & Bruno, J.P. (2000). Increases in cortical acetylcholine release during sustained attention performance in rat. *Cognitive Brain Research, 9*, 313-325.

Hoffman, H. S. & Ison, J. R. (1980). Reflex Modification in the Domain of Startle .1. Some Empirical-Findings and Their Implications for How the Nervous-System Processes Sensory Input. *Psychological Review, 87*, 175-189.

Hord LL, Booze RM, Mactutus CF. (2008, November). Ontogeny of prepulse inhibition of the acoustic startle response across modality. Abstracts: International Society for Developmental Psychobiology, 41st Annual Meeting, November 12-15, 2008 Washington D.C. *Developmental Psychobiology, 50*, 720-750.

Iudicello, J. E., Woods, S. P., Weber, E., Dawson, M. S., Scott, J. C., Carey, C. L. et al. (2008). Cognitive mechanisms of switching in HIV-associated category fluency deficits. *Journal of Clinical and Experimental Neuropsychology, 30,* 797-804.

Jackson, R. L., Greiwe, J. S., & Schwen, R. J. (2011). Emerging evidence of the health benefits of S-equol, an estrogen receptor beta agonist. *Nutrition Reviews, 69,* 432-448.

Jacome, L. F., Gautreaux, C., Inagaki, T., Mohan, G., Alves, S., Lubbers, L. S. et al. (2010). Estradiol and ER beta agonists enhance recognition memory, and DPN, an ER beta agonist, alters brain monoamines. *Neurobiology of Learning and Memory, 94,* 488-498.

Keeler, J. F. & Robbins, T. W. (2011). Translating cognition from animals to humans. *Biochemical Pharmacology, 81,* 1356-1366.

Keenan, P. A., Ezzat, W. H., Ginsburg, K., & Moore, G. J. (2001). Prefrontal cortex as the site of estrogen's effect on cognition. *Psychoneuroendocrinology, 26,* 577-590.

Kellendonk, C., Simpson, E.H., Polan, H.J., Malleret, G., Vronskaya, S., Winiger, V., et al. (2006). Transient and selective overexpression of dopamine D2 receptors in the striatum causes persistent abnormalities in prefrontal cortex functioning. *Neuron, 49,* 603-615.

Kerr, L. M., Ostapoff, E. M., & Rubel, E. W. (1979). Influence of Acoustic Experience on the Ontogeny of Frequency Generalization Gradients in the Chicken. *Journal of Experimental Psychology-Animal Behavior Processes, 5,* 97-115.

Koralnik, I. J., Beaumanoir, A., Hausler, R., Kohler, A., Safran, A. B., Delacoux, R. et al. (1990). A Controlled-Study of Early Neurologic Abnormalities in Men with Asymptomatic Human-Immunodeficiency-Virus Infection. *New England Journal of Medicine, 323,* 864-870.

Koutsilieri, E., Sopper, S., Scheller, C., ter Meulen, V., & Riederer, P. (2002). Parkinsonism in HIV dementia. *Journal of Neural Transmission, 109,* 767-775.

Kuiper, G. G. J. M., Carlsson, B., Grandien, K., Enmark, E., Haggblad, J., Nilsson, S. et al. (1997). Comparison of the ligand binding specificity and transcript tissue distribution of estrogen receptors alpha and beta. *Endocrinology, 138,* 863-870.

Kumar, A. M., Ownby, R. L., Waldrop-Valverde, D., Fernandez, B., & Kumar, M. (2011). Human immunodeficiency virus infection in the CNS and decreased dopamine availability: relationship with neuropsychological performance. *Journal of Neurovirology, 17,* 26-40.

Lalwani, A. K. & Sooy, C. D. (1992). Otologic and neurotologic manifestations of acquired-immunodeficiency-syndrome. *Otolaryngologic Clinics of North America, 25,* 1183-1197.

Lampe, J. W. (2009). Is equol the key to the efficacy of soy foods? *American Journal of Clinical Nutrition, 89,* S1664-S1667.

Lee, B., Groman, S., London, E.D., Jentsch, J.D. (2007). Dopamine D2/D3 receptors play a specific role in the reversal of a learned visual discrimination in monkeys. *Neuropsychopharmacology, 32,* 2125-2134.

Lu, S. M., Tremblay, M. E., King, I. L., Qi, J., Reynolds, H. M., Marker, D. F. et al. (2011). HIV-1 Tat-induced microgliosis and synaptic damage via interactions between peripheral and central myeloid cells. *Plos One, 6,* e23915. doi: 10.1371/journal.pone.0023915

Luine, V., Attalla, S., Mohan, G., Costa, A., & Frankfurt, M. (2006). Dietary phytoestrogens enhance spatial memory and spine density in the hippocampus and prefrontal cortex of ovariectomized rats. *Brain Research, 1126,* 183-187.

Maki, P. M., Cohen, M. H., Weber, K., Little, D. M., Fornelli, D., Rubin, L. H. et al. (2009). Impairments in memory and hippocampal function in HIV-positive vs HIV-negative women. *Neurology, 72,* 1661-1668.

Manes, F., Sahakian, B., Clark, L., Rogers, R., Antoun, N., Aitken, M., et al. (2002). Decision-making processes following damage to the prefrontal cortex. *Brain, 125,* 624-639.

Masliah, E., Heaton, R. K., Marcotte, T. D., Ellis, R. J., Wiley, C. A., Mallory, M. et al. (1997). Dendritic injury is a pathological substrate for human immunodeficiency virus-related cognitive disorders. *Annals of Neurology, 42,* 963-972.

Matas, C.G., Silva, S.M., Marcon, Bde A., & Goncalves, I.C. (2010). Electrophysiological manifestations in adults with HIV/AIDS submitted and not submitted to antiretroviral therapy. *Pro Fono: revista de atualizacao cientifica, 22,* 107-113.

McAlonan, K., & Brown, V.J. (2003). Orbital prefrontal cortex mediates reversal learning and not attentional set shifting in the rat. *Behavioural Brain Research, 146,* 97–103.

McArthur, J.C., Hoover, D.R., Bacellar, H., Miller, E.N., Cohen, B.A., Becker, J.T., et al. Dementia in AIDS patients: incidence and risk factors. Multicenter AIDS Cohort Study. *Neurology, 43,* 2245-2252.

McCarthy, M., Vidaurre, I., & Geffin, R. (2006). Maturing neurons are selectively sensitive to human immunodeficiency virus type 1 exposure in differentiating human neuroepithelial progenitor cell cultures. *Journal of Neurovirology, 12,* 333-348.

McGaughy, J., Kaiser, T., & Sarter, M. (1996). Behavioral vigilance following infusions of 192 IgG-saporin into the basal forebrain: selectivity of the behavioral impairment and relation to cortical AChE-positive fiber density. *Behavioral Neuroscience, 110,* 247-265.

McGaughy, J., Ross, R.S., & Eichenbaum, H. (2008). Noradrenergic, but not cholinergic, deafferentation of prefrontal cortex impairs attentional set-shifting. *Neuroscience, 153,* 63-71.

McGaughy, J., & Sarter, M. (1995). Behavioral vigilance in rats: task validation and effects of age, amphetamine, and benzodiazepine receptor ligands. *Psychopharmacology, 117,* 340-357.

McLean, S.L., Beck, J.P., Woolley, M.L., Neill, J.C. (2008). A preliminary investigation into the effects of antipsychotics on sub-chronic phencyclidine-induced deficits in attentional set-shifting in female rats. *Behavioural Brain Research, 189,* 152-158.

Mega, M.S., & Cummings, J.L. (1994). Frontal-subcortical circuits and neuropsychiatric disorders. *The Journal of Neuropsychiatry and Clinical Neurosciences, 6,* 358-370.

Melrose, R. J., Tinaz, S., Castelo, J. M. B., Courtney, M. G., & Stern, C. E. (2008). Compromised fronto-striatal functioning in HIV: An fMRI investigation of semantic event sequencing. *Behavioural Brain Research, 188,* 337-347.

Mendez, P., Garcia-Segura, L. M., & Muller, D. (2011). Estradiol promotes spine growth and synapse formation without affecting pre-established networks. *Hippocampus, 21,* 1263-1267.

Midde, N. M., Huang, X., Gomez, A. M., Booze, R. M., Zhan, C. G., & Zhu, J. (2013). Mutation of Tyrosine 470 of Human Dopamine Transporter is Critical for HIV-1 Tat-Induced Inhibition of Dopamine Transport and Transporter Conformational Transitions. *J.Neuroimmune Pharmacol., 8,* 975-987.

Milstein, J.A., Lehmann, O., Theobald, D.E., Dalley, J.W., & Robbins, T.W. (2007). Selective depletion of cortical noradrenaline by anti-dopamine beta-hydroxylase-saporin impairs attentional function and enhances the effects of guanfacine in the rat. *Psychopharmacology (Berl), 190*, 51–63.

Minassian, A., Henry, B. L., Woods, S. P., Vaida, F., Grant, I., Geyer, M. A. et al. (2013). Prepulse Inhibition in HIV-Associated Neurocognitive Disorders. *Journal of the International Neuropsychological Society, 19,* 709-717.

Monteiro, S. C., de Mattos, C. B., Ben, J., Netto, C. A., & Wyse, A. T. S. (2008). Ovariectomy impairs spatial memory: prevention and reversal by a soy isoflavone diet. *Metabolic Brain Disease, 23*, 243-253.

Moore, H., Fadel, J., Sarter, M., & Bruno, J.P. (1999). Role of accumbens and cortical dopamine receptors in the regulation of cortical acetylcholine release. *Neuroscience, 88*, 811-822.

Moran, L.M., Mactutus, C.F., & Booze, R.M. (2009, June). Generality of disruption of prepulse inhibition by the dopamine agonist apomorphine. Presentation at The College on Problems of Drug Dependence (CPDD), 71st Annual Meeting, Reno/Sparks, NV; CPDD Abstracts Program#25, 2009, p.54.

Moran, L. M., Booze, R. M., Webb, K. M., & Mactutus, C. F. (2013). Neurobehavioral alterations in HIV-1 transgenic rats: Evidence for dopaminergic dysfunction. *Experimental Neurology, 239,* 139-147.

Mozes, M. M., Bryant, J. L., Franks, R., Chan, C. C., & Kopp, J. B. (2002). Congenital nuclear cataracts and uveitis in HIV-transgenic mice. *Eye, 16,* 177-184.

Nath, A., Conant, K., Chen, P. Q., Scott, C., & Major, E. O. (1999). Transient exposure to HIV-1 Tat protein results in cytokine production in macrophages and astrocytes - A hit and run phenomenon. *Journal of Biological Chemistry, 274,* 17098-17102.

Neese, S. L., Bandara, S. B., Doerge, D. R., Helferich, W. G., Komi, D. L., & Schantz, S. L. (2012). Effects of multiple daily genistein treatments on delayed alternation and a differential reinforcement of low rates of responding task in middle-aged rats. *Neurotoxicology and Teratology, 34,* 187-195.

Neese, S.L., Wang, V.C., Doerge, D.R., Woodling, K.A., Andrade, J.E., Helferich, W.G., et al. (2010). Impact of dietary genistein and aging on executive function in rats. *Neurotoxicology and Teratology, 32,* 200-211.

Nomenclature and research case definitions for neurological manifestations of human immunodeficiency virus type-1 (HIV-1) infection. Report of a Working Group of the American Academy of Neurology AIDS Task Force. *Neurology, 41,* 778-785.

Ollo, C., Johnson, R., & Grafman, J. (1991). Signs of cognitive change in HIV disease - An event-related brain potential study. *Neurology, 41,* 209-215.

O'Neill, M., & Brown, V.J. (2007). The effect of striatal dopamine depletion and the adenosine A2A antagonist KW-6002 on reversal learning in rats. *Neurobiology of Learning and Memory, 88,* 75-81.

Pagano, M. A., Cahn, P. E., Garau, M. L., Mangone, C. A., Figini, H. A., Yorio, A. A. et al. (1992). Brainstem auditory evoked-potentials in human immunodeficiency virus-seropositive patients with and without acquired- immunodeficiency-syndrome. *Archives of Neurology, 49,* 166-169.

Pan, Y. L., Anthony, M., Watson, S., & Clarkson, T. B. (2000). Soy phytoestrogens improve radial arm maze performance in ovariectomized retired breeder rats and do not attenuate benefits of 17 beta-estradiol treatment
1. *Menopause-the Journal of the North American Menopause Society, 7,* 230-235.

Parasuraman, R., Davies, D.R. (1977). A taxonomic analysis of vigilance performance. In Mackie, R.R. (Ed.), *Vigilance: Theory, operational performance, and physiological correlates* (pp. 559-574). Plenum, NY: Springer.

Peng, J. S., Vigorito, M., Liu, X. Q., Zhou, D. J., Wu, X. W., & Chang, S. L. (2010). The HIV-1 transgenic rat as a model for HIV-1 infected individuals on HAART. *Journal of Neuroimmunology, 218,* 94-101.

Pfefferbaum, A., Rosenbloom, M.J., Rohlfing, T., Kemper, C.A., Deresinski, S., & Sullivan, E.V. (2009). Frontostriatal fiber bundle compromise in HIV infection without dementia. *AIDS, 23,* 1977-1985.

Phipps, A. J., Hayes, K. A., Buck, W. R., Podell, M., & Mathes, L. E. (2000). Neurophysiologic and immunologic abnormalities associated with feline immunodeficiency virus molecular clone FIV-PPR DNA inoculation. *Journal of Acquired Immune Deficiency Syndromes, 23,* 8-16.

Prospero-Garcia, O., Huitron-Resendiz, S., Casalman, S. C., Sanchez-Alavez, M., Diaz-Ruiz, O., Navarro, L. et al. (1999). Feline immunodeficiency virus envelope protein (FIVgp120) causes electrophysiological alterations in rats. *Brain Research, 836,* 203-209.

Purohit, V., Rapaka, R., & Shurtleff, D. (2011). Drugs of Abuse, Dopamine, and HIV-Associated Neurocognitive Disorders/HIV-Associated Dementia. *Molecular Neurobiology, 44,* 102-110.

Raymond, L. A., Wallace, D., Berman, N. E. J., Marcario, J., Foresman, L., Joag, S. V. et al. (1998). Auditory brainstem responses in a Rhesus Macaque model of neuro-AIDS. *Journal of Neurovirology, 4,* 512-520.

Reger, M., Welsh, R., Razani, J., Martin, D. J., & Boone, K. B. (2002). A meta-analysis of the neuropsychological sequelae of HIV infection. *Journal of the International Neuropsychological Society, 8,* 410-424.

Riazi, M., Marcario, J. K., Samson, F. K., Kenjale, H., Adany, I., Staggs, V. et al. (2009). Rhesus Macaque Model of Chronic Opiate Dependence and Neuro-AIDS: Longitudinal Assessment of Auditory Brainstem Responses and Visual Evoked Potentials. *Journal of Neuroimmune Pharmacology, 4,* 260-275.

Ridley, R.M., Haystead, T.A., & Baker, H.F. (1981). An analysis of visual object reversal learning in the marmoset after amphetamine and haloperidol. *Pharmacology, Biochemistry, and Behavior, 14,* 345-351.

Rodefer, J.S., Nguyen, T.N., Karlsson, J.J., & Arnt, J. (2008). Reversal of subchronic PCP-induced deficits in attentional set shifting in rats by sertindole and a 5-HT6 receptor antagonist: comparison among antipsychotics. *Neuropsychopharmacology, 33,* 2657-66.

Rowland, I. R., Wiseman, H., Sanders, T. A. B., Adlercreutz, H., & Bowey, E. A. (2000). Interindividual variation in metabolism of soy isoflavones and lignans: Influence of habitual diet on equol production by the gut microflora. *Nutrition and Cancer-An International Journal, 36,* 27-32.

Royal, W., Zhang, L., Guo, M., Jones, O., Davis, H., & Bryant, J. L. (2012). Immune activation, viral gene product expression and neurotoxicity in the HIV-1 transgenic rat. *Journal of Neuroimmunology, 247,* 16-24.

Rubel, E. W. & Rosenthal, M. H. (1975). Ontogeny of Auditory Frequency Generalization in Chicken. *Journal of Experimental Psychology-Animal Behavior Processes, 1,* 287-297.

Sarkaki, A., Amani, R., Badavi, M., Maghaddam, A.Z., Aligholi, H., Safahani, M. et al. (2008). Pre-treatment effect of different doses of soy isoflavones on spatial learning and memory in an ovariectomized animal model of Alzheimer's disease. *Pakistan Journal of Biological Science, 15,* 1114-1119.

Schroeder, M. M., Handelsman, L., Torres, L., Dorfman, D., Rinaldi, P., Jacobson, J. et al. (1994). Early and late cognitive event-related potentials mark stages of HIV-1 infection in the drug-user risk group. *Biological Psychiatry, 35,* 54-69.

Schroeder, M. M., Handelsman, L., Torres, L., Jacobson, J., & Ritter, W. (1996). Consistency of repeated event-related potentials in clinically stable HIV-infected drug users. *Journal of Neuropsychiatry and Clinical Neurosciences, 8,* 305-310.

Scott, J. C., Woods, S. P., Vigil, O., Heaton, R. K., Grant, I., Ellis, R. J. et al. (2011). Script Generation of Activities of Daily Living in HIV-Associated Neurocognitive Disorders. *Journal of the International Neuropsychological Society, 17,* 740-745.

Setchell, K. D. R., Borriello, S. P., Hulme, P., Kirk, D. N., & Axelson, M. (1984). Nonsteroidal Estrogens of Dietary Origin - Possible Roles in Hormone-Dependent Disease. *American Journal of Clinical Nutrition, 40,* 569-578.

Setchell, K. D. R., Brown, N. M., & Lydeking-Olsen, E. (2002). The clinical importance of the metabolite equol - A clue to the effectiveness of soy and its isoflavones. *Journal of Nutrition, 132,* 3577-3584.

Setchell, K. D. R., Clerici, C., Lephart, E. D., Cole, S. J., Heenan, C., Castellani, D. et al. (2005). S-Equol, a potent ligand for estrogen receptor beta, is the exclusive enantiomeric form of the soy isoflavone metabolite produced by human intestinal bacterial floral. *American Journal of Clinical Nutrition, 81,* 1072-1079.

Setchell, K. D. R. & Cole, S. J. (2006). Method of defining equol-producer status and its frequency among vegetarians. *Journal of Nutrition, 136,* 2188-2193.

Silvers, J. M., Aksenova, M. V., Aksenov, M. Y., Mactutus, C. F., & Booze, R. M. (2007). Neurotoxicity of HIV-1 tat protein: Involvement of D1 dopamine receptor. *Neurotoxicology, 28,* 1184-1190.

Sinclair, E., Gray, F., Ciardi, A., & Scaravilli, F. (1994). Immunohistochemical changes and PCR detection of HIV provirus DNA in brains of asymptomatic HIV-positive patients. *Journal of Neuropathology and Experimental Neurology, 53,* 43-50.

Smith, Y. R., Giordani, B., Lajiness-O'Neill, R., & Zubieta, J. K. (2001). Long-term estrogen replacement is associated with improved nonverbal memory and attentional measures in postmenopausal women. *Fertility and Sterility, 76,* 1101-1107.

Spreen, O., & Strauss, E. (1998). *A compendium of neuropsychological tests: Administration, norms and commentary*. (2 ed.) New York: Oxford University Press.

Taha, T. E., Kumwenda, J., Cole, S. R., Hoover, D. R., Kafulafula, G., Fowler, M. G. et al. (2009). Postnatal HIV-1 Transmission after Cessation of Infant Extended Antiretroviral Prophylaxis and Effect of Maternal Highly Active Antiretroviral Therapy. *Journal of Infectious Diseases, 200,* 1490-1497.

Tait, D.S., Brown, V.J., Farovik, A., Theobald, D.E., Dalley, J.W., & Robbins, T.W. (2007). Lesions of the dorsal noradrenergic bundle impair attentional set-shifting in the rat. *European Journal of Neuroscience, 25*, 3719-3724.

Tait, D.S., Marston, H.M., Shahid, M., & Brown, V.J. (2009). Asenapine restores cognitive flexibility in rats with medial prefrontal cortex lesions. *Psychopharmacology, 202,* 295-306.

Tardieu, M., Hery, C., Peudenier, S., Boespflug, O., & Montagnier, L. (1992). Human-immunodeficiency-virus type-1-infected monocytic cells can destroy human neural cells after cell-to-cell adhesion. *Annals of Neurology, 32,* 11-17.

Tees, R.C. (1976). Perceptual development in mammals. In Gottlieb, G. (Ed.), *Studies on the development of behavior and the nervous system* (pp. 281-326). New York: Academic Press.

Thomas, S. A. (2004). Anti-HIV drug distribution to the central nervous system. *Current Pharmaceutical Design, 10,* 1313-1324.

Thompson, P. M., Dutton, R. A., Hayashi, K. M., Toga, A. W., Lopez, O. L., Aizenstein, H. J. et al. (2005). Thinning of the cerebral cortex visualized in HIV/AIDS reflects CD4(+) T lymphocyte decline. *Proceedings of the National Academy of Sciences of the United States of America, 102,* 15647-15652.

Townsend, C. L., Cortina-Borja, M., Peckham, C. S., de Ruiter, A., Lyall, H., & Tookey, P. A. (2008). Low rates of mother-to-child transmission of HIV following effective pregnancy interventions in the United Kingdom and Ireland, 2000-2006. *Aids, 22,* 973-981.

Tunbridge, E.M., Bannerman, D.M., Sharp, T., & Harrison, P.J. (2004). Catechol-o-methyltransferase inhibition improves set-shifting performance and elevates stimulated dopamine release in the rat prefrontal cortex. *Journal of Neuroscience, 24,* 5331-5335.

Tozzi, V., Narciso, P., Galgani, S., Sette, P., Balestra, P., Gerace, C., et al. (1993). Effects of zidovudine in 30 patients with mild to end-stage AIDS dementia complex. *AIDS, 7,* 683-692.

Turner, J. V., Agatonovic-Kustrin, S., & Glass, B. D. (2007). Molecular aspects of phytoestrogen selective binding at estrogen receptors. *Journal of Pharmaceutical Sciences, 96,* 1879-1885.

UNAIDS (2011). UNAIDS World AIDS Day Report 2011. UNAIDS [On-line]. Available: http://www.unaids.org/en/media/unaids/contentassets/documents/unaidspublication/2011/JC2216_WorldAIDSday_report_2011_en.pdf

Vigliano, P., Boffi, P., Bonassi, E., Gandione, M., Marotta, C., Raino, E. et al. (2000). Neurophysiologic exploration: A reliable tool in HIV-1 encephalopathy diagnosis in children. *Panminerva Medica, 42,* 267-272.

Vivithanaporn, P., Heo, G., Gamble, J., Krentz, H. B., Hoke, A., Gill, M. J. et al. (2010). Neurologic disease burden in treated HIV/AIDS predicts survival A population-based study. *Neurology, 75,* 1150-1158.

Volmink, J., & Marais, B. (2008, Feb 5). HIV: mother-to-child transmission. *Clinical Evidence (Online),* Pii: 0909. PMID: 19450331.

Wallace, D. R., Dodson, S., Nath, A., & Booze, R. M. (2006). Estrogen attenuates gp120- and tat(1-72)-induced oxidative stress and prevents loss of dopamine transporter function. *Synapse, 59,* 51-60.

Wang, G. J., Chang, L., Volkow, N. D., Telang, F., Logan, J., Ernst, T. et al. (2004). Decreased brain dopaminergic transporters in HIV-associated dementia patients. *Brain, 127,* 2452-2458.

Wang, M., Ramos, B. P., Paspalas, C. D., Shu, Y. S., Simen, A., Duque, A. et al. (2007). alpha 2A-adrenoceptors strengthen working memory networks by inhibiting cAMP-HCN channel signaling in prefrontal cortex. *Cell, 129,* 397-410.

Wang, X. J., Chen, S. D., Ma, G. Z., Ye, M., & Lu, G. Q. (2005). Genistein protects dopaminergic neurons by inhibiting microglial activation. *Neuroreport, 16,* 267-270.

Warszawski, J., Tubiana, R., Le Chenadec, J., Blanche, S., Teglas, J. P., Dollfus, C. et al. (2008). Mother-to-child HIV transmission despite antiretroviral therapy in the ANRS french perinatal cohort. *AIDS, 22,* 289-299.

Watkins, J.M., Cool, V.A., Usner, D., Stehbens, J.A., Nichols, S., Loveland, K.A., et al. (2000). Attention in HIV-infected children: results from the Hemophilia Growth and Development Study. *Journal of the International Neuropsychological Society, 6,* 443-454.

Werner, H. (1948). *Comparative psychology of mental development*. Chicago: Follett.

Wiley, C. A., Masliah, E., Morey, M., Lemere, C., Deteresa, R., Grafe, M. et al. (1991). Neocortical damage during HIV-infection. *Annals of Neurology, 29,* 651-657.

Zhu, J., Mactutus, C. F., Wallace, D. R., & Booze, R. M. (2009). HIV-1 Tat Protein-induced rapid and reversible decrease in [H-3]dopamine uptake: dissociation of [H-3]dopamine uptake and [H-3]2 beta-carbomethoxy-3-beta -(4-fluorophenyl) tropane (WIN 35,428) binding in rat striatal synaptosomes. *Journal of Pharmacology and Experimental Therapeutics, 329,* 1071-1083.

Zhu, J., Ananthan, S., Mactutus, C. F., & Booze, R. M. (2011). Recombinant human immunodeficiency virus-1 transactivator of transcription(1-86) allosterically modulates dopamine transporter activity. *Synapse, 65,* 1251-1254.

Zink, M. C., Uhrlaub, J., DeWitt, J., Voelker, T., Bullock, B., Mankowski, J. et al. (2005). Neuroprotective and anti-human immunodeficiency virus, activity of minocycline. *Jama-Journal of the American Medical Association, 293,* 2003-2011.