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Review

# The HIV-1 transgenic rat model of neuroHIV

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ABSTRACT

Despite the ability of current combination anti-retroviral therapy (cART) to limit the progression of HIV-1 to AIDS, HIV-positive individuals continue to experience neuroHIV in the form of HIV-associated neurological disorders (HAND), which can range from subtle to substantial neurocognitive impairment. NeuroHIV may also influence substance use, abuse, and dependence in HIV-positive individuals. Because of the nature of the virus, variables such as mental health co-morbidities make it difficult to study the interaction between HIV and substance abuse in human populations. Several rodent models have been developed in an attempt to study the transmission and pathogenesis of the HIV-1 virus. The HIV-1 transgenic (HIV-1Tg) rat is a reliable model of neuroHIV because it mimics the condition of HIV-infected patients on cART. Research using this model supports the hypothesis that the presence of HIV-1 viral proteins in the central nervous system increases the sensitivity and susceptibility of HIV-positive individuals to substance abuse.

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## 1. Introduction

The human immunodeficiency virus (HIV) is a lentivirus (a member of the retrovirus family) that replicates by infecting and destroying primarily CD4+ T cells, a subset of lymphocytes that are essential for the normal function of the human immune system. As viral replication increases and the number of CD4+ T cells declines, progressive immune suppression occurs, resulting in extreme vulnerability to disease and opportunistic infections, such as pneumocystis pneumonia and toxoplasmic encephalitis, conditions rarely seen in people with healthy immune systems. The end stage of HIV viral progression is acquired immune deficiency syndrome (AIDS), identified when an individual's CD4+ cell count falls below 200, or when complicating infections occur (Health, 2014).

Anti-retroviral drugs have been developed to prevent viral replication, thereby stopping the virus from actively taking over the host's immune cells. Highly active anti-retroviral therapy (HAART) is the most commonly used form of HIV-1 treatment. This combination of drugs mitigates HIV-associated disease progression towards AIDS by reducing viral replication, resulting in a persistent, low-level infection. Advances in HAART have led to the current standard of care, more commonly referred to as combination anti-retroviral therapy (cART). While this treatment has successfully reduced the prevalence of AIDS, and HIV infection is now primarily managed as a chronic disease (Fauci and Folkers, 2012), a new spectrum of problems has emerged as a result of the prolonged presence of HIV-viral proteins in the central nervous system (CNS).

Before the progression to AIDS, HIV-positive individuals can experience neuroHIV, a collection of diseases, including neuropathy and dementia, that impacts the nervous system. The clinical manifestations of neuroHIV appear in the form of HIV-associated neurological disorders (HAND), which range from subtle neurocognitive impairment in asymptomatic HIV-seropositive patients, to more substantial neurocognitive impairment in patients treated chronically (Schouten et al., 2011). NeuroHIV may also directly and indirectly influence substance use, abuse, and dependence (Chang and Connaghan, 2014), behaviors that are closely intertwined with HIV infection. The spread of HIV is driven partly by injection of addictive drugs and by engaging in risky sexual behavior as a result of being under the influence of those drugs (Strathdee and Hallett, 2010). However, substance use plays more than a determining role in the acquisition and spread of HIV infection. Exacerbated neurodegeneration and neurocognitive symptoms are seen in chronic relapsing substance-abusing HIV patients compared to non-substance-abusing HIV-infected patients (Nath et al., 2002; Byrd et al., 2012). The interactive effects of substance abuse and HIV infection occur because both substances of abuse and HIV-1 viral proteins target common neural systems, such as the dopaminergic pathway (Theodore et al., 2007). As a result, it has been hypothesized that HIV-1 alters responsivity to psychoactive drugs

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and increases vulnerability to substance abuse (Chang and Vigorito, 2006; Chang and Connaghan, 2014). Understanding the interaction between HIV and substance abuse is made even more complicated by mental health co-morbidities.

### 1.1. HIV-associated neurocognitive disorder (HAND)

The brain is one of the first targets of HIV-1; thus, clinical assessment of neurocognitive function is recommended for all HIV-1 positive patients regardless of symptoms or patient risk factors for HAND (Mind Exchange Working, 2013). A presumptive clinical diagnosis of HAND is indicated when there is evidence of an acquired difficulty with everyday functioning as determined by questionnaires and other screening tools (Vance et al., 2014).

Using the current nosology of HAND (Antinori et al., 2007), a decline in everyday functioning is diagnosed as HIV-associated mild neurocognitive disorder (MND). The incidence of MND has increased and the prevalence has remained stable despite the introduction of cART (Cysique et al., 2004). In contrast, the incidence of HIV-associated dementia (HAD), a diagnosis applied to patients with severe limitations in day-to-day functioning, declined with the advent of cART (Mocroft et al., 2000). HAD is an end-stage complication of HIV that was seen in the pre-cART era due primarily to opportunistic infection rather than to direct HIV-induced CNS neuroinflammation and toxicity.

Performance-based neurocognitive assessment of HIV patients on cART revealed an increasing incidence of asymptomatic neurocognitive impairment (ANI), defined as mild neurocognitive impairment in the absence of limitations in everyday functioning (Robertson et al., 2007). Although there are no widely agreed upon clinical measures of daily functioning (Marcotte, 2009), ANI appears to put the individual at risk for subsequent deficits in day-to-day functioning (Doyle et al., 2013).

Despite extensive clinical work and research on neuroHIV, the pathogenesis of HAND is still not well understood. Studies rooted in cognitive psychology and cognitive neuroscience as well as behavioral and neural plasticity are a promising approach to test hypotheses regarding the neural mechanisms underlying HAND (Woods et al., 2009).

### 1.2. NeuroHIV, substance abuse, and alterations in behavior

The acquisition and maintenance of learned behavior depends on cognitive processes, such as attending, manipulating, and remembering information relevant to a particular task, and adjusting to the consequences of actions. However, some learned behaviors may transition away from reliance on cognitive processes into an automatic habitual performance that becomes difficult to undo, marking the loss of behavioral plasticity (Balleine and O'Doherty, 2010). Because of these connections, behavioral studies can be useful in gaining insight into less explicit neurological changes.

Another complicating factor associated with HIV-1 infection is the high prevalence of substance use and abuse among HIV-1 infected individuals (Chander et al., 2006). In addition to learning, age, and natural environmental events such as the effects of repeated exposure to stressors, substance abuse also often results in neurological and behavioral changes. There is a growing consensus among researchers that substance-use disorders are acquired brain disorders which alter the individual's response to motivationally relevant stimuli on both neurological and behavioral levels (Kalivas and O'Brien, 2008; Lewis, 2011; Torregrossa et al., 2011). Repeated exposure to psychoactive substances can result in a substance-induced maladaptive change in behavior that is mediated by underlying disordered brain processes in areas of the brain that are responsible for reward and decision making (Robinson and Berridge, 2000; Koob, 2006; Russo et al., 2010).

As a chronic acquired brain disorder, substance-induced behavioral plasticity shares characteristics with other acquired brain injuries. For example, HIV-1, whether treated with cART or left untreated, often results in some form of HAND. Currently, substance use among HIV positive individuals is treated as a co-morbidity that confounds research on HAND and impacts negatively on HIV treatment outcomes. A broader theoretical approach that addresses both HAND and HIV-associated substance use/abuse as an alteration in behavioral and neural plasticity may better inform and enhance neuroHIV research.

Research also supports that HIV-1 infection may directly or indirectly affect substance use and abuse by altering brain function (Chang and Connaghan, 2012). Interestingly, HIV-induced neuroinflammation has been shown to impact similar brain regions causing neuronal cell death and altered neurotransmission (Li et al., 2013; Chang and Connaghan, 2014). To date, very few studies have evaluated the possible causal role of HIV-1-induced neuroinflammation on substance use and abuse (Chang and Vigorito, 2006).

## 2. Animal models of HIV-1

Animal models of HIV-1 can play a major role in the investigation of the pathogenesis of neuroHIV and the complex interactions between HIV-1 infection and substances of abuse. The human immunodeficiency viruses type 1 (HIV-1) and type 2 (HIV-2) resulted from a cross-species (zoonotic) infection, with origins in a small number of non-human primates (Gao et al., 1999). HIV infection and replication is species-specific and only occurs in humans and chimpanzees; the progression of HIV to AIDS only occurs in humans. To better understand the transmission and progression of the HIV virus, researchers recognized that an animal model of HIV-induced pathology was necessary. Thus, the problem of species tropism needed to be circumvented, and several approaches were implemented. We review briefly some of these approaches before expanding upon the HIV-1 transgenic rat model.

### 2.1. Naturally occurring species-specific lentiviruses

One approach was to find similar lentiviruses specific to other species that also cause profound immunodeficiencies as a result of affinity for CD4+ T cells and macrophages, e.g., simian immunodeficiency virus in monkeys, bovine immunodeficiency virus in cows, and feline immunodeficiency virus in cats (Olmsted et al., 1989; Matthew et al., 1994; Schmitz et al., 1999). Despite the similarities to HIV-1, these viruses have species-specific differences in their gene products and their impact on the pathogenesis of disease in their unique hosts. Unfortunately, it was also costly to work with these large animals. To better evaluate the clinical manifestations associated with HIV-1 infection, murine models were developed and have proven useful in providing future directions for understanding viral determinants of pathogenicity (VandeWoude and Apetrei, 2006).

### 2.2. Expression of human HIV-1 receptors in rodent cells

A second approach was to genetically engineer rodents so that their cells express the human version of the CD4 receptor and the chemokine co-receptors to which HIV-1 binds to enter target cells (Nischang et al., 2012). The envelope glycoprotein 120 (gp120) on the surface of the HIV-1 virus interacts with target cell receptors to begin the cell entry process. Transgenic mice that expressed the CD4 receptor and a co-receptor were successfully produced. However, HIV-1 gp120 did not successfully bind to CD4-expressing T cells and, therefore, the target cells were not successfully infected (van Maanen and Sutton, 2003).

### 2.3. Production of infectious chimeric viruses

The problem of viral entry restriction was overcome by making changes in the HIV-1 virus rather than in the murine host cell. By replacing the gp120 coding region of the HIV-1 virus with the gp80 coding region of the murine leukemia virus, the newly created chimeric HIV-1 clone could infect conventional mice cells, but not human cells.

Several chimeric HIV-1 clones were developed to investigate HIV-1 infection and to test anti-retroviral drugs (Potash et al., 2005; Hadas et al., 2007), but these models failed to produce the kind of neuroHIV disease progression seen in humans.

### 2.4. Infection and immune responses in humanized mice

Humanized mouse models first became possible with the development of severe combined immunodeficiency (scid) mice. Lacking T and B lymphocytes, scid mice are unable to activate a full adaptive immune response. These early scid mice were 'humanized' with either human hematopoietic stem cell transplants to form human blood and immune cells, or with tissues from human fetal liver and thymus (Zhang and Su, 2012). Early models of HIV-1 infection in humanized mice had several limitations in reconstituting human cells with immune function in the animal host, but many significant advances were made. All of the immune cells required for a human adaptive immune response in HIV-1 pathogenesis can now be reconstituted in humanized mice (Zhang and Su, 2012) such that HIV-1 can now establish an infection in these models that results in CD4+ T cell depletion, a generalized immune activation, and immunopathology that mimics HIV-1 infection in humans (Denton and Garcia, 2011; Poluektova and Makarov, 2014).

Humanized mouse models are currently used in many lines of investigation, including the mechanisms of immune responses, mucosal transmission and prevention, immune activation and pathogenesis, and anti-viral drug development. However, there continues to be some sub-optimal human-like immune responses in these humanized mouse models that are being addressed for further development (Akkina, 2013).

### 2.5. HIV-1 transgenic (HIV-1Tg) rodent models

A different strategy was used to bypass the infectious step altogether and to incorporate the HIV-1 genome directly into a murine model so that it was expressed in many tissues. By excluding genes essential for the production of virions, this type of model would be non-infectious and would not require expensive high-level biosafety laboratories. This model is not useful to study the initial infection stage of HIV or the progression towards AIDs, but is suitable for elucidating the mechanisms underlying HIV-associated disease progression caused by the accumulation of viral gene products.

The HIV-1 transgenic (HIV-1Tg) model was developed from an infectious clone of an integrated proviral plasmid (pNLS-3). An overlapping fragment containing 2 of the 9 viral genes, the *gag* gene at the 3′ region and the *pol* gene at the 5′ region, was deleted, resulting in a non-infectious provirus (pEVd1443). The first animal that was created from the non-infectious HIV-1 provirus was the transgenic mouse. Although the HIV-1 transgenic mouse expresses the transgene, the distribution of the transgene is atypical and HIV-associated clinical manifestations are limited primarily to the skin (Kopp et al., 1993).

HIV-1 transgenic mice also show inefficient *tat* transactivation (Wei et al., 1998; Reid et al., 2001). The HIV-1 gene, *tat*, regulates HIV gene expression by producing the Tat protein, which controls the elongation mechanism of the transcription process and, therefore, ensures high levels of HIV viral protein production once transcription is activated. It appears that, in these transgenic mice, Tat does not effectively interact with its viral RNA target and, therefore, fails to effectively regulate transcription (Wei et al., 1998; Reid et al., 2001).

A much more successful HIV-1 transgenic rodent model was created using rats. To create this model, the non-infectious provirus was microinjected into a fertilized egg derived from a Fisher/NHsd (F344) rat and a Sprague Dawley rat to produce a female transgene founder. The transgene was incorporated into only one copy of the two alleles of an HIV-1 transgenic animal (they are hemizygous). Therefore, when mated with a wild type inbred F344 rat, the offspring are either HIV-1 transgenic (HIV-1Tg) or wild type (Tg-wild type). The HIV-1 transgene was incorporated into all cells (20–25 copies) of the HIV-1Tg rat, which results in an overtly identifiable phenotype by the presence of opaque cataracts (Reid et al., 2001). Tg-wild type littermates (commercially available from Harlan, Inc., Indianapolis, IN) are typically used in control comparison groups, but standard F344 rats can also be used as non-Tg wild type controls.

The HIV-1 transgene expresses a non-replicative provirus under its own viral promoter (i.e., a DNA sequence that activates transcription). As a result, the provirus encodes for only one (*env*) of the three genes (*gag*, *pol*, and *env*) needed to produce viral particles, plus all of the regulatory (*tat*, *rev*) and supplementary (*vif*, *vpr*, *vpu*, and *nef*) genes (Fig. 1).

All models prior to the HIV-1Tg rodent models attempted to produce infection in the host animal. The transgenic approach uses a unique strategy that does not look at infection, but, instead, focuses on the presence of viral proteins in the periphery and CNS. Because the transgenic mouse exhibits limited viral effects, this review focuses on characterization of the HIV-1Tg rat model and the effects of HIV-1 viral proteins in the CNS.

### 2.6. Gene expression in the HIV-1Tg rat

Although there is no viral replication in the HIV-1Tg rat, viral proteins are continually expressed throughout the animal's life (Peng et al., 2010; Abbondanzo and Chang, 2014). This is similar to HIV-1-infected patients receiving cART in which viral replication is substantially suppressed, but viral proteins continue to have an impact (Letendre, 2011). The HIV-1 non-infectious transgene carries the *env* gene and six supplementary genes, *tat*, *rev*, *vif*, *vpr*, *vpu*, and *nef*. The *env* gene encodes for the viral glycoprotein, gp160, which is responsible for forming the viral envelope. Gp160 is subsequently cleaved into gp120 and gp41. Gp120 binds to CD4+ receptors, permitting the virus to enter immune cell targets, such as macrophages and helper T cells. *Tat* and *rev* are regulatory genes that encode for two regulatory proteins essential for the transcription process. The Rev protein is necessary to transport the viral mRNA transcripts from the nucleus to the cytoplasm.

Gp120 and the other viral proteins are expressed in the blood, lymph nodes, and spleen of the HIV-1Tg rat and in the CNS (Reid et al., 2001; Peng et al., 2010). Higher levels of gp120, Tat, Nef, and Vif are expressed in the spleen of young HIV-1Tg rats (2–3 mo old) compared to older (10–11 mo old) HIV-1Tg rats. The drop in viral protein expression in the spleen of older rats is most likely due to the loss of T lymphocytes and increased apoptosis (Reid et al., 2001, 2004; Yadav et al., 2006) rather than to an overall decrease in viral protein expression since viral protein expression increases with age in some areas of the CNS (Peng et al., 2010).

## 3. The HIV-1Tg rat as a model of neuroHIV

The HIV-1Tg rat is a non-infectious model and, thus, it is not suitable for studies investigating viral progression or replication,

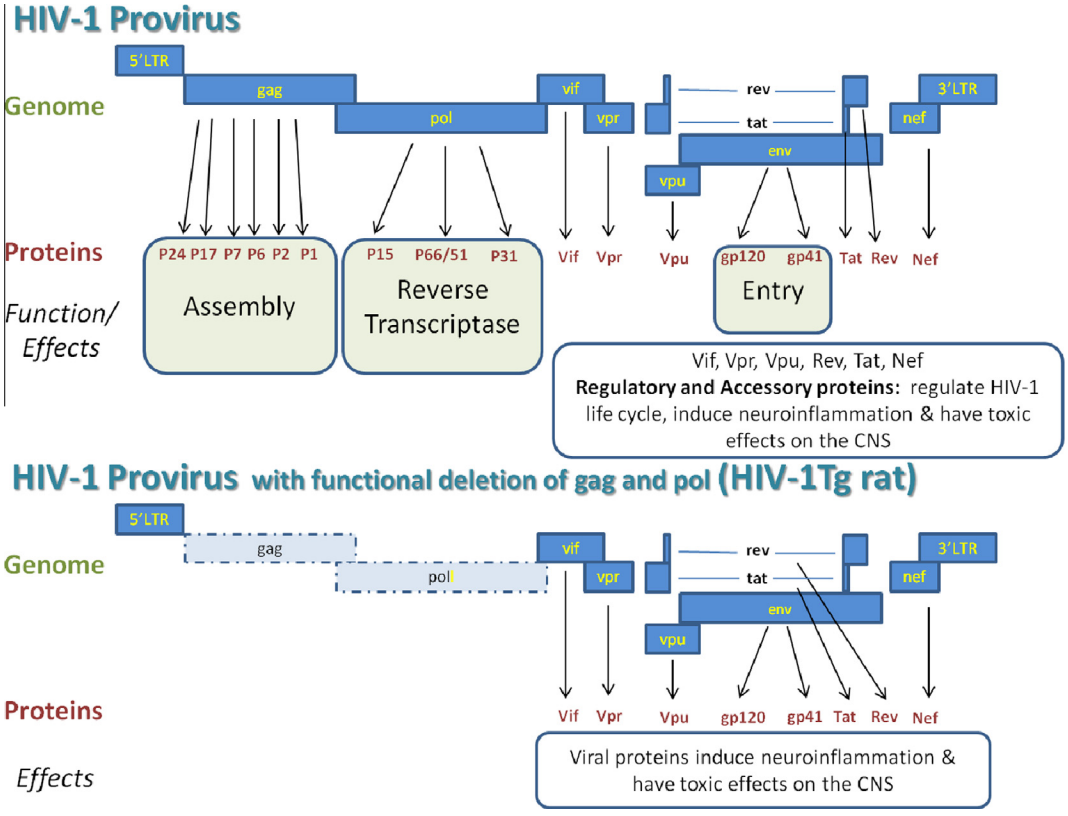


**Fig. 1.** Structural organization of the genes of the HIV-1 genome and provirus with functional deletion of *gag* and *pol* genes. The protein products encoded by the retroviral genes support the HIV-1 life cycle and contribute to the pathological conditions associated with HIV-1 infection. Although the functional deletion of the *gag* and *pol* genes renders the HIV-1Tg rat non-infectious, the production of the regulatory and accessory gene protein products results in neuroinflammation in the CNS (Royal et al., 2007; Rao et al., 2011; Homji et al., 2012a; Royal et al., 2012).

or for studying the impact of cART on viral replication. This model is, however, ideal for investigating the efficacy of therapeutic treatments which reduce neurological dysfunction in HIV-infected individuals in the post-cART era. The use of this rodent model for investigating neurologically related issues has been well established (Royal et al., 2007, 2012; Kass and Chang, 2010; Moran et al., 2012). Moran et al. (2012, 2013) used this model to test alterations in sensorimotor gating and behavior resulting from HIV-1 infection, including changes in dopamine (DA) function (Moran et al., 2012, 2013). Royal et al. (2007) and Royal et al. (2012) used the HIV-1Tg rat to test the effects of vitamin A deficiency on HIV-1-associated neuroinflammation and mu opioid receptor (MOR) expression as well as peripheral and CNS immune responses in HIV infection (Royal et al., 2007, 2012). Rao et al. (2011) found that HIV-1Tg rats exhibit neurological markers for neuroinflammation that are associated with cognitive impairment, and they identified neuroinflammation as a target for treatment of such impairment in HIV-positive populations (Rao et al., 2011). Taken together, these studies suggest that HIV-induced neuroinflammation results in alterations in neurological function and may be responsible for the many behavioral alterations reported in HIV-1Tg rats as well as other animal models of HIV.

From a very early age and throughout life, HIV-1Tg rats gain less body weight than control animals (Peng et al., 2010). This difference in body weight gain, however, is not due to reduced motivation to eat, or to illness, but appears to be due, in part, to reduced build-up of lean body mass (skeletal muscle) rather than fat mass (Pruznak et al., 2008). The HIV-1Tg rat is generally healthy, showing no evidence of anhedonia or severe behavioral deficits during the first year of life, a condition that seems to mimic asymptomatic HIV-positive patients on cART. The organ pathologies eventually take their toll, however, and eventual organ failure leads to an earlier death in the second year of life of the HIV-1Tg rats compared to controls (Peng et al., 2010; Moran et al., 2014). The general good health of the HIV-1Tg rat in the first year of life provides the opportunity to investigate the neurocognitive deficits that may result from prolonged exposure to the viral proteins. These studies would not be possible in a sick animal experiencing the confounding effects of illness and wasting.

The CNS is an early target of HIV-1. Shortly following infection, the virus infiltrates the brain through infected monocyte-derived macrophages, the so-called 'Trojan-horse' mechanism (Cavrois et al., 2008). Once in the brain, the virus causes the release of cytokines, which recruit more monocytic cells from the systemic immune system causing progressive neuroinflammation as resident perivascular macrophages and microglia are also infected. HIV-1 viral proteins, such as gp120 and Tat, also facilitate brain inflammation by impairing the structure of the blood–brain barrier [BBB] (Resnick et al., 1988). Studies with transgenic mice confirmed that soluble gp120 (Toneatto et al., 1999) and Tat (Avraham et al., 2004) change the integrity of the BBB. Neurons are not infected by HIV-1, but the expression of cell-surface receptors (e.g., CCR5 and CXCR4 chemokine receptors, NMDAR, LRP, and Da transporter) makes them vulnerable to the effects of viral proteins as well as to the increased levels of pro-inflammatory cytokines and other immune cell products (e.g., nitric oxide and arachidonic acid). The neuroinflammation caused by these HIV-1-induced events results in neuropathology and the development of HAND (Rao et al., 2014).

Despite the absence of infection, the HIV-1 transgene produces many of the same neuroinflammatory events in HIV-1Tg rats that occur during human HIV-1 infection. This is not the case in mice

because the same transgene is expressed only in the periphery, mostly in skin and muscle tissue (Dickie et al., 1991). HIV-1 protein mRNA is detectable in the brain of HIV-1Tg rats as young as 2–3 mos of age and increases or decreases with age, depending on the specific brain area (Peng et al., 2010), in a pattern consistent with autopsy results in humans (Wiley et al., 1999). HIV is associated with volumetric loss of specific brain areas, especially subcortical, in HIV-1 seropositive patients.

The presence of viral proteins in the HIV-1Tg rat suggests that it is the viral products that mediate these brain changes rather than any possible toxic effects of anti-retroviral drugs. This hypothesis is supported by a recent report that volumetric brain changes in HIV patients occur independent of the presence or absence of anti-retroviral treatment (Ances et al., 2012). The reason for changes in selective brain areas is unknown, but may be due to brain-region specific differences in penetration by HIV-1-infected monocytes and differential susceptibility to the toxic effects of viral proteins. The HIV-1Tg rat model can be instrumental in elucidating the role of the persistent presence of viral proteins in brain changes and the subsequent disruption in psychological and behavioral processes independent of infection.

### 3.1. Immunodeficiency in the HIV-1Tg rat

Soon after the creation of the HIV-1Tg rat, several studies were conducted in order to characterize immune changes in this animal. Reid et al. (2001) determined that, in addition to developing symptoms of HIV such as skin lesions and wasting, HIV-1Tg rats also express viral transcripts in their lymph nodes, thymus, liver, spleen, and kidneys (Reid et al., 2001). Further, these animals express gp120 in B and T cells, both of which are critical in immunity (Reid et al., 2001, 2004). Similar to HIV-1 infected patients, the HIV-1Tg rat shows a reduction in the overall number of CD4+ cells, increased susceptibility of T cells to apoptosis, and alterations in T effector functions (Reid et al., 2004). In a subsequent study, Yadav et al. (2006) found that reduction of CD4+ cells results in immune dysfunction via dysregulation of T helper 1 (Th1) effector cells (Yadav et al., 2006).

The body's defense mechanism is mediated by early non-specific innate immunity, followed by specific adaptive immunity. The principle cells of the innate immune system are monocytes and monocyte-derived macrophages, which are targets of HIV-1. The HIV-1 viral products impact on macrophages in the HIV-1Tg rat, and viral gp120 is expressed and shed into the circulating blood (Reid et al., 2001). Macrophages contain pattern recognition receptors (PRR) that serve as a second line of innate defense by patrolling the physical barriers of the body, such as the mucosal epithelia of the lung. The alveolar macrophages in the HIV-1Tg rat exhibit reduced phagocytosis and decreased expression of the hematopoietic cytokine, GM-CSFRb, which may contribute to the pulmonary hypertension (Joshi et al., 2008) as well as to the pneumocystis pneumonia infection seen in the HIV-1Tg rat model (Ateh et al., 2014).

Macrophages also play an important role in humoral (B lymphocytes) and cell-mediated (T lymphocytes) responses of adaptive immunity. There is considerable evidence that adaptive immune responses are abnormal in the HIV-1Tg rat. The proliferation and differentiation of effector (i.e., activated) T lymphocytes, CD4+ T helper (Th) cells, and CD8+ cytolytic T lymphocytes (CTL), are reduced in both young and mature rats, and older rats also show deficits in CD4+ and CD8+ memory cells that mediate rapid responses to subsequent antigens (Reid et al., 2004).

CD4+ Th cells are classified into subtypes depending on the type of cytokines produced. Th1 cells participate primarily in cell-mediated immunity and inflammation by releasing interferon gamma (IFN-$\gamma$), interleukin-2 (IL-2), and tumor necrosis factor-alpha (TNF-$\alpha$), whereas Th2 cells participate primarily in humoral immunity (i.e., antibody mediated; anti-inflammatory), and release IL-4, IL-5, IL-6, and IL-10. HIV-1Tg rats have deficits in IFN-$\gamma$ production by peripheral blood Th1 cells due, in part, to defects in antigen-specific signaling and increased activation-induced apoptosis of CD4+ T lymphocytes (Reid et al., 2004; Yadav et al., 2006).

Macrophages and dendritic cells bridge the gap between innate and adaptive immunity by serving as antigen presenting cells (APC). After detecting an evolutionarily conserved microbial pathogen with toll-like receptors (TLRs), macrophages and dendritic cells capture and digest the pathogen and load the detected antigen onto their cell surface Class II Major Histocompatibility Complex (MHC II), then migrate to lymph nodes to present the antigen to naïve CD4+ T cells. Stimulation of the TLRs also induces the release of cytokines (e.g., IL-12) that favor the production of Th1 cells over Th2 cells. The dendritic cells in the HIV-1Tg rat exhibit a defective response to TLR-stimulation that impacts the subsequent adaptive immune response (Yadav et al., 2009).

The inflammatory response is one of the principle mechanisms used by the innate immune system to defend against invading pathogens. The purpose of the early inflammatory response by macrophages is to eliminate invading microbes by recruiting leukocytes to the site of infection and to initiate a balance of pro- and anti-inflammatory cytokines. In the HIV-1Tg rat, the TLR-stimulated immune response to the bacterial endotoxin, lipopolysaccharide (LPS), is altered (Chang et al., 2007a,b). LPS fails to initiate the adhesion of leukocytes to the endothelial cell walls, the first step by which leukocytes migrate through the endothelium of post-capillary venules to the site of infection (Chang et al., 2007b). Blood plasma levels of TNF-$\alpha$ and IL1-$\beta$ are greater in the HIV-1Tg rat compared to control animals (Chang et al., 2007a), and the balance between pro- and anti-inflammatory cytokines and chemokines is substantially altered in response to an endotoxin challenge in the HIV-1Tg rat (Homji et al., 2012a). Monocytes and macrophages are also the principle cells responsible for endotoxin tolerance (Cavaillon and Adib-Conquy, 2006). Stimulation by LPS causes a substantially greater IFN-$\gamma$, TNF-$\alpha$, and IL1-$\beta$ response in HIV-1Tg rats, even after induction of endotoxin tolerance (Homji et al., 2012a).

The spleen is an important immune organ of the lymphatic system. The level of TNF-$\alpha$ is also greater in splenic monocytes and macrophages in the HIV-1Tg rat following an LPS challenge (Royal et al., 2012). Baseline IFN-$\gamma$ levels in the spleen (Homji et al., 2012a) and in splenocytes following T cell CD3/CD28 receptor stimulation are lower in HIV-1Tg rats compared to controls (Royal et al., 2012).

HIV-associated diseases begin with an acute HIV-1 infection that is temporarily controlled by the innate immune response, only to ultimately lose the battle when the adaptive immune system fails. AIDS occurs as a result of massive depletion of CD4+ T cells. In the HIV-1Tg rat, however, the viral genes are a part of the genome of all the cells and so the viral proteins are not identified as foreign. Thus, early mobilization of the innate immune system is absent in the HIV-1Tg rat.

Immunodeficiencies are prevalent in the HIV-1Tg rat and are most likely due to the chronic presence of viral proteins. Viral proteins, such as gp120 and Tat, produce inflammation by inducing cytokine production in the periphery and CNS (D'Aversa et al., 2005). The cytokines and viral proteins enter the brain by means of blood-independent viral transport and appear to have toxic effects on neurons (Banks et al., 2006). Although infection results in an adaptive inflammatory response, inflammation can also emerge independent of infection, and chronic inflammation can lead to neurodegenerative disorders (Viviani et al., 2014). As noted by Rao et al., 2011, HIV-1Tg rats show significantly higher mRNA and protein levels of the inflammatory cytokines, IL-1$\beta$ and TNF-$\alpha$, as well as a decrease in the neuroprotective brain-derived

neurotrophic factor (BDNF), among other changes in regulatory factors and signaling (Rao et al., 2011). Similarly, Royal et al. (2012) determined that pro-inflammatory cytokines, IFN-γ, TNF-α, and IL-1β, are up-regulated in the HIV-1Tg rat, which is, in part, responsible for the neurodegeneration seen in HIV-infected individuals (Royal et al., 2012).

Because the immune deficiencies and direct expression of HIV-1 viral proteins in several organs can cause damage, the HIV-1Tg rat is a useful model for studying HIV-1 in the era of cART (Peng et al., 2010), and, in particular, for investigating the pro-inflammatory and toxic effects of viral proteins in the absence of viral infection (Joshi and Guidot, 2011). However, the success of the HIV-1Tg rat as a model for HIV-associated pathology is not consistent. Substantial progress has been made using this model to study the effects of the HIV-1 transgene transcripts on cardiovascular function (Kline et al., 2008; Otis et al., 2008; Hag et al., 2009) and on lung (Fan et al., 2011, 2013) and liver function (Joshi and Guidot, 2011), but the HIV-1Tg rat has been less useful in mimicking HIV-1-associated skin pathology (Cedeno-Laurent et al., 2011). The skin pathology in the HIV-1Tg rats that is reported by laboratories that maintain HIV-1Tg rats other than those commercially available from Harlan, Inc. has been rarely observed by our laboratory or by others (Peng et al., 2010; Moran et al., 2014; Nemeth et al., 2014).

With the prolonged life expectancy of individuals with HIV-1 in the post-cART era, research into the effects of the HIV-1 virus over time is essential. Both cART patients and the HIV-1Tg rat experience age-related immune-response alterations (Reid et al., 2001), T-cell abnormalities (Reid et al., 2004), kidney failure (Ray et al., 2003), changes in behavior, and neuropathology (Reid et al., 2001). HIV-1Tg rats exhibit learning deficits even before they develop symptomatic signs of HIV infection (Vigorito et al., 2007; LaShomb et al., 2009), which is consistent with the cognitive impairment reported in HIV-1 patients receiving cART, and suggests that, despite the lack of viral replication, the persistent presence of HIV-1 viral proteins continues to affect brain and immune cells, causing neurological and immunological damage (Rao et al., 2011).

Recent evidence indicates that both the baseline and LPS-challenged immunophenotype of the HIV-1Tg rat continue to change with advancing age (Abbondanzo and Chang, 2014). Treatment with LPS mimics bacterial infection and allows researchers to identify changes in immune responses during the aging process. As a result of aging, HIV-1Tg rats exhibit several immunological changes not seen in control rats (Abbondanzo and Chang, 2014). Following an LPS challenge, HIV-1Tg rats exhibit a significant decrease in the percentage of T cells and an increase in T helper cells, neutrophils, and monocytes compared to F344 control rats. There is also a pronounced increase in the pro-inflammatory cytokines, IL-6 and TNF-α, and the chemokine, KC/GRO, in older HIV-1Tg rats. These findings suggest that HIV-1 initiates and/or impacts aging-associated immune alterations.

Early studies aimed at characterizing immunity in the HIV-1Tg rat were conducted using HIV-1Tg rats from the University of Maryland. More recently, Abbondanzo and Chang (2014) compared immune factors between HIV-1Tg rats and F344 control rats purchased from Harlan Laboratories (Abbondanzo and Chang, 2014). Their analyses determined that, whereas F344 rats show a relatively stable number of T cells from 2 to 16 mos of age, HIV-1Tg rats exhibit a decline in T cells starting around 6 mos. By 16 mos, HIV-1Tg rats have a significantly lower number of T cells compared to healthy controls. This is similar to what was reported by Reid et al. (2004). Abbondanzo and Chang (2014) also found that T helper cells remain elevated in HIV-1Tg rats compared to F344 rats (Abbondanzo and Chang, 2014). The elevation in T helper cell levels reaches significance at 6 mos of age and remains significantly higher until 18 mos. This may be related to the dysregulation in Th1 effector cells reported in 2006 by Yadav et al. (2006). HIV-1Tg rats provided by Harlan Laboratories also exhibit significantly elevated T cytotoxic cells at 6, 12, and 18 mos of age, significantly elevated neutrophils at 18 mos, and significantly elevated monocytes at 2, 6, and 12 mos (Abbondanzo and Chang, 2014). Immunity in HIV-1Tg rats was further dysregulated by alterations in the pro-inflammatory cytokines, IL-6 and TNF-α, and an increase in the chemokine, KC/GRO (Abbondanzo and Chang, 2014).

While animals from both the University of Maryland and Harlan Laboratories appear to have comparable immunity, Reid et al. (2001) reported that animals from the University of Maryland begin to exhibit clinical manifestations of AIDS by 5–9 mos of age (Reid et al., 2001). These symptoms include weight loss, neurological abnormalities, respiratory difficultly, and skin lesions. Aside from behavioral abnormalities, these symptoms are not seen until later in HIV-1Tg rats (e.g., 18 mos or older) purchased from Harlan Laboratories (Moran et al., 2012, 2013; Vigorito et al., 2013; Moran et al., 2014).

### 3.2. Neuroinflammation in the HIV-1Tg rat

In the past, the brain was described as 'disconnected' from the systemic immune system and as having an 'immune privileged' status in order to evade immune recognition and be protected from potential damage from excessive inflammation. The brain parenchyma is indeed an immunosuppressive microenvironment compared to the supporting non-parenchymal structures (ventricles, choroid plexus, meninges, and circumventricular organs) and peripheral organs, but the blood brain barrier permits a dynamic interaction between the brain and the immune system (Banks, 2015) and once inflammation occurs, this privileged state is severely compromised (Galea et al., 2007). Because of the development of neuroimmunology, immunopharmacology, immunophysiology, psychoneuroimmunology, and neuroimmunopharmacology in the last three to four decades, the bidirectional communication between the brain and the immune system has been a focus of considerable research. Both immune and neuronal products co-exist in lymphoid and neuronal tissues (Tomaszewska and Przekop, 1997; Aller et al., 2001). Various immune cells have been shown to produce hormones and neuropeptides, including ACTH (Ottaviani et al., 1999), endorphins (Morch and Pedersen, 1995), enkephalin (Wybran, 1985; Jankovic and Radulovic, 1992), prolactin (Gala, 1991; Yu-Lee, 1997, 2002), growth hormone (Gala, 1991; Meazza et al., 2004), catecholamines (Elenkov, 2007), and acetylcholine (Hosoi and Nomura, 2004), and the release of various neurotransmitters in immune system organs is triggered by nerve impulses from the CNS to peripheral tissue (Qiu et al., 1996; Pacheco et al., 2010).

In contrast, cytokines, such as IL-6, normally produced by immune cells, have been shown to be synthesized by and secreted from anterior pituitary cells when stimulated by LPS (Fuchs et al., 2013). Receptors for cytokines, hormones, neurotransmitters, and neuropeptides are found in both immune and brain cells and include opionergic, dopaminergic, cholinergic, and cannabinoid receptors. The chemokine, CCR5, is elevated in HIV-1 infected patients (Giovannetti et al., 1999) and has been identified as a target for gene therapies aimed at reducing the deleterious effects of the HIV-1 virus (Nazari and Joshi, 2008; Nazari et al., 2008). However, CCR5 deficiency has also been linked to modifications in the dopaminergic reward pathway (Choi et al., 2012).

Deregulation of immune mediators in the brain, including, but not limited to, increased cytokine exposure during brain development, acts as a "vulnerability" factor for later brain pathology, leading to behavior disorders. For example, there is strong evidence correlating early CNS infection with the development of schizophrenia (Suvisaari et al., 2003). Infections early in life can alter cytokine expression and glial activation in response to a subsequent immune challenge in adulthood (Bland et al., 2010), and a

neonatal *Escherichia coli* infection has been shown to impair memory formation in adulthood in the presence of a subsequent immune challenge with LPS (Bilbo and Schwarz, 2009).

A number of studies have shown that injection of LPS leads to deregulation of cytokine and chemokine expression and secretion in the CNS, which is enhanced in the morphine tolerant state (Staikos et al., 2008) or in the persistent presence of HIV-1 viral proteins (Chen et al., 2005; Homji et al., 2012a). Viral infections, including HIV infection, can lead to CNS inflammation, which subsequently causes alterations in neurotransmitter-dependent pathways associated with compulsive behavior (Lindl et al., 2010).

Evidence from studies employing different experimental strategies strongly support the hypothesis that viral proteins produce toxic effects that cause neuroinflammation and neurological dysfunction. The viral protein, gp120, binds to the chemokine receptor, CXCR4, an HIV-1 co-receptor involved in AIDS, that is expressed in most cells of the CNS, including neurons, to guide brain development (Tran and Miller, 2003). Gp120 stimulates CXCR4 and activates pro-apoptotic pathways in human neuroblastoma cells (Bardi et al., 2006). Gp120 also induces apoptosis through its interaction with the CCR5 receptor (Catani et al., 2000). These neurotoxic effects of gp120 on neurons appear to be mediated through activation of a p38-MAPK signaling cascade (Kaul and Lipton, 1999).

Neuronal injury and apoptosis also result from gp120 stimulation of the N-methyl-D-aspartate receptor (NMDAR). Excessive stimulation of NMDAR causes a large influx of $Ca^{2+}$ and subsequent generation of free radicals (e.g., NO) and reactive oxygen species during the process of necrosis or neural apoptosis (Lannuzel et al., 1995; Rao et al., 2014). The role of gp120 in inducing oxidative stress is also supported by the demonstration that genes involved in an antioxidant response are expressed in human astrocyte cultures treated with gp120 (Reddy et al., 2012).

In addition to its apoptotic effects, gp120 induces activation of the inflammatory cytokines, IL-1β, TNF-α, IL-6, IL-8, and CCL5, in rats and humans (Ronaldson and Bendayan, 2006; Shah and Kumar, 2010; Shah et al., 2011a,b). The impact of gp120 on brain function was demonstrated by experiments using the exogenous administration of gp120 to rodents and the expression of shedable gp120 in transgenic mice. Toggas et al. (1994) developed transgenic mice that contain astrocytes that express gp120 under the control of the promoter of glial fibrillary acidic protein and that develop CNS damage consistent with HIV-1-associated neuropathology (Toggas et al., 1994). Those transgenic mice exhibit changes in neural function in brain structures [e.g., neocortex and hippocampus (HIP)] associated with HIV-1 brain deficits (Krucker et al., 1998; Maung et al., 2012) and age-related changes in species-typical behaviors, cognitive performance, and responses to substances of abuse (D'Hooge et al., 1999; Roberts et al., 2010; Kesby et al., 2012; Henry et al., 2013). Exogenous gp120 also disrupts rodent performance of learning and memory tasks (Glowa et al., 1992).

The supplementary HIV-1 proteins also produce neurotoxicity. Similar to gp120, the viral protein, Tat, can lead to apoptosis by excessively stimulating NMDA receptors, and by interacting with the low-density lipoprotein (LDL) receptor gene family expressed in neurons and astrocytes (Liu et al., 2000; Rao et al., 2014). Changes in neuronal circuitry also occur in response to the presence of Tat (Hargus and Thayer, 2013; Shin and Thayer, 2013), as do deficits in learning and memory following exogenous Tat (Li et al., 2004) as well as transgenically expressed Tat (Carey et al., 2012; Fitting et al., 2013).

Other viral proteins, such as Vpr and Vpu, have neurotoxic effects, produce inflammatory cytokines (Patel et al., 2000; Trillo-Pazos et al., 2000), and affect CNS function and behavior, including memory performance of standard learning tasks (Acharjee et al., 2010; Chompre et al., 2012; Torres and Noel, 2014).

Chang and Connaghan (2012) proposed the possibility that a positive feedback interaction exists between opioid receptor-dependent pathways and HIV progression and that this interaction is, at least partly, moderated by HIV-induced neuroinflammation (Chang and Connaghan, 2012).

The hypothesis that decline in cognitive performance is at least partially caused by neuroinflammation is also supported by various clinical investigations. For example, one study looked at factors associated with cognitive decline following ischemic stroke and found that, among the 231 patients, 83 patients showed signs of impairment within 48 h of the stroke event; plasma levels of IL-6 and TNF-α were significantly higher in these individuals compared with those who did not exhibit such clinical deterioration (Vila et al., 2000). While more research is needed to determine the relationship between inflammation and cognitive dysfunction in autism spectrum disorder (ASD), patients with ASD were found to have significantly elevated TNF-α levels (Zimmerman et al., 2005). In a longitudinal study of cognitive impairment which spanned 20 yrs, IL-6 and TNF-α levels were collected from 1947 participants. Analyses revealed that those with high levels of both pro-inflammatory agents throughout the duration of the study, or increasing levels of IL-6 throughout the study, were at greater risk of cognitive impairment (Wichmann et al., 2014). Similar results have been reported in patients with type 2 diabetic encephalopathy (Diaz-Gerevini et al., 2014) and with Alzheimer's disease (Bettcher and Kramer, 2014).

## 4. Behavioral alterations in the HIV-1Tg rat

The modulating influence of immune function on behavioral and neural plasticity is well established (Yirmiya and Goshen, 2011). Thus a better understanding of the impact of HIV-1 infection on behavioral and neural plasticity can provide insights into neurodegenerative and neuropsychiatric diseases in general.

Animal models have been invaluable in evaluating the causal interactions between substance use, learning and memory, and the impact of HIV-induced brain injury. Such models allow for greater control in isolating HIV-1-induced changes in brain plasticity from co-morbidities that cause non-HIV-associated brain injury and in the detection of possible neurodegenerative disorders associated with aging in HIV patients. As an animal model of HIV-1-induced neuroinflammation, the HIV-1Tg rat shows alterations in both experience- and substance-induced behavioral plasticity.

Although HIV-1Tg rats have consistently lower body weight than control rats, they gain weight at the same rate as controls, indicating a normal motivation for and consumption of food (Peng et al., 2010; Midde et al., 2011; Moran et al., 2013; Nemeth et al., 2014), and they exhibit no anhedonia to sucrose solutions (Peng et al., 2010; Nemeth et al., 2014).

In a standard running wheel, HIV-1Tg rats run as much, or more, than controls during daily 20 min sessions (Chang and Vigorito, 2006) and do not differ from controls in swim speeds during a water maze task (Vigorito et al., 2007; Lashomb et al., 2009). This suggests that adult transgenic rats with the *gag-pol* deleted HIV-1 provirus exhibit good motor competence, consistent motivated behaviors, and no illness behavior that could confound assessments of learning and cognition. The observed differences in motivated behaviors suggest that the persistent presence of HIV-1 proteins alters the neural circuitry and the resulting behavioral phenotype. Although rodents engage in species-typical exploratory behavior, like humans, they utilize cognitive information such as memory of locations recently visited to guide their behavior. The altered exploratory behavior in HIV-1Tg rats could reflect similar deficits in cognition (Repunte-Canonigo et al., 2014) that underlies the decline in everyday functioning that defines HIV-associated mild neurocognitive disorder in human HIV patients.

When tested using an open-field test, a common assessment of general locomotor activity, unconditioned motivated behavior, and behavioral plasticity (Walsh and Cummins, 1976), HIV-1Tg rats demonstrate robust locomotor activity, but somewhat less overall activity than F344 controls (June et al., 2009; Midde et al., 2011; Moran et al., 2013; Nemeth et al., 2014). Similar changes in open field behavior have also been noted in transgenic mice expressing gp120 (D'Hooge et al., 1999). Locomotor activity in an open field partly reflects the strong motivation to explore unfamiliar environments. There is some evidence that unconditioned exploratory strategies are altered in the HIV-1Tg rat. For example, in a circular open field, HIV-1Tg rats spent significantly more time in the periphery when tested repeatedly at 6, 7, and 11 mos of age (Moran et al., 2013). In a more structured test environment, such as a T-maze, rats have a very strong tendency to alternate arm choices as they explore across trials. This spontaneous alternation behavior is significantly reduced in HIV-1Tg rats (Repunte-Canonigo et al., 2014).

The lower locomotor activity and altered exploratory behavior of the HIV-1Tg rats is not caused by reduced motor competence because, in a rotarod test of balance and coordination, HIV-1Tg rats perform as well as control animals during initial training; although, in more challenging tests (faster rod rotation rates), performance is poorer than controls (June et al., 2009). These results suggest good basic motor competence, but deficits in more complex tasks that may involve motor planning, a neurocognitive deficit characteristic of neuroHIV. It is possible that the reduction in locomotor activity seen in HIV-1Tg rats is, in part, due to neuroinflammation caused by circulating HIV-1 viral proteins. Similar reductions in locomotor activity have been noted in mice treated with LPS, which is known to induce peripheral cytokine production and, consequently, dysregulated behavior (Kozak et al., 1994).

Habituation, a fundamental form of learning that involves the progressive decline in a response to a repeated stimulus, results from synaptic plasticity rather than sensory adaptation to the stimulus or motor fatigue (Rankin et al., 2009). Functionally, habituation allows an individual to ignore stimuli that have no motivational significance, allowing attentional resources to be directed towards other potentially meaningful stimuli and to reduce (and eventually discontinue) unnecessary responses to repetitive stimuli.

The behavioral characteristics of short- and long-term habituation (Rankin et al., 2009) involve different neural and molecular mechanisms (Typlt et al., 2013). Although most studies of habituation focus on reflexive behaviors, such as orienting and startle responses, even motivated behaviors are modulated by habituation processes. For example, voluntary wheel running behavior and operant responding in rodents are modulated by habituation and sensitization processes (Aoyama and McSweeney, 2001; McSweeney and Murphy, 2009). Reduced habituation is correlated with several neurodegenerative disorders, including chronic schizophrenia and autism spectrum disorder. Such disturbances in habituation reflect cognitive deficits (e.g., pre-attentional and attentional processes) that contribute to the neurocognitive symptoms of the correlated neurodegenerative disorders (Akdag et al., 2003).

Few studies have examined habituation in HIV-1Tg rats. We have observed in HIV-1Tg rats more persistent wheel running behavior during daily 30 min tests than control rats, indicating that HIV-1Tg rats show less habituation of a motivated behavior within a running session (Chang and Vigorito, 2006). HIV-1Tg rats also show less habituation of locomotor activity in the periphery of a circular open field compared to controls (Moran et al., 2013), but do not demonstrate any change in the rate of short-term (intrasession) habituation of a reflexive startle response to a repetitive auditory stimulus (Moran et al., 2014). More studies on the habituation of reflexive and motivated behaviors in HIV-1Tg rats are needed before any conclusions about the effects of the transgene on habituation can be formed.

Deficits in executive function are commonly observed in human neurological disorders, such as Alzheimer's, Parkinson's, and neuroHIV. Symptoms of disturbances in executive function are often first noted by patients in the form of concerns about subjective experiences, which suggests a decline in cognition and sensorimotor capacity. However, not all patients are aware of their cognitive decline until more severe symptoms occur. It is possible to detect deficits in executive function pre-symptomatically in neuroHIV.

Animal models have been important tools for the investigation of disorders of executive function in that these models allow for a focus on the experimental manipulation of the frontal-striatal circuit, which is essential for executive function in humans (Chudasama, 2011) and which is believed to be disrupted by HIV infection (Chang et al., 2002). Animals models can also help to break down the broader area of executive function into specific psychological constructs that support and mediate behavioral plasticity and that can be objectively measured in animals and humans (Chudasama, 2011). The data from the few studies targeting specific psychological constructs of executive function in HIV-1Tg rats are encouraging.

Prepulse inhibition (PPI) is an operational measure of sensorimotor gating. In this paradigm, the magnitude of a defensive startle reflex (usually to a loud auditory stimulus) is inhibited by the presentation of a weaker 'prepulse' prior to the startle-eliciting stimulus. PPI, therefore, reflects the ability to isolate salient and potentially relevant stimuli from the flood of trivial, irrelevant stimuli. Deficits in PPI suggest a loss of sensorimotor gating and/or impaired behavioral plasticity in the form of reduced response inhibition (Braff et al., 2001).

Moran et al. (2013) assessed PPI with both auditory and visual cues in HIV-1Tg female rats from 2 to 8 mos of age. The magnitude of PPI is influenced by the interval between the prepulse stimulus and a subsequent startle-eliciting stimulus. Although HIV-1Tg and control rats both exhibited PPI, the HIV-1Tg rats were less sensitive to the manipulation of the interval between the stimuli at all ages except the youngest, suggesting a disturbance in sensorimotor gating and/or plasticity of defensive startle behavior. Mice exposed to LPS-induced pro-inflammatory cytokines *in utero* show similar alterations in sensorimotor gating, which indicates that neuroinflammation is likely involved in this change in behavior (Tsakok et al., 2012).

Similar alterations in the sensitivity of PPI to interval manipulation have been demonstrated in adult female rats following Tat and gp120 injection into the HIP (Fitting et al., 2006a,b). PPI deficits have also been reported in gp120 transgenic mice, although these impairments appear to differ between males and females (Henry et al., 2014). Interestingly, a recent study demonstrated PPI deficits in HIV-positive individuals with a diagnosis of HAND, but not in cognitively asymptomatic HIV-positive participants (Minassian et al., 2013). These results, together with the fact that the PPI is reduced following treatment with dopaminergic agonists such as amphetamines, suggest that alterations in PPI may be caused by viral protein-induced changes in the dopaminergic system of the HIV-1Tg rat (Moran et al., 2013).

PPI and sensorimotor gating have also been extensively applied to translational studies on cognitive fragmentation in schizophrenia (Swerdlow and Geyer, 1998).

The construct of working memory in humans involves three principal subsystems, including a 'central executive', which is postulated to be flexible in its control and regulation of cognitive processes (Baddeley, 1996). Whereas working memory in rodents differs from that of humans in that it is less explicit and is typically conceptualized as a transient mental representation or short-term memory for a stimulus, object, location, or event, rodent models are still able to shed light on HIV-induced alterations in basic short-term working memory.

Another approach to assessing working memory is to take advantage of a rodent's natural, species-typical tendency to spontaneously choose alternative locations when exploring and searching in mazes. To choose to enter an alternate arm of a maze during a test session, it is necessary for the animal to maintain the location of its prior response in working memory. Thus, spontaneous alternation is supported by the capacity of working memory. In a recent study, HIV-1Tg rats showed a marked decrease in spontaneous alternation in a T-maze compared to control animals, indicating disruption in working memory (Repunte-Canonigo et al., 2014). Moreover, this deficit was correlated with the expression of HIV-1 proteins in the HIP as well as gene expression that is consistent with neuroHIV. The HIP is one of the brain regions shown to be a target of HIV-induced neuroinflammation, which suggests that, similar to the aforementioned alterations in behavior, deficits in working memory in the HIV-1Tg rat may also be related to increased inflammation in the brain (Li et al., 2013).

Moran et al. (2014) further explored the impact of HIV on executive functioning by looking at performance on signal detection, discrimination learning, and reversal learning tasks (Moran et al., 2014). They found that the HIV-1Tg rats displayed impairment in attention, flexibility, and inhibition as evidenced by a lower number of hits, misses, and correct rejections, but a comparable number of false alarms as controls in a signal detection task, slower acquisition of criterion and response rate in discrimination learning, and difficulty learning a novel stimulus–response contingency in reversal learning. These findings are consistent with the cognitive decline seen in HIV-positive individuals, suggesting that the persistent presence of HIV-1 in the CNS results in various cognitive impairments.

Even in the era of cART, HIV-1-positive individuals still experience neurocognitive impairment, including deficits in attention, memory, psychomotor functioning, and behavioral flexibility associated with subcortical and frontal-striatal brain damage (Becker et al., 2011). The Morris water maze is a procedure used for investigating learning, memory, and behavioral flexibility in rodents. Although the task is simple – the animals must locate a hidden platform to escape water – there are several strategies that rats can use to search for and remember the location of the platform for an efficient escape. Rats appear to have a preference for the use of visual landmark cues when available (Hodges, 1996), but they can learn the task even in the dark (Rossier et al., 2000). Because HIV-1Tg rats are born with opaque cataracts, we conducted several studies investigating the performance of HIV-1Tg rats in a modified water maze that minimizes visual cues. The tests were conducted under dim red light, and non-visual cues were added (olfactory and tactile intra-maze cues and an auditory extra-maze cue) to encourage the use of non-visual landmark cues (an allocentric strategy) rather than an egocentric swim strategy that did not rely on any available cues. HIV-1Tg rats consistently exhibit a deficit in the acquisition of the water maze task, either when the start location is varied from day-to-day (Vigorito et al., 2007, 2013), or when the start location is not varied (Lashomb et al., 2009).

After acquisition of the task, when the platform is removed on probe tests, the HIV-1Tg rats behave similar to control rats by persistently searching in the former platform location. Thus, once the task is learned, the HIV-1Tg rats remember the location of the platform and use an allocentric strategy to locate it. These findings indicate that HIV-1Tg rats experience deficits in psychomotor functioning and cognition that retards acquisition of the water maze task. The behavioral inflexibility that is characteristic of neuroHIV is also evident in the HIV-1Tg rats. When the location of the platform is changed after initial acquisition, the HIV-1Tg rats show very clear deficits in adjusting their behavior to locate the newly located platform (Lashomb et al., 2009).

### 4.1. Substance-induced behavioral alterations in the HIV-1Tg rat

Patients on cART continue to experience neurological impairment, especially in the HIP and temporal cortex, regions associated with memory, spatial recognition, motivation, and movement (Brew et al., 2009). Thus, HIV-induced inflammation in the CNS may be the key mechanism underlying the use of addictive substances.

In addition to changes in unconditioned motivated behavior, psychomotor functioning, attention, and learning, HIV-1Tg rats also exhibit more profound substance-induced behavioral plasticity as evidenced by changes in behavior in response to substances of abuse, such as methamphetamine (METH), morphine, alcohol, and nicotine. Research investigating the neurological mechanisms underlying substance abuse and addiction has strongly supported the belief that chronic use of addictive substances is linked to neural plasticity or abnormalities in neurotransmitter-dependent pathways, such as the mesolimbic dopaminergic reward pathway and the endogenous opioid system [EOS] (Herz, 1998). Moreover the delicate balance that exists between central immune cells (microglia & astrocytes), peripheral immune cells (T cells and macrophages) and neural pathways in modulating normal behavioral and neural plasticity (Yirmiya and Goshen, 2011) may be disrupted by HIV-1 viral products, facilitating substance-induced behavioral and neural plasticity. Neuroinflammation, for example, also leads to alterations in neurotransmitter-dependent pathways associated with addictive behavior (Trigo et al., 2010; Chang and Connaghan, 2012; Homji et al., 2012a; Li et al., 2013). Neuroinflammation can cause neuronal damage leading to deregulation of the dopaminergic system, and inflammatory cytokines have been shown to alter the expression of the MOR, an important component of the endogenous opioid system (EOS). Recently, the EOS was shown to interact with another reward system, the endogenous cannabinoid system [ECS] (Fattore et al., 2004).

#### 4.1.1. Methamphetamine

Repeated exposure to METH causes behavioral changes, such as increased locomotor activity (rearing) and stereotypic behavior (repeated head movements) in rats. Such changes are caused by behavioral sensitization, which indicates neuronal adaptation associated with substance addiction and dependence (Robinson and Berridge, 2008). METH increases the number of rearings and head movements in both HIV-1Tg and F344 rats; however, HIV-1Tg rats exhibit significantly more extreme behavioral sensitization as evidenced by a significantly higher number of rearings and head movements compared to F344 rats treated with the same dose of METH (Liu et al., 2009).

#### 4.1.2. Morphine

Similar changes in behavior have been reported in HIV-1Tg rats treated with morphine. HIV-1Tg rats exhibit significantly longer tail flick latencies after a significantly lower dose of morphine than control animals (Chang and Vigorito, 2006). These findings indicate that there is a greater anti-nociceptive effect in HIV-1Tg rats from the same dosage of morphine as normal animals and that HIV-1Tg rats may be more susceptible to the potentially rewarding effects of opioids such as morphine.

Conditioned place preference (CPP) is a paradigm used in behavioral pharmacology research to determine the rewarding properties of substances of abuse. In the initial pre-conditioning stage, animals are tested for baseline place preference, which is determined by the amount of time spent in one of two distinctly different chambers in the CPP box. Subsequently, the chamber that is not preferred is paired with the substance under investigation over several trials in the conditioning stage, and place preference is re-evaluated. If the substance produces a reward, animals will

spend more time in the chamber that has been paired with the substance. When animals continue to spend more time in the substance-paired chamber, despite several unpaired exposures during the extinction stage, drug seeking behavior is indicated.

When CPP was used to compare reward and drug seeking in HIV-1Tg and F344 rats, all rats administered morphine showed a preference for the morphine-paired chamber (Homji et al., 2012b). However, F344 rats receiving morphine showed preference extinction by Day 7, whereas HIV-1Tg rats continued to show place preference until Day 14 of testing, suggesting that the rewarding effects of morphine are more profound in HIV-1Tg rats and that HIV-1Tg rats are more likely to partake in drug seeking behavior despite exposure to un-reinforced drug cues.

Ruzicka et al. (1996) and Vidal et al. (1998) were among the first to link inflammatory cytokines to the effects of MOR ligands such as morphine (Ruzicka et al., 1996; Chang et al., 1998). This discovery opened up the field of neuroimmunology by demonstrating that molecules that are mainly linked to the immune system can also interact and modulate expression of neurotransmitter receptors directly related to the effects of addictive substances. Not only do cytokines and chemokines mediate effects of MOR ligands, but these ligands also interact with the function and binding of cytokines and chemokines.

There have been many studies which report that morphine use increases the progression of HIV-1 infection to AIDS through its interaction with cytokines, chemokines, and their receptors (Hahn et al., 2010; El-Hage et al., 2011). The relationship between morphine and cytokine/chemokine receptors has been demonstrated *in vivo* as well as *in vitro*. Mahajan et al. (2002) suggested that morphine acts to both inhibit and enhance gene expression of certain chemokines in a concentration-dependent manner (Mahajan et al., 2002). Morphine induces heterogeneous desensitization by inhibiting the migration of chemokines to CXCR1 and CXCR2 receptors via phosphorylation of these receptors (Grimm et al., 1998).

Conversely, morphine tends to up-regulate the expression of chemokine receptors, CCR5, CCR3, and CXCR4 (Mahajan et al., 2002; Rogers and Peterson, 2003). This is significant for the HIV-1 infected population because CCR5 and CXCR4, along with CCR3 and CXCR2, while primary receptors for particular chemokines, are also major co-receptors for the HIV-1 virus (Horuk et al., 1997). These receptors are located throughout the CNS on microglia, astrocytes, neurons, and vascular endothelial cells (Horuk et al., 1997).

Since morphine inhibits the production and gene expression of IL-8 and the expression of macrophage inflammatory protein-1β (MIP-1β) in astroglial cells (Mahajan et al., 2002), it is likely that the use of morphine and other MOR ligands is involved in HIV-1 viral progression. Both IL-8 and MIP-1β act to suppress HIV-1 infection by blocking HIV-1 receptors, and, thus, by inhibiting the proper function of IL-8 and MIP-1β, morphine enhances the possibility of HIV-1 binding to these receptors.

Even at basal levels, MOR expression is significantly higher in HIV-1Tg rats. LPS was used to induce IL-1β, IL-10, and TNF-α, all of which have been shown to modulate MOR expression (Borner et al., 2004; Kraus, 2009). TNF-α and IL-1β levels were increased 7- and 38-fold, respectively, by LPS in HIV-1Tg rats compared to control rats (Chang et al., 2007a). MOR expression was also examined in response to LPS treatment. LPS induced MOR expression in both HIV-1Tg and F344 rats, but the increase in expression was significantly higher in HIV-1Tg rats (Chang et al., 2007a).

In animal models, chronic exposure to morphine causes desensitization of the HPA axis (Chang et al., 1996a,b; House et al., 2001) and inhibits the release of the final product of the HPA axis, glucocorticoid. Inhibition of this endogenous anti-inflammatory molecule in individuals addicted to opioids could be one of the factors responsible for the high susceptibility of these individuals to opportunistic infections (Ocasio et al., 2004).

Morphine increases the expression of chemokine receptors (Mahajan et al., 2002; Rogers and Peterson, 2003), which are major co-receptors for the HIV-1 virus (Horuk et al., 1997), thereby increasing the susceptibility of HIV-infected individuals to opportunistic infections. Chang and Connaghan (2012) proposed the possibility that a positive feedback interaction exists between the MOR and HIV progression, and that this interaction is, at least partly, moderated by HIV-induced neuroinflammation (Chang and Connaghan, 2012).

Using tail-flick latency as a measure of the anti-nociceptive effects of morphine, Chang and Vigorito (2006) demonstrated that tail flick latencies are significantly longer in the HIV-1Tg rat following treatment with morphine, and the ED50 of morphine is lower in HIV-1Tg rats than in control rats (Vigorito and Chang, 2006), indicating that the HIV-1Tg rat is more prone to the anti-nociceptive and possibly the rewarding properties of opioids. One of the possible molecular mechanisms underlying the increase in tail-flick latency is the increased expression of the MOR in the HIV-1Tg rat (Vigorito and Chang, 2006; Chang et al., 2007a).

When CPP testing was used to more directly evaluate the rewarding properties of morphine, HIV-1Tg rats continue to show a preference for the morphine-paired compartment for up to 14 day, whereas this preference extinguished over a 7-day period in F344 control animals. These data suggest that HIV-1Tg rats are more likely to partake in drug seeking behavior despite repeated exposure to unreinforced drug cues (Chang and Connaghan, 2012).

#### 4.1.3. Alcohol

Changes in behavior in the HIV-1Tg rat have been noted in the context of alcohol-related locomotor activity. In one study, locomotor activity was assessed in adult HIV-1Tg and F344 control rats administered 20% ethanol (EtOH) intragastrically (i.g.) twice daily for three consecutive days (Sarkar et al., 2013). Twenty-four hours following the last EtOH treatment, the rats were placed in an open-field chamber and locomotor activity was assessed based on the distance traveled for 25 min in the open field chamber. Locomotor activity was decreased in HIV-1Tg rats given water compared to F344 control rats given water, and was significantly decreased in both the HIV-1Tg and F344 rats treated with EtOH compared to the water-controls. The decrease in locomotor activity was significantly more profound in the HIV-1Tg rats compared to the F344 rats, suggesting that the presence of HIV-1 viral proteins may enhance the effects of EtOH.

In addition to behavioral changes, Clary et al. (2011) found that, in HIV-1Tg rats, chronic alcohol consumption accentuates skeletal muscle atrophy and decreases expression of anabolic factors, CT-1 and CNTF, indicaing that alterations in these signaling mechanisms may be involved in the loss of muscle mass associated with alcoholism, especially in HIV-infected populations (Clary et al., 2011).

Excess alcohol consumption also impacts lung liquid clearance and alveolar epithelial paracellular permeability in HIV-1Tg rats, which may be related to the decrease in expression of nuclear factor-erythroid 2-related factor 2 (Nrf2) seen in the animals (Fan et al., 2011). In addition, while treatment with gp120 alone results in barrier dysfunction in the lung epithelium, co-treatment with alcohol exacerbates this effect (Fan et al., 2011).

#### 4.1.4. Nicotine

Acetylcholine has been associated with learning and behavioral plasticity as well as reward (Crespo et al., 2006). Further, nicotine, a nicotinic acetylcholine receptor (nAChRs) agonist, produces neuroprotective effects in cognitive disorders such as dementia and depression (Picciotto et al., 2002; Picciotto and Zoli, 2002). A toll-like receptor pathway that plays a key role in innate immunity

and in the production of pro-inflammatory cytokines has been shown to be modulated by nicotine through α7nAChRs (Cui et al., 2012, 2013).

Given that nicotine can ameliorate HIV-induced deficits in event-related potentials (Gonzalez-Lira et al., 2006) that are associated with cognition, it is possible that cholinergic receptor function is altered by the HIV-1 virus. Deep-sequencing analysis of RNA transcripts revealed differentially altered gene expression in cortical, HIP, and STR brain regions in the HIV-1Tg rat compared to control (Cao et al., 2013). When treated with nicotine, approximately 20% of the altered gene expression in each brain region is restored (Li et al., 2013). The most profound restoration was observed in the Wnt/b catenin and ephrin B signaling pathways in the prefrontal cortex (PFC), cAMP responsive element-binding protein signaling and glutathione metabolism pathways in the HIP, and the tricarboxylic acid cycle and calcium signaling pathway in the STR. These data suggest that cholinergic-modulators may be useful in buffering the neurological deficits resulting from HIV-1 infection.

Using RNA deep sequencing, Li et al. (2013) sequenced 72 RNA samples from the PFC, HIP, and STR of HIV-1Tg and F344 control rats (Li et al., 2013). Following deep-sequencing analysis of 50-bp paired-end reads of RNA-Seq, Bowtie/Tophat/Cufflinks suites were used to align these reads against the Rn4 rat reference genome and to quantify the relative abundance of each transcript. Statistical and bioinformatics analyses of each brain region between the two strains revealed that immune response-related pathways are altered in the HIV-1Tg rat, with brain region differences, indicating that the persistent presence of HIV viral proteins causes inflammation in the brain of the HIV-1Tg rat (Li et al., 2013).

In addition, analysis of serum cytokine levels revealed that, while LPS induces a significant increase in TNF-α and IL-1β in both F344 and HIV-1Tg rats, the increase in these cytokines is significantly greater in the HIV-1Tg rats (Rao et al., 2011; Chang and Connaghan, 2012).

Li et al. (2013) compared PFC, HIP, and striatial (STR) gene expression in HIV-1Tg rats using deep-sequencing analysis of RNA transcripts in brain regions related to learning and memory (Li et al., 2013). They found that there are differences in neural pathways related to immune responses, neuronal health, and neurotransmission in HIV-1Tg rats (Li et al., 2013). In a subsequent study, RNA deep-sequencing analysis was also used to determine whether the altered gene expression observed in the HIV-1Tg rats could be corrected by nicotine. Cao et al. (2013) found that nicotine restores expression of about 20% of the altered genes in each brain region and modulates distinct pathways in different brain regions (Cao et al., 2013). The most significantly restored pathways are the Wnt/b catenin signaling and ephrin B signaling pathways in the PFC, cAMP-responsive element-binding protein (CREB) signaling and glutathione metabolism pathways in the HIP, and the tricarboxylic acid (TCA) cycle and calcium signaling pathway in the STR. These findings suggest that cholinergic modulators, such as nicotine, can have beneficial effects on HIV-1-induced neurologic deficits.

## 5. Conclusions

Several animal models have been developed in recent years in an attempt to characterize and study the HIV-1 virus. Despite the absence of infection, the presence of viral proteins in the HIV-1Tg rat causes immune deficiencies and neuroinflammation similar to those seen in HIV-1-infected humans. HIV-induced neuroinflammation causes neurocognitive deficits that emerge early in humans. HIV-1Tg rats show similar deficits as evidenced by a decline in their performance on tasks that require some basic cognitive capacities and behavioral flexibility. The data suggest that the presence of viral protein-induced neuroinflammation alters behavior and causes HIV-1-induced neurological deficits, which may be among the causes for the increased incidence of substance abuse in HIV-positive individuals.

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