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Spatial Learning Deficit in the HIV-1 Transgenic Rat: Discerning Place From Strategy Learning

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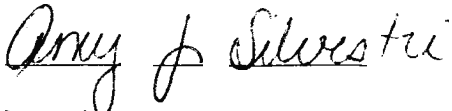

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Abstract

HIV-1 infection is associated with a constellation of cognitive and motor deficits collectively termed HIV associated dementia. With the onset of HAART treatment, these cognitive effects have become more mild, but also more prevalent. The lack of a proper animal model to study these cognitive effects of HIV has lead to the development of a new transgenic rat. This rat expresses the HIV-1 genome with functional tat and gp 120 viral proteins. These proteins are linked with direct neural toxicity as well as the induction of cytokines and other indirect means of neuronal damage. As part of the transgenic process, however, the HIV Tg rats express prominent cataracts, which can hinder cognitive testing that relies on visual stimuli. In order to test the spatial ability of these rats, a modified Morris water maze was developed that does not require the use of visual cues. A combination of olfactory, auditory, and tactile cues compensate for the lack of visual cues in this modified maze. First, a pilot study was performed to test the validity of the modified maze. Second, HIV Tg rats as well as F Tg littermate controls and normal F controls were run in the modified Morris water maze to test for spatial deficits. Lastly, a third experiment was performed, again with HIV Tg, F Tg, and Frats to determine the nature of the spatial deficit in these rats as well as test reversal and strategy learning. Results of all three experiments indicate that rats are able to solve a modified Morris water maze, and that HIV-1 Tg rats display deficits in place learning, reversal learning, and strategy learning compared to controls. These results parallel effects seen in the human population and implicate the HIV-1 Tg rat as a good model to explore potential treatment of the cognitive effects associated with HIV-1 infection.

Introduction

Human immunodeficiency virus type 1 (HIV-1) in its most advanced state, acquired immunodeficiency syndrome (AIDS), was first identified in 1981, and quickly developed into a world wide epidemic (Simon, Ho, & Karim, 2006; Lewthwaite & Wilkins, 2005; Glasner & Kaslow, 1990). Its history can be traced to a retrovirus transmitted from chimpanzees to humans, although variant strains have originated in other non-primate species (Simon, Ho, & Karim, 2006). The first documented case of HIV-1 infection occurred in 1959 and by 2005 more than 40 billion human beings world wide were diagnosed HIV positive, with 25 million lives already claimed (Simon, Ho, & Karim, 2006; Lewthwaite & Wilkins, 2005). Nearly twenty years after its identification, researchers continue to search for a cure. Numerous advancements in understanding HIV-1 infection have lead to antiretroviral therapies that combat the virus on the molecular level, but infection continues to persist in a dormant state (Simon, Ho, & Karim, 2006).

HIV-1 infection follows a rather predictable progression to full blown AIDS. The initial response to HIV-1 infection is a sharp drop in CD4 type T-lymphocytes corresponding with high viral replication and subsequent load (Simon, Ho, & Karim, 2006; Lewthwaite & Wilkins, 2005). CD4 immune cells are the target cells of the virus upon entry into the body. The glycoprotein 120 (gp 120) and the intramembrane protein gp 41 on the surface of the virus interact with the CD4+ receptor on certain T-cells, the "middlemen" of the immune system (Alberts et al., 1994). Further interaction with the chemokine co-receptors on the surface of the CD4 cells allows the virus to release its

viral core into the immune cell and begin reverse transcription of its genetic material (Simon, Ho, & Karim, 2006).

HIV is a retrovirus, so named because it contains viral RNA that is turned into DNA after infection of a healthy cell by the specific enzyme reverse transcriptase (Simon, Ho, & Karim, 2006). This viral DNA can then be inserted into the cell's nucleus and incorporated into the genome of the cell itself. This turns the once healthy cell into a potential virus producer (Simon, Ho, & Karim, 2006). HIV can lie dormant in this state for up to 10 years (Simon, Ho, & Karim, 2006; Lewthwaite & Wilkins, 2005; Glasner & Kaslow, 1990). During this time, the immune system kicks in to combat the presence of HIV-1 in the body. Viral load decreases and CD4 T cell count rebounds, but never reaches pre-infection levels (Simon, Ho, & Karim, 2006; Lewthwaite & Wilkins, 2005). Anti-retroviral treatments prolong the amount of time the virus remains in this dormant state by cutting down viral replication (Simon, Ho, & Karim, 2006, Gonzalez-Scarano & Martin-Garcia, 2005). Yet the virus remains present in hidden viral reservoirs, such as long-lived memory CD4 T cells (Simon, Ho, & Karim, 2006). Highly active antiretroviral therapy (HAART) is a drug regimen composed of at least three different drugs that target both the proteins that allow HIV to bind and fuse with CD4 cells and the enzymes that allow the virus to replicate (Cressy & Lallement, 2007). By targeting these viral components, HAART can cut down on viral replication, thus allowing the immune system a chance to rebound (Cressy & Lallement, 2007). To HAART, hidden viral reservoirs are no different from resting cells, and thus go unchecked (Simon, Ho, & Karim, 2006).

Neuropathology of HIV-1

One possible "anatomical sanctuary" for HIV is the brain (Gonzalez-Scarano & Martin-Garcia, 2005). HAART treatments do not cross the blood brain barrier, and thus do not affect replication in this area (Gonzalez-Scarano & Martin-Garcia, 2005). In addition, the virus enters the brain early in its pathology, usually within the first 2 weeks of infection (Zink et al., 1999; Paul et al., 2002). Since infected individuals rarely develop antibodies to the virus until after 4-6 weeks of infection, knowledge of contracting HIV usually takes at least this long (Lewthwaite & Wilkins, 2005). By this time, the virus has undoubtedly infected the resident immune cells of the brain, macrophages and microglia (Lawrence & Major, 2002). In order to infect these cells, however, the virus must first cross the blood brain barrier (BBB) (Nottet & Gendelman, 1995; Gonzalez-Scarano & Martin-Garcia, 2004). This can occur through direct infection of the cells that compose the BBB or through the more likely "Trojan horse" method where infected macrophages carry it across (Nottet & Gendelman, 1995; Gonzalez-Scarano & Martin-Garcia, 2004). Due to the selectively permeable nature of the BBB, immune cells such as macrophages can cross it easily as part of immune surveillance (Gonzalez-Scarano & Martin-Garcia, 2004). The resident macrophages and microglia interact with the virus once across the BBB, and can likewise become infected (Gonzalez-Scarano & Martin-Garcia, 2004; Lawrence & Major, 2002). It is thought that microglia cells carry the highest load of HIV of any other cells in the brain (Goodkin & Asthana, 1997). This infection can lead to neurological deficits through both direct and indirect effects of viral presence (Gonzalez-Scarano & Martin-Garcia, 2004).

Viral proteins such as the coat glycoprotein, gp120, the transcriptional transactivator protein (tat), or the viral protein R, are shed or secreted by an infected cell, and can consequently infect other cells or directly damage neurons (Gonzalez-Scarano & Martin-Garcia, 2004; Lawrence & Major, 2002). In addition, multiple infections (e.g. HIV and hepatitis) can increase viral shedding to levels similar to those seen during acute infection (Simon, Ho, & Karim, 2006).

Neuron function can also be affected by general inflammation caused by the secretion of cytokines or chemokines by non-infected activated macrophages to fight the infected cells (Gonzalez-Scarano & Martin-Garcia, 2004). Cytokines and chemokines are important mediators and modulators of many physiological systems, including immunological processes and inflammatory responses (Asthana & Fletcher, 1997). Cytokines regulate and control the intensity and length of the immune response by influencing T and B cell activation, proliferation, differentiation, and cytotoxicity (Asthana & Fletcher, 1997). These substances can also stimulate other cells to release cytokines (Asthana & Fletcher, 1997). Chemokines are potent chemotactic agents for leukocytes and can induce several biological processes including cellular activation, division, and cytokine induction (Asthana & Fletcher, 1997). Cells infected with HIV have been known to spontaneously release these substances, presumably due to stimulation by viral proteins (Gonzalez-Scarano & Martin-Garcia, 2005). Cytokines are also known to further stimulate macrophages and microglia to produce cellular toxins (Lawrence & Major, 2002).

It is most likely that it is through the indirect mechanisms of cytokine induction and oxidative stress that HIV causes its neurodegenerative effects. While viral proteins

can be directly toxic to cells, current research seems to support the idea that chronic activation of the immune system results in the gradual decline in cognitive abilities seen in infected individuals. The cytokine Tumor Necrosis Factor alpha or TNF-α, has been related to dymyelination of neuronal axons, thus degrading the signaling capability of affected cells (Goodkin & Asthana, 1997). Demyelination also results from the effects of TNF-α on oligodendrocytes, the cells responsible for myelinating axons (Nottet & Gendelman, 1995; Price et al., 2005). TNF-α is also known to cause activation of substances that mediate apoptosis, or programmed cell death (Goodkin & Asthana, 1997). Apoptosis occurs when TNF-α stimulates the voltage dependent Ca^{2+} ion channels of neurons, causing increased release of glutamate which in turn leads to the activation of the caspase enzyme apoptosis pathway (Gonzalez-Scarano & Martin-Garcia, 2004). TNF- α also stimulates the development of astrocytosis, the increased number and size of astrocytes. Astrocytes are known to suppress the neurotoxic effects of immune activated macrophages, even when the overactive macrophages are induced by HIV-1. However, HIV-1 also infects and disables astrocytes, eliminating this protective mechanism against the overactive macrophages (Nottet & Gendelman, 1995). Additionally, TNF-α enhances replication of HIV in chronically infected immune cells through induction of further substances that bind to the promoter region of the HIV-1 genome (Goodkin & Asthana, 1997). Similarly, the cytokine interleukin-1 or IL-1 has been shown to stimulate viral replication through identical mechanisms (Goodkin & Asthana, 1997). Both TNF-α and IL-1 can induce the expression of each other through positive feedback loops, increasing the potency of each substance (Goodkin & Asthana, 1997). IL-1 is induced by a variety of immune cells including macrophages and astrocytes, both cells known to be involved

in the neuropathology of HIV infection (Goodkin & Asthana, 1997; Gonzalz-Scarano & Martin-Garcia, 2005). IL-1 itself is cytotoxic to certain cells in either its membrane bound form (α) or its secreted form (β). Over-expression and chronic exposure of either form of IL-1 can lead to progressive neurodegeneration (Goodkin & Asthana, 1997). In fact, this is a mechanism similar to that seen in Alzheimer's disease (Goodkin & Asthana, 1997). In addition to cytokines, chemokines are also known to be expressed by microglia and astrocytes (Goodkin & Asthana, 1997). Induction of these substances is thought to play a role in the compromised integrity of the BBB which can lead to the crossing of additional viral molecules into the CNS, thus proliferating the pathogenic process (Goodkin & Asthana, 1997).

Proteins shed by HIV-1 add to and augment the degenerating effects of cytokines and chemokines. For example, tat can up-regulate the transcription of certain genes to speed the death of oligodendrocytes, compounding the effects of TNF-α on these cells (Lawrence & Major, 2002). Tat also has the capacity to both directly and indirectly induce neuron apoptosis, or the programmed death of cells (Lawrence & Major, 2002). Tat has also been linked with disruption of brain function in areas necessary for learning and memory (Behnisch et al 2004). In addition to tat, gp120 is often shed after cell entry or by an infected cell (Nottet & Gendelman, 1995). Once it is inside the CNS, gp 120 binds to an activation receptor on glial cells, which causes the release of neurotoxic substances, such as or IL-1β (Barak, 2002; Pugh et al., 2000). Gp120 has been shown to activate microglia and astrocytes to release IL-1β when injected directly into the brain of rats and when applied to human cell cultures (Pugh et al., 2000; Merrill et al., 1992). Furthermore, astrocytes exposed to gp 120 exhibit both cytoskeletal and functional

changes that could impair the ability of these cells to protect other neurons from damage (Lawrence & Major, 2002).

HIV-1 Dementia and Cognitive Deficits

Together, these neurotoxic effects of the presence of HIV in the brain contribute to a constellation of neurological deficits that emerge as a consequence of HIV infection. AIDS dementia complex (ADC) and HIV associated dementia (HAD) are neurological disorders that manifest typically in the late stages of the disease (Nottet & Gendelman, 1995; Gonzalez-Scarano & Martin-Garcia, 2005; Lawrence & Major, 2002; Pugh et al., 2000; Barak, 2002). However, milder forms of these disorders, such as minor cognitive motor disorder (MCMD), may occur at earlier stages of infection and demonstrate a gradual decline of cognitive ability (Gonzalez-Scarano & Martin-Garcia, 2005; Lawrence & Major, 2002). HAD and its related variations are all associated with profound motor, cognitive, and behavioral deficits directly attributable to HIV-1 infection of the central nervous system (CNS) (Nottet & Gendelman, 1995; Gonzalez-Scarano & Martin-Garcia, 2005; Lawrence & Major, 2002; Barak et al., 2002). These deficits include impaired short term memory and concentration, slower processing speed, verbal abstraction, decreased learning efficiency, and leg weakness (Gonzalez-Scarano & Martin-Garcia, 2005; Paul et al., 2002). Specific behavioral markers of disease usually accompany these neurological symptoms including withdrawal, apathy, and personality changes (Gonzalez-Scarano & Martin-Garcia, 2005). Neuronal injury and loss usually follow the high levels of HIV replication observed in the brain during the later stages of infection (Nottet & Gendelman, 1995). Cortical and sub-cortical atrophy in HIV-1 patients is not

uncommon, with the highest concentration of viral proteins being found in the globus pallidus, caudate nucleus, and deep white matter (Paul et al., 2002).

HAART has been successful in cutting down the severity of HAD symptoms in many patients by acting through indirect means of cutting down peripheral replication so less of the virus enters the brain (Lawrence & Major, 2002; Gonzalez-Scarano & Martin-Garcia, 2004). Before the implementation of the HAART treatment regimen, 20-30% of infected individuals developed HAD/ADC-like symptoms (Gonzalez-Scarano & Martin-Garcia, 2005). Yet more than 85% of AIDS patients worldwide do not have access to HAART, and 20-50% of patients on the complex regimen develop HIV infection resistant to the treatment (Lawrence & Major, 2002). Moreover, HAART does not directly act in the brain, lending support to the theory that the brain may serve as a reservoir for dormant HIV. Inaccessibility to the brain also implies that HAART leads to decreased incidences of HAD by cutting down peripheral replication of the virus so that less HIV can cross the BBB (Gonzalez-Scarano & Martin-Garcia, 2005).

HAART treatment, for those with access to its effects, has turned HIV into a chronic disease. With increased life spans due to effective treatment, MCMD, the more subtle form of cognitive dysfunction, has become more prevalent (Gonzalez-Scarano & Martin-Garcia, 2005). While the prevalence of HAD and ADC closely parallel the levels of the HIV virus, and thus develop late in infection, there are several mechanisms through which the virus leads to neurodegeneration (e.g. the induction of cytokines and shedding of viral proteins) and MCMD may be more closely related to these actions (Goodkin & Asthana, 1997). After the establishment of the HAART regimen, incidence of HAD and ADC declined to less than 10% of the infected population (Gonzalez-Scarano & Martin-

Garcia, 2005). MCMD, however, is far more common at approximately 30% (Gonzalez-Scarano & Martin-Garcia, 2005). MCMD closely parallels the pathological changes in the CNS characteristic of HIV invasion, and is associated with overall worse prognosis for patients (Gonzalez-Scarano & Martin-Garcia, 2005). One potential explanation for the increasing incidence of MCMD is that low viral replication established by HAART leads to slow, but progressive neurodegeneration (Gonzalez-Scarano & Martin-Garcia, 2005). This is consistent with evidence of longer life spans of individuals treated with HAART, as well as the low penetration of antiviral therapies into the CNS.

Experimental Evidence of HIV-Associated Cognitive Deficits

Human Studies

Clinical evidence of MCMD, as indicated by slow response times to test stimuli, is seen in patients expressing HIV symptoms and in patients not showing symptoms (Hinkin et al., 1999; Sorensen, Martin, & Robertson, 1994; Martin et al., 1993). Many early studies of HIV associated cognitive deficits were focused on predicting the onset of HAD or ADC. These experiments usually divided HIV subjects into subgroups of "good" and "poor" learners (e.g. Martin et al., 1993). Longitudinal studies were necessary to determine the outcome of such predictions based on the preliminary separations (Martin et al., 1993; Sorensen, Martin, & Robertson, 1994). Yet these initial studies were enough to demonstrate the existence of cognitive deficits early in disease progression, and certainly before the onset of AIDS (Hinkin et al., 1999; Sorensen, Martin, & Robertson, 1994; Martin et al., 1993). Effects on cognitive performance in attention tasks demonstrated that deficits in varying aspects of selective attention depend on the demands of the task (Sorensen, Martin, & Robertson, 1994). Easier tasks that

require only automatic attentional abilities are not affected by early HIV-1 infection, but more difficult tasks that place higher demands on attentional resources are (Hinkin et al., 1999; Sorensen, Martin, & Robertson, 1994; Martin et al., 1993). This demonstrates the subtle effect of a milder cognitive impairment that may persist early in infection.

One such task that places high demands on attention is the Stroop task. This task requires individuals to say the ink color of a printed word instead of reading the meaning of the word. This task proves particularly challenging when the meaning of the words are for colors that are inconsistent with the ink color of the printed word themselves. That is, subjects are to say "green" when the word "blue" is written in green ink (Hinkin et al., 1999). HIV-1 positive subjects that do not show symptoms of AIDS show slower decision making speed and greater interference in a Stroop task versus normal control subjects (Martin, Sorensen, Edelstein, & Robertson, 1992). These results lead to the hypothesis that HIV infected individuals are more impaired on tasks that require controlled processing versus automatic processing (Martin, Sorensen, Edelstein, & Robertson, 1992).

Controlled processing is involved when more voluntary control is necessary for a task, where automatic processing requires the opposite, that is, no voluntary control (Martin, Sorensen, Edelstein, & Robertson, 1992; Hinkin et al., 1999). These types of processing correspond to the attentional demands of a particular task, and demonstrate the subtlety in attentional deficits associated with the early stages of HIV-1 infection. The Stroop task relies heavily on executive functions of the frontal lobes, an area that has been demonstrated to be dysfunctional in HIV subjects suffering from dementia (Hinkin et al., 1999). Executive functions are responsible for two key components necessary for

successful completion of the Stroop task, controlled attentional processing and inhibition of proponent responses (Hinkin et al., 1999). HIV-1 positive individuals show deficits in both of these components of executive function as measured by increased response times in the Stroop task (Sorensen, Martin, Edelstein, & Robertson, 1992; Hinkin et al., 1999).

Animal Studies

Given the variability of incidence of cognitive deficits in human HIV-1 patients, and the potential for confounds in longitudinal and correlation studies, many researchers have turned to animal models to study the progression of infection and associated cognitive-motor function. Animal models have proven helpful in studying the effects of HIV-1 since better controlled studies can be done. However, animals cannot be infected with HIV-1 directly, and as a result, different strategies have evolved to simulate the effects of HIV in animal models. One strategy has been to identify similar diseases that occur naturally in animals. Feline immunodeficiency virus (FIV) results in decreased CD4 T cell count, and parallels HIV infection (Levine et al., 2005). Likewise, simian immunodeficiency virus (SIV) closely simulates HIV infection without complications of HAART treatment and co-infections or drug use (Horton et al., 2002). Research with these animal versions of HIV-1 infection has lead to further understanding of viral mechanisms as well as implications for treatment (Levine et al., 2005; Horton et al., 2002). Since the focus of animal studies in this paper will be on rodent models, further work with FIV and SIV will not be addressed here.

In rodents, two main strategies for studying HIV infection have emerged. Since rodents cannot be infected with HIV directly, researchers test the effects of the direct and indirect products of HIV-1 infection on various aspects of behavior and biology. In

addition to the higher cognitive deficits seen in human subjects, several rodent models of HIV have demonstrated that HIV associated proteins and the acute and chronic presence of cytokines can have negative effects on various forms of cognitive function (Li et al., 2004; Gibertini, Newton, Friedman, & Klein, 1995; Oitzl et al 1993; Shaw et al 2001; Gibertini, 1998; Hanisch et al 1997; Shaw et al 2005; Sanchez-Alavez 2000; Barrientos, 2002; Pugh et al., 1997; Pugh et al., 1999; Pugh et al., 2000). Animal research focusing on the acute or chronic presence of cytokines has revealed mixed results depending on the tasks and parameters involved. Some studies inject cytokines such as IL-1β either directly into the brain or into the peritoneal cavity. Others inject various bacterial endotoxins to induce the presence of cytokines which are released in response to an infection. For example, some researchers have found that injection of *legionella pneumophila*, an endotoxin, causes impairment in rats in a Morris water maze visual-spatial task (Gibertini et al., 1995). Using the same task, Oitzl et al. found that injections of IL-1β produced similar spatial navigation impairments (Oitzl et al., 1993). Other researchers choose to inject lipopolysaccharide or LPS, a non-infectious component of the Gram-negative bacterial cell wall known to strongly activate the immune system, including cytokine induction (Sparkman et al., 2005; Shaw et al., 2001).

Experiments using LPS injections have revealed a complexity of interactions between LPS, cytokine induction, motor effects, stress effects, and effects of 'sickness behavior' (Shaw et al., 2001). Sickness behavior includes a repertoire of effects such as reduced activity and exploration, decreased social interaction, fever, decreased food and water intake, hypersomnia, activation of the hypothalamic-pituitary-adrenal axis, and increased activation of the sympathetic nervous system (Shaw et al., 2001). In addition

to these effects, LPS is also thought to influence learning and memory. LPS is thought to block long term potentiation in the hippocampus, and thus affect hippocampal dependent functions, such as spatial learning (Shaw et al., 2001). The mixed results from behavioral studies with LPS injections have created a confusing picture of cytokine involvement in learning and memory. Some researchers find acute administration of LPS to impair acquisition of a spatial learning task (Shaw et al., 2001). Chronic LPS injections also impaired acquisition of spatial navigation, but these effects were difficult to differentiate from performance effects caused by the motor impairments of sickness behavior (Sparkman et al., 2005). Studies injecting cytokines directly (instead of inducing their release) have had more consistent results. These studies link IL-1β and TNF-α, in particular, to learning and memory deficits as measured by the Morris water maze (Oitzl et al., 1993). Other tasks have also been used to link these substances with learning and memory deficits. Injections of IL-1β after fear conditioning training trials showed a disruption in consolidation of memory for the context in which training was administered (Pugh et al., 1999). Like spatial learning and memory, the hippocampus is thought to be involved in memory for specific contextual information (Pugh et al., 1999)

Other studies have more directly linked learning and memory deficits with HIV-1 by examining the effects of the products shed by the virus. Injections of gp 120 and tat have shown these viral proteins to be involved in the learning and memory deficits associated with HIV (Li et al., 2004; Sanchez-Alavez et al., 2000; Glowa et al., 1992; Pugh et al., 2000). Rats treated with an intracerebroventricular (i.c.v) infusion of gp 120 showed increased latencies to solve a Barnes maze task, a task dependent upon both search strategies and spatial navigation (Sanchez-Alavez et al., 2000). The gp 120 treated

rats used a random search strategy throughout the experiment whereas control groups (given denatured gp 120 or saline) successfully decreased latencies over time by switching to a serial or spatial search strategy (Sanchez-Alavez et al., 2000). The same study measured the effects of gp 120 on hippocampal LTP in rat brain-derived hippocampal slices (Sanchez-Alavez et al., 2000). Corresponding with the impaired learning in the Barnes maze, gp 120 was shown to cause a decrement in LTP of the dentate gyrus (Sanchez-Alavez et al., 2000). A similar study was conducted using i.c.v. injections of tat (Li et al., 2004). Also similarly to the gp 120 study, tat was found to impair spatial memory and attenuate LTP (Li et al., 2004). Rats injected with tat showed greater errors of arm entries in a radial arm maze and inhibition of hippocampal LTP (Li et al., 2004). Some researchers have used transgenic mice over-expressing gp 120 (Krucker et al., 1998; Toggas et al., 2004). These studies have also demonstrated detriments in hippocampal LTP associated with actions of gp 120 (Krucker et al., 1998). In addition, gp 120 mRNA levels were found to be in the highest concentration in the neocortex, the olfactory bulb, and the hippocampus in these rats (Toggas et al., 2004). The use of animal studies to better understand the source of HIV related cognitive deficits can help illuminate courses of treatment and prevention for ADC, HAD, and MCMD.

The HIV Tg Rat

Thus far, studies of the effects of HIV-I on learning and memory have been limited to studies with infected humans, which are difficult to interpret because of numerous potential confounds (e.g., co-infection, treatment regimens, and use/abuse of psychoactive drugs), and to experiments with animals, which are under better experimental control but do not involve actual infection with HIV-I. To minimize

confounds and enhance experimental control better animal models are needed. However HIV-1 cannot naturally infect rodents, therefore an animal model that allows for direct study of HIV-1 in the brain was previously unavailable. In 2001, however, Reid and colleagues developed an HIV-1 transgenic (Tg) rat that displays many aspects of HIV-1 pathology(Reid et al., 2001). HIV-1 infects human cells by incorporating its own genetic code into the DNA of the host cell. The virus is unable to enter the cells of rodents, but by using modern genetic engineering methods Reid and colleagues were able to artificially insert the HIV-1 DNA (or genes) into the fertilized egg of a rat to create a HIV Tg rat model. In this process, however, they excluded the *gag* and *pol* genes, two of the seven genes of the HIV-1 genome. These two genes are needed by the virus to replicate and spread infection, thus their deletion has rendered the HIV Tg rat as noninfectious. More importantly, the remaining five genes are sufficient to produce functional tat and gp120 viral proteins.

When female HIV Tg rats give birth to a litter, some of the pups will have inherited the HIV-1 transgene and some will have not. A convenient visual marker for the presence of the HIV-1 transgene is the presence of cataracts. Thus, rats born with pronounced cataracts are identified as HIV Tg rats and their littermates born with no cataracts are identified as littermate controls. Experimental studies with these rats can also include normal control animals. Because the original HIV Tg rat was created from the Sprague-Dawley and Fisher rat lines, normal Sprague-Dawley or normal Fisher rats can serve as additional controls to compare with the HIV Tg rats and their littermate controls.

Studies with the HIV Tg rat have found several aspects of HIV-1 pathology typically seen in humans including T-cell abnormalities, wasting, respiratory difficulties, mild and severe skin lesions, and most importantly for the study of NeuroAIDS, neurological signs (Reid et al., 2001; Reid et al., 2004). These neurological signs were not profound in the HIV Tg animals, as inspection of brain tissue did not reveal gross differences (Reid et al., 2001). However, microscopic hemorrhaging and cell apoptosis was observed upon close inspection (Reid et al., 2001). In addition, neuronal cell death was noted, particularly in animals with clear clinical signs of disease progression (Reid et al., 2001). For example, rats displaying motor deficits showed abnormalities in the caudate putamen and substantia nigra, with severity of differences corresponding to severity of symptoms (Reid et al., 2001). No prior behavioral tests to determine the learning and memory capabilities of these transgenic animals have been performed.

The Morris Water Maze

A common thread of many animal experiments investigating the effects of HIV-1 in the brain is the use of the Morris water maze as a test of spatial navigation, learning, and memory. The Morris water maze is named for its creator, Richard G. M. Morris. Morris created the maze in 1981 to study spatial localization in the rat (Morris, 1981). The procedure was designed in order to test the then new claims about the neural underpinnings of spatial learning and memory (Morris, 1984). A book that spawned over 2 decades of research, *The Hippocampus as a Spatial Map*, by O'Keefe and Nadel (1978) lead to controversy over the idea that learning about spatial relationships may differ from the traditional measures of associative learning (Morris, 1984). In order to study spatial learning and memory more precisely, Morris developed the water maze in which rats are

placed into a pool of water and required to locate a hidden platform (Morris, 1984). Althoughrats are good swimmers, they experience the water as aversive and quickly learn to escape the water by locating and sitting on the platform. In traditional learning terms the learning that is observed in the water maze procedure can be described as an example of negative reinforcement (improved performance as a consequence of the removal of an aversive stimulus). However, negative reinforcement as an explanation does not indicate precisely what behavior is being reinforced and it does not require inferences of cognitive factors such as spatial maps or memory. The growing interest in animal cognition in general, and in cognitive maps in particular, lead Morris to develop his new procedure.

In the original Morris water maze, the platform is hidden by being located just below the surface of the water, which is made opaque by the addition of either powdered milk or a non-toxic paint (Morris, 1982; Morris, 1984). When implemented, Morris soon found that the rats quickly learned the task swimming directly to the hidden platform with little training, even when dropped from varying start locations around the perimeter of the pool (Morris, 1984). In addition to the lack of extensive training, the Morris water maze provided an advantage in that the rats did not need to be deprived of food or water during the course of testing (Morris, 1984). The accurate directionality displayed by the rats in the maze was evidence to many researchers that the rats were learning and remembering a spatial location of the platform relative to available distal visual cues (Morris, 1984). While the nature of these cues varied greatly between studies in different laboratories (e.g. curtains, walls, cabinets, sinks, posters, even experimenters), the performance of the rats was replicated again and again (D'Hooge & De Deyn, 2001). Furthermore, rats

treated with brain manipulations (e.g. drugs or lesions) could still learn the maze fairly quickly, yet the task was sensitive to differences between treated and untreated groups (Morris, 1984; D'Hooge & De Deyn, 2001).

If the rats tested in the water maze task were simply swimming towards a stimulus that precisely marked the location of the escape platform, then it would not be necessary to infer a memory process or a cognitive map to explain the learning of the maze. To ensure that the successful performance in the maze was not dependent on the use of unseen local cues (that is, cues belonging to the goal object itself), or unanticipated landmark cues (cues that reliably "flagged" the location of the platform), a set of visual platform trials typically served as a control (Morris, 1984; Morris, 1981). In these trials, a black platform protruded just above the water surface so that it was visible to the rat (Morris, 1984; Morris, 1981). The rats were tested in the same manner as during the hidden platform trials, and performance on each set of trials was compared (Morris, 1984; Morris, 1981). Rats in the visible platform trials quickly learned to swim directly to the platform, while rats in the hidden platform trials initially swam along the edge or randomly about the pool (Morris, 1984; Morris, 1981). With additional training, the rats in the hidden platform trials would learn that the platform is in a particular location relative to distal visual cues (Morris, 1984; Morris, 1981). The rats were eventually able to use these cues to swim directly to the platform, showing performance similar to rats in the visible platform trials (Morris, 1984; Morris, 1981). In fact, with extensive training, there were no differences between the performance of the rats in the hidden versus the visible platform trials (Morris, 1984; Morris, 1981). This control measure ensured that the rats were not seeing, hearing, smelling, or otherwise sensing the goal platform in

another way besides spatial localization and that rats were as efficient in using a spatial method to solve the maze as the use of a visible local cue (Morris, 1981).

According to O'Keefe and Nadel's spatial mapping theory an expected characteristic of rats relying on a cognitive map to solve a task is behavioral flexibility. If a rat has a cognitive representation of the environment surrounding the water maze and it remembers where in the cognitive map the platform is located, then it should not matter where in the maze it starts from. Experiments of the behavioral flexibility indicative of O'Keefe and Nadel's spatial mapping theory was tested by dropping the animals into the pool from the same start location, which presumably does not rely on spatial orientation (Morris, 1981). After the animals have had extensive experience with a single start location, the start location is randomized as in the original paradigm (Morris, 1981). If, even from this new start location, the animals are able to locate the platform quickly and efficiently, then this is demonstration of behavioral flexibility, and presumably evidence for a spatial map (Morris, 1981). Various control groups are used to ensure that the animals are using the required cues to find the platform and not some unknown strategy (Morris, 1981). Current researchers do not typically include these control groups unless explicitly examining the use of alternative strategies.

Another way of confirming that rats formed a memory of the platform location is to introduce probe trials. Probe trials are introduced after some training on the water maze task such that the platform is removed and the rats allowed to swim freely in the pool for anywhere from 30-180 seconds (D'Hooge & De Deyn, 2001). Time spent swimming in each quadrant is measured during the probe trails and is considered a measure of reference memory for the previous platform location (Morris, 1981; D'Hooge

& De Deyn, 2001). Although successful probe tests demonstrate that the rats formed a memory of the platform location, it does not prove that the animals are relying on this memory to find the location of the platform during training. The reliance on memory may vary during the course of training as other strategies are realized. This issue will be discussed later.

Probe trials are effective because rats tend to persist with their search in the remembered location. At some point, however, the rats will give up and search in other locations. Reversal learning is measured in the Morris water maze by placing the platform in the opposite quadrant as during training and measuring time spent in the previous quadrant as well as how long it takes to learn the new location (Morris, 1981). Once familiar with the pool and the task, rats show flexibility in discovering the new platform location as well (Morris, 1981). Rats with various lesions or drug treatments may have deficits in any number of the spatially dependent behaviors measured by the Morris water maze.

In addition to its ability to accurately and reliably measure the spatial abilities of animals, both normal and with various experimental manipulations, the water maze also measures the ability of rats to learn, implement, and alter various behavioral, non-spatial strategies (Morris, 1981; Morris, 1982; Baldi, Lorenzini, & Bucherelli, 2003; Choi et al., 2006; Blokland, Geraerts, & Been, 2004; Michaeu et al., 2004; de Bruin, Swinkels, & Brabander, 1997; Mackintosh, 2002). Recently, research with the Morris water maze has centered around a blend of behavioral and cognitive factors that together lead to the reliable and seemingly spatial performance of rats. Certain behavioral strategies must first be learned upon a rat's first few encounters with the maze (Baldi et al., 2003). These

behavioral or procedural strategies include learning that escape is possible, learning how to efficiently climb onto the platform and remain there until removed by the experimenter, learning that the platform is the only form of refuge, and learning to suppress swimming near the pool edge (Baldi et al., 2003). Typically, upon initial experience in the maze, rats will swim around the edge of the pool, a strategy known as thigmotaxis, because the wall serves as the only means of visual escape (Choi et al., 2006). Once the animals learn that escape is not possible along the edge of the pool, they will begin to search the rest of the pool, until by chance they locate the platform (Choi et al., 2006). Once the procedural/behavioral strategies are learned, the rat can begin to learn that there are other, more efficient ways to solve the maze. The spatial localization that O'Keefe and Nadel originally proposed to rely on a spatial map is considered an allocentric or place strategy where animals use the available cues to represent a specific location in space to which they can reliably orient themselves and navigate (O'Keefe & Nadel, 1978; de Bruin et al., 1997). A second commonly used strategy in the maze, however, is a response learning strategy (de Bruin et al., 1997; Baldi et al., 2003; Michaeu et al., 2004; Mackintosh, 2002). This strategy is also known as a taxon system, praxis or position-response navigation, or an egocentric spatial localization (de Bruin et al., 1997). These two strategies, which will now be referred to only as place learning and response learning, have emerged as the dominant strategies of interest by researchers using in the water maze. A researcher may try to encourage place learning over response learning, or vice versa, by making various modifications to the water maze procedure, but generally it is thought that these strategies both contribute in parallel to successful

navigation of the Morris water maze (Michaeu et al., 2004; Baldi et al., 2003; Choi et al., 2006).

The discovery of these harmonious strategies has complicated results obtained in the maze, and current research has focused on how to dissociate the two in order to attribute the contribution of certain brain areas and/or neurotransmitter paths to certain aspects of solving and navigating the water maze (Michaeu et al., 2004; Grannon & Poucet, 1995; Day et al., 1995: Miranda et al., 2006; Choi et al., 2006; Compton, 2004; Da Cunha et al., 2006; de Bruin et al., 1997; Pouzet et al., 2002). Evidence has emerged that the striatum and medial prefrontal cortex in rats are necessary for response learning, while the hippocampus and parietal areas are necessary for place learning (Michaeu et al., 2004; Grannon & Poucet, 1995; Day et al., 1995: Miranda et al., 2006; Choi et al., 2006; Compton, 2004; Da Cunha et al., 2006; de Bruin et al., 1997; Pouzet et al., 2002). It is difficult to dissociate these strategies, however, as response learning can be isolated from place by removal of the extramaze cues, but there is still no way to truly isolate spatial learning (Pouzet et al., 2002; de Bruin et al., 1997). This is in part because rats can compensate for any lack of spatial ability by employing response strategies such as sub-circular patterns, and this can mask any deficit due only to place learning strategies (Baldi et al., 2003).

The successful navigation of the maze is attributed to an evolution of strategies (de Bruin et al., 1997). First, rats use a thigmotaxic strategy, second, they use a place strategy, and third, they use a response strategy (de Bruin et al., 1997). This progression of strategies reflects a progression of learning in the maze, specifically, the learning of the relation between the platform location and the available extramaze visual cues. What

happens, however, when the cues are unavailable or the animals are unable to use them due to poor visual acuity? Several experiments have shown that rats are capable of solving the Morris water maze even in the absence of visual cues (Linder et al., 1997; Prusky et al., 2000; Baldi et al., 2003). In fact, blind rats have been shown to successfully navigate the maze, and to be indistinguishable from cognitively impaired rats (Linder et al., 1997). Blind rats actually performed better than atropine treated rats and performed as well or better than the worst control rat (Linder et al., 1997). In a probe trial animals with reduced visual acuity spent significantly more time in the target quadrant and did not differ from normal controls (Prusky et al., 2000). These results show the importance of response learning in the maze, and demonstrate how use of only these strategies can lead to performance that parallels that of controls using a combination of response and place learning. In addition, cues other than visual cues have been shown to support place learning (Rossier, Haeberli, & Schenk, 2000). These experiments show the diversity and complexity of the Morris water maze.

Performed Studies: Pilot, Experiment 1, & Experiment 2

Due to functional gp 120 and Tat in the HIV Tg rat, cytokine induction and direct viral effects should result in certain neurological deficits in these rats. Using a traditional task of spatial navigation and memory, the MWM, the HIV Tg rat should display deficits in acquisition of the task. The MWM also provides the opportunity to test various aspects of learning and memory in these animals. The flexibility and richness of testing using the MWM allows for a comprehensive test of abilities and is easily modified to fit specific needs and aims. The first experiment examined if HIV Tg rats show deficits during the acquisition of the water maze and during probe tests of place learning.

Reversal learning and strategy learning, which may depend on different brain areas than place learning, as well as behavioral flexibility, were examined in Experiment 2. Evidence for viral effects on multiple brain areas suggests a distributed presence of the virus in the brain that would result in general learning and memory impairments. Therefore, the HIV Tg rats were expected to be impaired in several aspects of MWM learning and navigation.

As a consequence of the transgenic process, however, the rats have profound opaque cataracts that make the behavioral testing of these animals challenging in any task that requires the use of visual cues (Reid et al., 2001). In order to test the spatial learning and memory abilities of these rats in the Morris water maze, certain modifications had to be made. In order to keep the task dependent upon spatial navigation, cues (not belonging to the goal platform itself) must be used to guide the rats through the maze. Since it has already been shown that auditory cues in conjunction with visual cues (but not auditory cues alone) are sufficient to support successful navigation of the maze, a single auditory cue was placed from the NW quadrant of the pool (Rossier et al., 2000). In addition, olfactory cues were placed around the perimeter of the pool to differentially mark the northern and southern quadrants. The same olfactory cue was used in each half of the pool (two distinct scents, mint and vanilla) so that one particular scent could not be associated with the target quadrant, and thus serve as a beacon or landmark (Mackintosh, 2002). A pilot study using normal Sprague-Dawley rats was performed to test these modifications of the MWM prior to testing of the HIV-1 Tg rats. Three groups of six rats each were tested either (1) in the light with visual cues available (2) in the dark with the auditory and olfactory cues available or (3) in the dark without any cues available. These

groups were termed Light, Dark+ Cues, and Dark, respectively. Based on results from the pilot study and to further encourage the use of the place learning cues and not other response strategies, a third tactile cue was placed in the western quadrants for Experiments 1 and 2. The tactile cue consisted of fishing wire hanging just above the surface of the water so that the rats' heads hit the wire as they swam by. The unique combination of cues in each quadrant made each a distinct place that the rats could distinguish from each other in order to orient and navigate within the maze. Experiment 1 used this three-nonvisual cue water maze to test the HIV Tg rats as well as the F Tg littermate controls, and F normal controls in spatial navigation. This first study showed that the HIV Tg rats have a general overall deficit during training and in a probe test of the MWM. In Experiment 2, emphasis was placed on dissociating place and response strategies, to determine more precisely the nature of the HIV Tg rats' deficit. Thus protocols were employed that tested each strategy explicitly and required rats to switch from one strategy to another during the course of testing. To keep the control rats from using visual cues, all testing was done under red light illumination.

Pilot Study

Method

Subjects

Eighteen Sprauge-Dawley rats were used in the pilot study. Animals were approximately 5 months of age when used in the experiment. All rats were double housed and maintained on a 12 hour light-dark schedule. Rats were provided ad-libitum food and water during the course of the experiment. All procedures were in accordance with the Institutional Animal Care and Use Committee.

Apparatus

The apparatus used in the pilot study was a black round tub 130 cm in diameter and 52.5 cm in depth. The tub was filled with water to 30 cm in depth. The surface area of the platform was 15.2 cm square and the whole platform measured 28 cm in height. The top surface of the escape platform sat 2 cm below the water surface. The surface of the water was covered in packing peanuts to hide the location of the platform (Cain et al., 1993). For the rats in the Dark + Cues group, the spatial navigation cues consisted of two olfactory cues and one auditory cue. For the olfactory cues, pipe cleaners dipped in mint scented mouthwash were placed on the rim of the pool in the northern quadrants and pipe cleaners dipped in vanilla extract were placed on the rim of the pool in the southern quadrants. A metronome (Seiko Quartz Metronome SQ50) set at 96 beats per min, 55 cm away from the outer wall of the NW quadrant and 21 cm above the rim of the pool, provided a single auditory cue. Rats in the Light group used the visual cues that were provided around the room. A white curtain extended past the Western quadrants of the pool, an experimenter stood near a large metal sink to the South of the pool, and two

posters of vertical or horizontal black and white lines and a picture of Edward Tolman were hanging on the wall in the Eastern and Northern quadrants, respectively. The rats in the Dark group received no cues at all, and were run only in the dark under red light illumination.

Procedure

Three groups of six rats each were tested either (1) in the light with visual cues available (2) in the dark with the auditory and olfactory cues available or (3) in the dark without any cues available. These groups were termed Light, Dark + Cues, and Dark, respectively. Red light illumination was used in the dark conditions to aid the experimenter, but was not visible to the rats. The rats were given seven days of training, 4 trials each day. On the first day of training, the rats received a 60 second pre-training trial in which the rat was simply placed on the platform. Pre-training allows the rats to orient themselves and acclimate to the test environment. Trial one began immediately following the pre-training trial. The rats were dropped into the pool from randomized start locations and the platform remained in a fixed location in the NE quadrant. Latencies for the rat to find the hidden platform and climb up onto it were measured via stopwatch and recorded. All trials and days were video taped for later analysis. On days 3, 5, and 7 of training, rats in all groups were given a single 90 second probe trial immediately following trial 4. The platform was removed and the rats dropped from a randomized start point into the pool as during a normal trial. Videos of the probe trials were viewed and scored for time spent in each quadrant. On day 8, the cues used by each of the three groups were removed. The rats in the Light group were run in the dark with the olfactory and auditory cues (visual cues removed), the rats in the Dark+ Cues group

were run in the dark with no cues available, and the Dark group of rats were run in the light (visual cues available). Latencies for the rats to find the platform were recorded as in training. This Cue Rotation test was used to demonstrate the usefulness of the available cues to the rats when solving the maze. If the rats are highly dependent on the available cues, their removal would significantly impact the rats' latencies.

Data Analysis

Training and Cue Rotation. For training latencies, a 3 x 7 x 4 three factor (group x day x trial) mixed ANOVA was performed with group (Light/Dark/Dark+ Cues) as the between subjects factor and days and trials as within subjects factors. Pairwise comparisons were performed using least-significant difference (LSD) post-hoc tests. The cue rotation on day 8 was analyzed using a 3 x 4 two factor (group x trial) mixed ANOVA. This day (day 8) was also compared to the last day of training (day 7), when the rats had the greatest amount of exposure to the cues and had reached optimal performance in solving the maze using a 3 x 2 x 4 three factor (group x day x trial) mixed ANOVA.

Probe Trials. Probe trials were scored for time spent in each quadrant during the entire 90 second duration. The water maze was divided into 8 quadrant locations, northeast inner (NEi), northeast outer (NEo), northwest inner (NWi), northwest outer (NWo), southwest inner (SWi), southwest outer (SWo), southeast inner (SEi), and southeast outer (SEo). Video tapes were viewed with a template of the quadrant designations marked on a television screen. Using the program Etholog, time spent in each quadrant location was recorded based on the video tape recordings (Ottoni, 2000). New quadrant location entries were determined as when the head of any rat passes

through the designated quadrant boundaries. Time spent in each quadrant (inner and outer) for the probe data were analyzed using a 3 x 8 two factor mixed ANOVA (group x quadrant) with group (Light/Dark/Dark+ Cues) as the between subjects factor and quadrant as the within subjects factor. Further two factor ANOVAs were performed to analyze general time spent in each quadrant (collapse across inner and outer) and general time spend in the inner and outer annuli of the pool (collapse across quadrants). A 3 x 4 two factor (group x quadrant) mixed ANOVA was used to analyze general time spent in each quadrant. A 3 x 2 two factor (group x area) mixed ANOVA was used to analyze time spent in the inner and outer areas of the pool.

Results

Training and Cue Rotation. The mean escape latencies of the Sprague-Dawley rats in the Light, Dark, and Dark + Cues groups during the 7 days of training are shown in Figure 1. The latencies are averages of the 4 trials conducted each day. These latencies indicate that animals in all groups were able to learn the task as it took less time for the rats to find the escape platform over days, $F(96, 12) = 22.8$, $p = .00$. The Light group appears to show fastest latencies compared to the Dark and Dark + Cues groups on days 5 to 7, with the Dark+ Cues group showing intermediate latencies and the Dark group showing the longest latencies. However the main effect of Groups, $F(2, 15) < 1$, and the Groups x Days interaction failed to be significant even on those days, $F(12, 90) < 1$. Latencies were higher on the first trial and tended to decrease on subsequent trials in all groups, $F(3,45) = 9.2$, $p = .00$, (data not shown). This effect of trials suggests that following the initial trial, the rats improved in performance each day. On Day 8, the cues available to each group of rats were switched so that each group received different cues

than those used during the first seven days of training. The insert in Figure 1 shows the group latencies for each trial during the cue rotation. Latencies from day 7 were included for comparison purposes. Removal of the cues had a greater effect on the Light group than on either the Dark or the Dark + Cues groups, with the disruptive effect occurring on the first trial in the Light group only, [3-way interaction, $F(6,45) = 2.5$, $p = .04$].

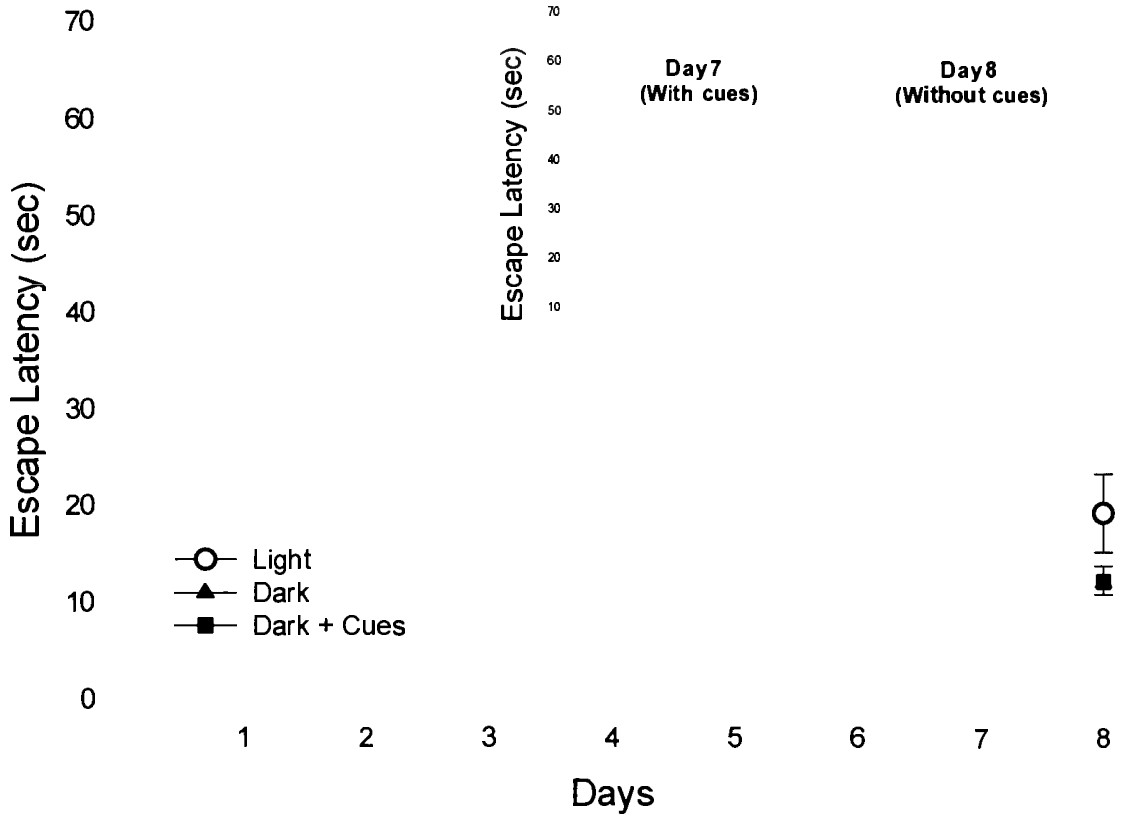


Figure 1. Acquisition curves for the pilot experiment with normal Sprauge-Dawley rats. The cues were removed on Day 8. The inset shows trial-by-trial escape latencies on the last day of training with cues present (Day 7) and on the day the cues were removed (Day 8).

Probe Trials. Analysis of the percentage of time spent in the training quadrant (NE) during the three probe tests (Figure 2 top) yielded a significant groups x probes interaction, $F(2,15) = 3.4$, $p = .02$. Further analysis revealed that the Light group spent more time in the training quadrant than the Dark + Cues group on probe days 3 and 5 but by probe day 7 the Dark + Cues group increased their time to equal the Light group. The Dark group was inconsistent, spending less time in the training quadrant compared to the Light group on Day 5 only.

Further analysis of the time spent in the quadrant divisions of the pool during the probe tests suggests that the cues differently effected strategy development in the three groups. The percentage of the time spent in the inner annulus is shown in Figure 2 (bottom). The animals with the cues added in the dark spent much more of their time searching in the inner annulus on all three probe tests, whereas the rats in the other two groups spent significantly less time in the inner annulus early in training (probe 3). In later probe trials, these groups reached levels of inner annulus search similar to that seen in the Dark+ Cues group, [group x probe, $F(4, 30) = 3.1$, $p = .03$]. An evaluation of the searching time in the two northern quadrants revealed that all groups spent an equal amount of time in the inner annulus of the NW and NE quadrants (data not shown), but the groups differed in the outer annulus, [groups x probes, $F(4, 30) = 6.7$, $p = .001$]. As can be seen in Figure 3 (top), the Light group tended to avoid the outer annulus of the NW quadrant and spend more time in the NE quadrant (inner or outer) on all three probe

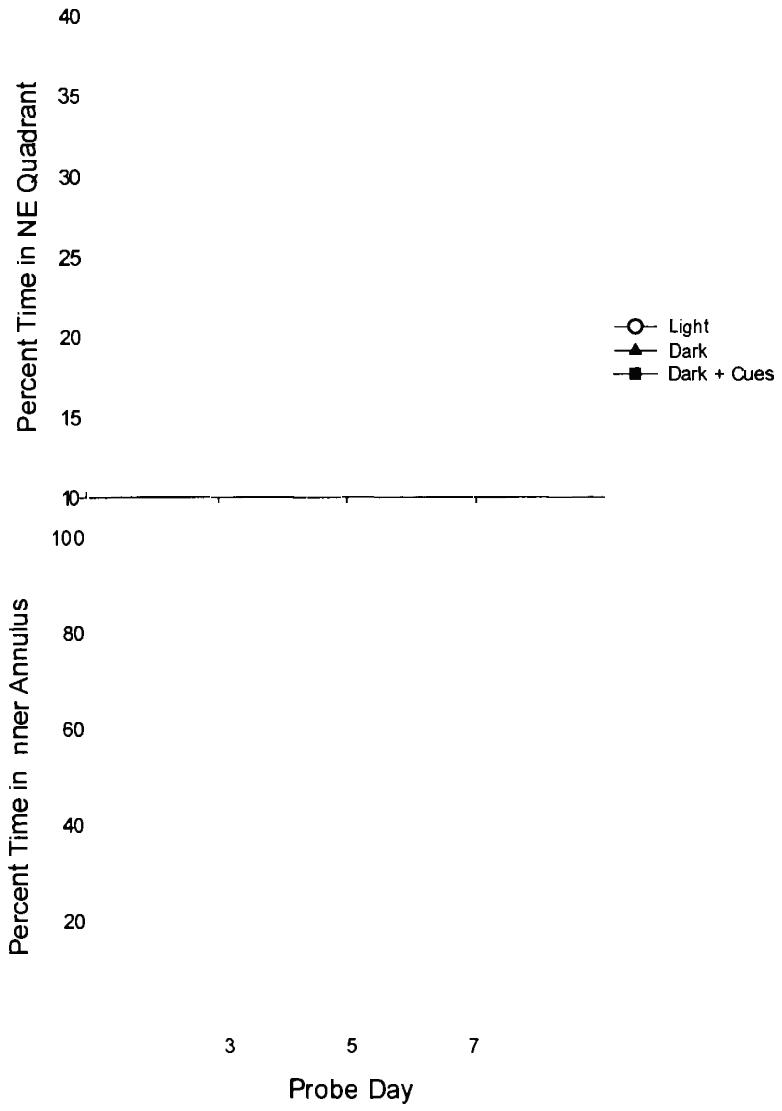


Figure 2. Probe data from pilot experiment with normal Sprauge-Dawley rats. Probe trials were performed on days 3, 5, and 7 of training. The percent time the rats were in the NE quadrant is plotted in the top graph and the percent time in the inner annulus is plotted in the bottom graph. The diagram in the lower graph shows the location of the escape platform in relation the quadrants and inner/outer annulus.

in the dark showed a pattern similar to the Light group with increased time spent in the NE quadrant and decreased time spent in the NW outer area (Figure 3, middle). The groups also differed in the development of searching patterns while in the NE quadrant, [groups x probes, $F(4, 30) = 5.7$, $p = .001$]. The Dark group spent more time in the outer annulus than inner annulus of the NE quadrant on probe day 3, but on the next probe the pattern reversed (Figure 3, middle). The Light group had a similar pattern except that the time spent searching in the inner and outer annulus did not differ on probe days 5 and 7 (Figure 3, top). The Dark+ Cues group spent more time in the inner quadrant on all probe days (Figure 3, bottom).

Discussion

The results of the pilot study confirm the findings of previous studies that rats can learn to solve a Morris water maze even in the absence of visual cues (Prusky et al., 2000; Linder et al., 1997). In spite of the ability of these rats to solve the maze, the addition of the visual or non-visual navigation cues did not reliably improve performance during training. However, the probe tests showed that the rats in the Dark+ Cues group showed some benefit of the available nonvisual cues at least early in training, since their pattern of searching differed from the Light and Dark groups. In the probe trial, the Dark and Light groups showed similar patterns of searching, suggesting that these rats were using similar strategies. Since the Light group had visual cues available to them throughout training, this result suggests the possibility that these rats were not optimally using the cues available to them. The removal of the cues on Day 8 indicates that the rats in the Light group were more dependent on the available cues than the other two groups as their performance was dramatically disrupted on the first trial. The quick recovery of

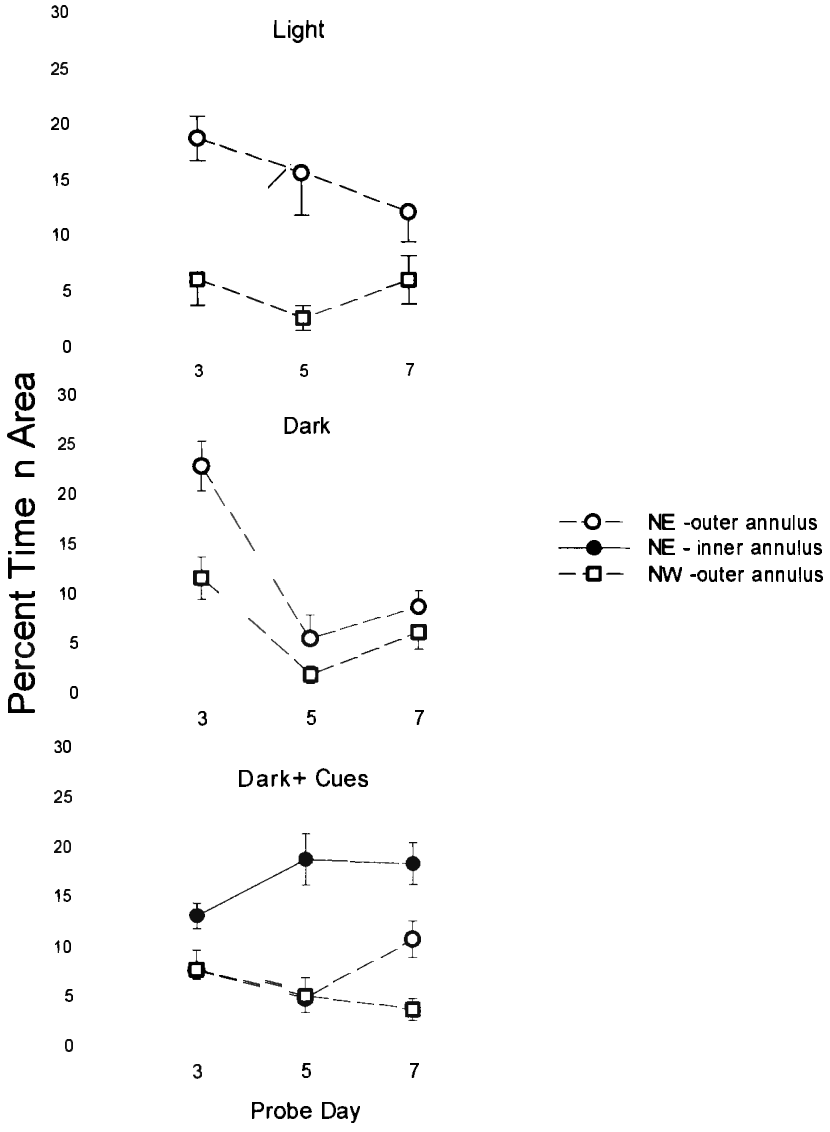


Figure 3. Percent Time in the NE inner, NE outer, and NW outer areas during the three probe tests of the pilot study.

performance on the remaining trials on day 8 suggests that the Light group was able to switch to another strategy to find the platform, perhaps the same strategy being used by the Dark group. This strategy may also have been used early in training, and explains why the data for the Light and Dark groups looks similar in the probe trials. It is also possible, however, that the disruption of performance on the first trial of Day 8 in the Light group was due to the abrupt difference in testing conditions and context (i.e. from the light to the dark). However, this nonspecific effect seems unlikely since the Dark group did not show a similar performance disruption, yet they also experienced a sudden change in the environment, being trained in the dark and abruptly tested in the light on day 8. Likewise, removal of the non-visual cues in the Dark+ Cues group did not disrupt performance in the MWM. This suggests that the added cues may have influenced the initial strategies learned to find the location of the platform, but may not be necessary for continued performance in well trained animals (Rossier, 2000). For example, during training the swimming rats may use cues unknown to the experimenters (e.g., changes in the echo of the splashing sound as they approach a wall or a change in how the water bounces off a wall or the platform while swimming) or they may develop a searching strategy that continues to be effective even when the original cues are removed (e.g. overlapping circles, loops, or arcs) (Baldi et al., 2003).

The probe data also showed that the rats in all three groups learned how to find the platform as well as specifically where the platform was located. Overall, the addition of the non visual cues in the Dark + Cues group appears to have benefited the rats in this group. The Dark+ Cues group spent an average of 30.7% of the time in the target quadrant by day 7, rather than the 25% that would be expected if the rats randomly

searched all four quadrants. Collectively, these results confirm previous studies that visual cues are not necessary to learn the water maze and behavior is sufficiently flexible to adapt to changes of the available cues (Rossier et al.2000, Linder et al., 1997; Prusky et al., 2000).

Experiment 1

Method

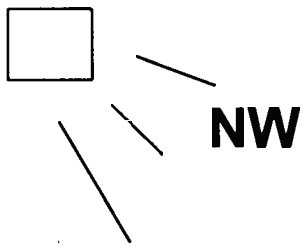
Subjects

Eleven HIV Tg rats, nine Fischer 344 Tg (F Tg) littermate controls, and ten Fischer 344 (F) normal controls were used in Experiment 1. Animals were approximately 5 months of age when used in the experiment. All rats were single housed and maintained on a 12 hour light-dark schedule. Rats were provided ad-libitum food and water during the course of the experiment. All procedures were in accordance with the Institutional Animal Care and Use Committee.

Apparatus

The pool apparatus used in both Experiments 1 and 2 was the same black round tub used in the pilot study, 130 cm in diameter and 52.5 cm in depth. The dimensions for the water in the tub and the platform used are also identical to that used in the pilot study. Two of the spatial navigation cues used in this experiment were the same as those used in the pilot with the Sprauge-Dawley rats. The two olfactory cues, mint and vanilla scented pipe cleaners in the Northern and Southern quadrants, respectively, were the same in the pilot and Experiment 1. Also, the auditory cue was the same and in the same location. In addition to these cues, however, a tactile cue was added in Experiment 1 based on the results of the pilot that showed relatively little cue dependence in the probe tests and in the Cue Rotation. Lengths of fishing line were spaced 10 centimeters apart and tied to four plastic poles. These poles were laid across the western quadrants of the pool so that the fishing line brushed the surface of the pool. The lines thus brushed across the tops of the rats' heads as they swam in the western half of the pool, providing a tactile cue.

Thus, although none of the three cues provided an exact location of the escape platform, they served as relative navigational cues (Figure 4). All rats were run under red light illumination.



Inner

SE

Figure 4. Modified Morris Water Maze. The northern quadrants were marked with a distinctly different smell from the southern quadrants. A tactile stimulus touching the heads of the swimming rats as they swam in the western quadrants distinguished the western from the eastern quadrants of the pool. A single metronome provided an auditory cue from the NW direction. There was no physical demarcation of the inner and outer annulus; this imaginary division was made to facilitate scoring of swim patterns.

Procedure

Rats were run in squads of six for eight days, with rats from all three groups (HIV Tg, F Tg, F) in each squad. On day 1, the rats were given a 60 second pre-training trial immediately before the first trial. Rats were then placed, one by one, into the maze at randomized start locations. Rats were always dropped in the maze facing the maze wall. Each day consisted of 4 consecutive trials where each rats' latency to find the platform was recorded via stopwatch. The time was only recorded once the rats' full body was on the platform. The platform location was fixed in the northeast quadrant of the maze, 22 centimeters from the pool wall. If the rat did not find the platform within 90 seconds, the trial was terminated and the rat placed on the platform. Rats remained on the platform for 10 seconds before being removed from the pool.

Probe trials were performed after the last trial (trial 4) on days 3, 5, and 7 of training. During probe trials, the platform was removed and the rats allowed to swim freely in the maze for 90 seconds. Video recordings of the probe trials were scored for total amount of time spent in each quadrant of the pool. Probe trials were used to validate learning and test for reference memory.

Days 1-7 were training days where rats were placed in the pool at randomized start locations and latency to find the platform was measured. On day 8, the olfactory, auditory, and tactile cues were removed for all rats. The rats were then placed, one by one, into the pool at randomized start locations. The platform location remained in the northeast quadrant of the pool, 22 centimeters from the wall. Latency to reach the

platform for each rat was measured via stopwatch. Cue removal was used to determine the extent to which pool navigation was dependent upon the available cues.

All trials were video taped, and swim paths were traced using the video tapes and tracing paper on a television screen. Pictures were taken of the traced swim paths and path lengths in cm were measured using Image J software to determine distance traveled (Rasband, 1997-2005). This value was divided by the latencies for each rat to calculate swim speed.

Data Analysis

Training and Cue Removal. For training latencies, a 3 x 7 x 4 three factor (group x day x trial) mixed ANOVA was performed with strain (HIV Tg/F Tg/F) as the between subjects factor and days and trials as within subjects factors. Pairwise comparisons were performed using least-significant difference (LSD) post-hoc tests to determine where significant differences found in the ANOVA test are between groups, days, and trials in all statistical analyses where appropriate. The cue removal on day 8 was analyzed separately using a 3 x 4 two factor (group x trial) mixed ANOVA. Cue removal (Day 8) was also compared to the last day of training (Day 7) using a 3 x 2 x 4 three factor (groups x days x trials) mixed ANOVA to examine the effect of the cue removal relative to the optimal performance of the rats on the last day of training. If the HIV Tg rats differ in their dependence on the cues, there should be a significant interaction with group and days or trials in this analysis.

Probe Trials. Probe trials were scored for time spent in each quadrant during the entire 90 second duration. The water maze was divided into 8 quadrant locations, as in the pilot study, inner and outer NE, NW, SE, and SW. Once again, video tapes were

viewed with a template of the quadrant designations marked on a television screen. Using the program Etholog, time spent in each quadrant location was recorded based on the video tape recordings (Ottoni, 2000). Criterion for quadrant entry did not differ from that used in the pilot study. Time spent in each quadrant (inner and outer) for the probe data were analyzed using a 3 x 8 two factor mixed ANOVA (group x quadrant) with strain (HIV Tg/F Tg/F) as the between subjects factor and time spent in each quadrant as the within subjects factor. Further two factor ANOVAs were performed to analyze general time spent in each quadrant (collapse across inner and outer) and general time spend in the inner and outer annuli of the pool (collapse across quadrants). A 3 x 4 two factor (group x quadrant) mixed ANOVA was used to analyze general time spent in each quadrant. A 3 x 2 two factor (group x area) mixed ANOVA was used to analyze time spent in the inner and outer areas of the pool.

Path Lengths and Swim Speeds. The swim paths of the animals were traced from video tape recordings using tracing paper placed on a television screen. Digital pictures of the drawn swim paths were then uploaded onto a computer and imported into the program ImageJ (Rasband, 1997-2005). Paths were re-traced in ImageJ, which has a tool for measuring the length of the traced line. This length is given in centimeters and can then be divided by the recorded latencies to determine the swim speed of each animal in em/s. Path lengths and swim speed were analyzed using three factor (group x day x trial) ANOVAs with strain as the between subjects factor and all other factors within subjects. Analysis of path lengths and swim speeds accounted for motor effects, and helped rule this out as a potential confound to explain significant differences between groups in acquisition of the task.

Results

Training. As can be seen in Figure 5 the rats in all three groups (HIV Tg, F Tg, and F) showed decreasing latencies to find the platform over training days, $F(6,162) = 62.0$, $p = .00$. Latencies tended to decrease over trials during training (data not shown) although by the last day of training this within-day improvement in performance was no longer evident, [days x trials: $F(18, 486) = 2.9$, $p = .000$]. A significant main effect of group, $F(2, 27) = 4.07$, $p = .03$, indicated that the HIV Tg group took significantly longer to find the platform. There were no significant interactions between groups and any other factor.

Probe Trials. There were no differences between groups regarding percentage of time spent in the training quadrant (NE) during the three probe tests, [groups: $F(2, 27) < 1$; groups x probes $F(4, 54) = 1.87$, $p > .1$], and no effect of probe tests, $F(2, 54) < 1$ (Figure 6). The groups also did not differ in the percentage of time spent in the inner annulus, $F(4, 54) < 1$, averaging across probe days. The HIV Tg rats spent 69.0% of their time in the inner annulus while the F Tg rats spent 65.2% and the F rats spent 64.4%. Because there were no interactions between probe and any other factors, the percentage of time in the four quadrants was averaged over the 3 probe days. As can be seen in Figure 6 all three groups searched more in the NE training quadrant compared to the other 3 quadrants, $F(3, 81) = 56.7$, $p = .000$, and the groups did not differ, $F(6, 81) < 1$.

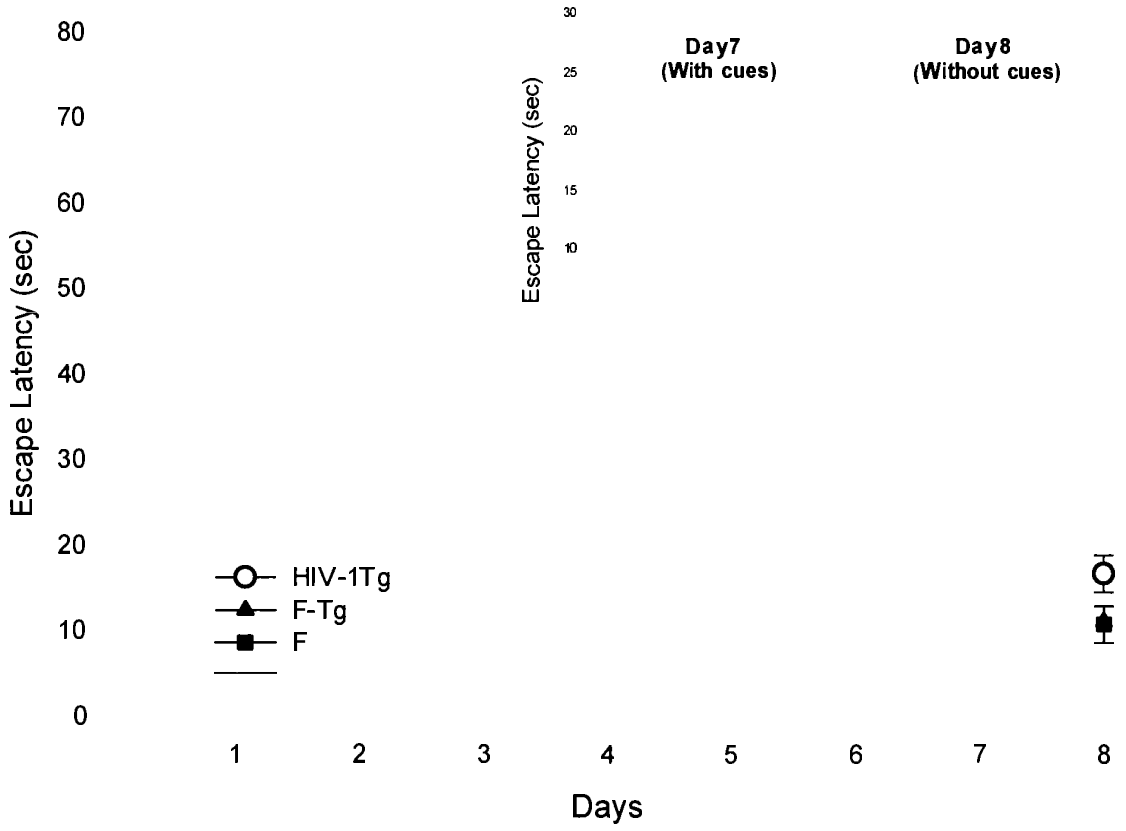


Figure 5. Mean training latencies of the HIV-1 Tg, F Tg, and F 344 rats in Experiment 1. Insert graph shows trial-by-trial latencies on day 7 (the last day the cues were present) compared with day 8 when the cues were removed.

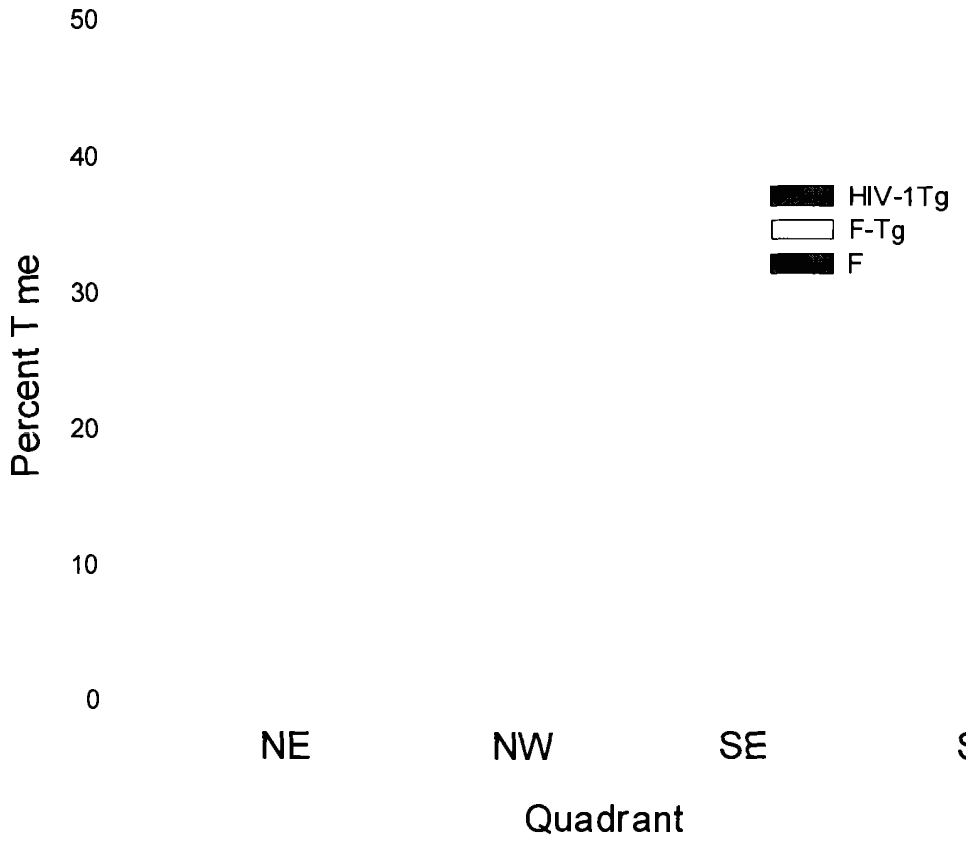


Figure 6. Experiment 1 probe data averaged across all three probe days for groups HIV Tg, F Tg, and F.

Cue Removal. On Day 8 the auditory, olfactory and tactile cues were removed and the rats placed into the pool from random start locations with the platform still located in the NE quadrant. The latencies on Day 8 were compared with the previous day, when the cues were still present (see insert graph in Figure 5). There was only a significant effect of groups in this test, indicating that the HIV Tg rats continued to display higher escape latencies compared to the other two control groups during the test, $F(2, 27) = 4.9$, $p = .02$.

Path Lengths and Swim Speed. To determine if the group differences in latencies could be explained by motor effects on swimming ability in the HIV Tg rats the mean path lengths (cm) were measured and swim speeds (cm/sec) were calculated on days 3, 4 and 5, when the group differences were most obvious (see Figure 5). All groups swam decreasing distances across trials, $F(3, 81) = 5.3$, $p = .00$, and over days, $F(2, 54) = 5.7$, $p = .00$, but the HIV Tg group showed significantly longer paths than the two control groups (data not shown), which did not differ, $F(2, 27) = 5.2$, $p = .01$. A marginally significant three-way interaction, $F(12, 162) = 1.8$, $p = .07$, was due to poor performance of the F controls on the first trial of day 4, the reason for which is unknown. The swim speed for rats in all three groups did not differ over days, $F(2, 54) = 1.1$, $p = .4$, although it tended to decrease by the last trial on each day, $F(3, 81) = 3.2$, $p = .02$. A significant effect of groups, $F(2, 27) = 3.4$, $p = .05$, and a three-way interaction, $F(2, 162) = 1.82$, $p = .05$, suggested differences in swim speed between groups. Post-hoc analysis indicated that the HIV Tg group generally swam slower than the F Tg controls during the first 3 trials but not on the 4th trial when the control animals may have been showing some

fatigue. The F control animals did not differ from the HIV Tg rats on trial 3 of day 4 (Figure 7).

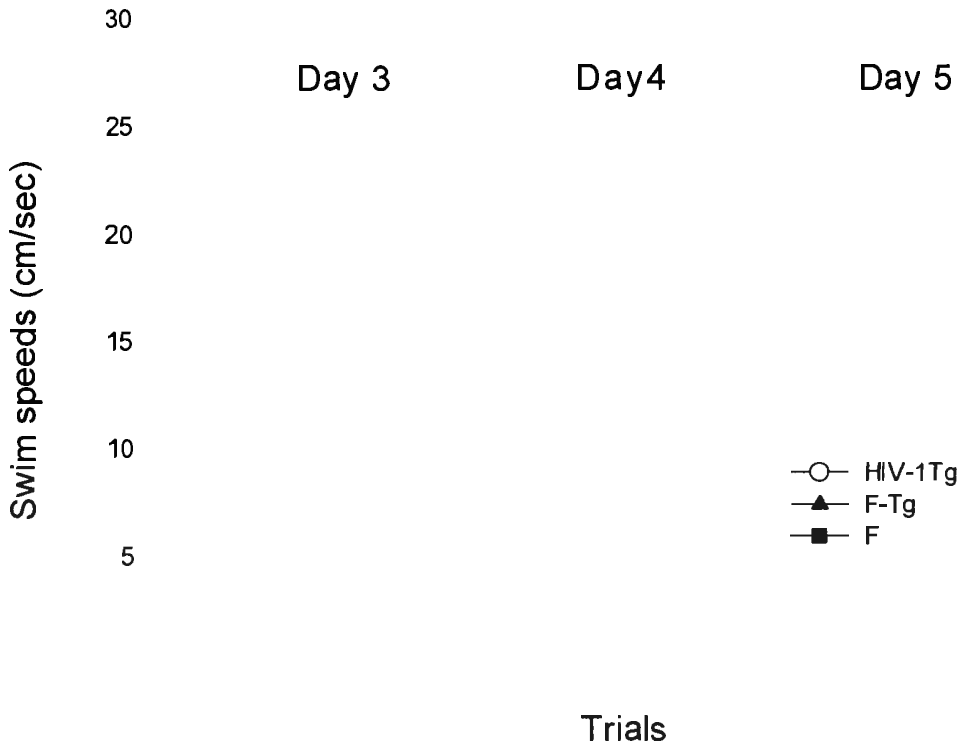


Figure 7. Swim speeds for the HIV Tg, F Tg, and Frats during training days 3, 4 and 5 in Experiment 1.

Discussion

This study showed that the addition of the tactile cue in combination with to the two non-visual cues used in the pilot study benefited the animals in all three groups. Experiment 1 showed that rats tested with the added tactile cue spent 43.7% of the time in the target quadrant on the first probe trial on day 3, compared to the 30.7% observed in the Sprauge-Dawley rats by the 7th day. In spite of the benefits from this added cue, the HIV Tg rats still showed overall longer latencies to reach the platform than the control

groups. The longer path lengths of the HIV Tg rats indicate that the poorer performance was not due to a general motor deficit, but a result of longer search times. The HIV Tg rats did swim slightly slower than controls in the analyzed sessions, but this effect was due to the increased swim speed in the control groups in earlier trials which generally declined by later trials to match speeds of the HIV Tg rats. The HIV Tg rats were very consistent in their swim speed across all trials and seemed to show no evidence of fatigue. The poorer performance of the HIV Tg rats, therefore, does not suggest a reduced ability to swim. Results of Experiment 1 provide evidence for the role of chronic viral effects on spatial learning as measured by the MWM. These results have implications for the potential use of the HIV Tg rat to uncover the relationship between HIV-1 brain effects and learning impairments.

Experiment 2

Method

Subjects

Thirteen Fischer 344 HIV Tg rats, thirteen Fischer 344 Tg littermate controls, and thirteen normal Fischer 344 control rats were tested in Experiment 2. Animals were approximately 5 months of age when used in the experiment. The animals were double or triple housed in standard shoebox cages and provided free access to food and water for the duration of the experiment. The rats were maintained on a 12-hr light-dark schedule.

Apparatus

The pool, platform, and cues used in Experiment 2 were identical to those used in Experiment 1, and were in the same configuration (See Figure 1).

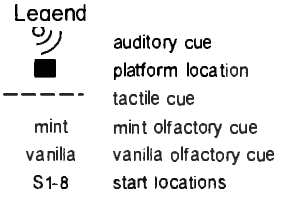
Procedure

All rats were run in squads of 6, with rats from all three groups (HIV Tg, F Tg, F) in each squad. The squads were run consecutively over the course of 19 consecutive days with each rat receiving four trials per day. Water temperature was taken daily and maintained at approximately 20±1°C. As in Experiment 1, rats were held in wire hanging cages before the start of Trial 1 and between trials. Before phase 1, all rats were given a pre-training trial in which they were placed on the platform for 60 seconds. Rats that did not stay on the platform during pre-training were repeatedly placed back onto the platform. The experiment proceeded in three distinct phases (See Figure 8).

Figure 8. Procedures for all phases and tests of Experiment 2. Phase 1 tested response learning, Test 1 tested for behavioral flexibility, Test 2 tested for quadrant search strategy, Phase 2 tested quadrant search strategy and reversal learning, Cue Rotation tested for dependence on the cues, and Phase 3 tested for perimeter search strategy.

Procedures of Experiment 2

Procedure	Description			
Phase 1	Fixed platform (NE), fixed start			
			S1	
Probe	No platform, fixed start	S2		
			S1	
			S5	
Test 1	Fixed platform (NE), variable start	S7		S3
			S1	
			S5	
Test 2	Variable platform (NE), variable start	S7		S3
			S1	
			S5	
Phase 2	Variable platform (SW), variable start			S3
Cue Rotation	Variable platform (SW), variable start, cued rotated 90°	S7		S3
			S1	
			S5	
Phase 3	Variable platform (perimeter), variable start	S7		S3
			S1	



In Phase 1, the platform was held in a fixed location for all days and all trials. The platform remained in the Northeast (NE) quadrant, 22 cm from the pool wall and 2 cm below the water surface. The rats were dropped into the pool from a fixed start location that was counter balanced between rats. Half of the rats were started from the Southern (S) start point where a left turn leads to the platform, while the other half of the rats were started from the Western (W) quadrant where a right turn leads to the platform. This counter balance of start locations was done to eliminate the potential confound of turning preference that may be present in the HIV Tg rats. According to the distributor, the HIV Tg rats may have a tendency to rotate in a particular direction. For example, if the rats have a tendency to turn right, then starting them from the left side of the pool may put these rats at a disadvantage since swimming to the right would take longer to reach the platform than swimming to the left. Since there is no knowledge of whether this turning preference transfers to performance during swimming, the platform location remained fixed for all rats, but the start location (left or right side) was varied among rats. If the HIV Tg rats have a turning preference, this counter balancing may balance out the effect of such a preference on performance. Latencies to find the platform were measured with a stopwatch for each rat on each trial. Once the rats reached an average criterion of less than 20 seconds to find the platform over 4 trials (5 days), the rats were given a 60 second probe trial where the platform was removed and time spent in each quadrant was recorded. If the rats learned and remember the general location of the platform they should persist searching in the target area during the probe test. To test for behavioral flexibility, the rats were given one day of variable start locations with the

platform in the same fixed location as previous trials (NE). Latency to find the platform was recorded. This single day of variable start location in Phase 1 was termed Test 1. If the rats remember the general location of the platform using the available non-visual cues they should be able to find the target area even when they begin their swim from new start locations. If the HIV Tg rats are not able to display such behavioral flexibility, it may indicate that the rats are impaired in learning the general location of the platform, or it may be that the rats are using a response learning strategy rather than a place learning strategy. Since this initial phase of the experiment is relatively easy, the animals may be learning that a simple response (swim to the right or swim to the left) is all that is necessary to solve the maze. If the HIV Tg rats are using such a strategy, differences in latencies may not be evident during training trails, but would be revealed during the test phase where start locations are randomized, and a place learning strategy is now required to find the platform.

To examine search strategy within the original target quadrant, a second test (Test 2) was done the next day. The platform location remained in the NE quadrant, but the platform was no longer fixed at 22 cm from the wall. While the platform was always located in the same quadrant, it was moved randomly within that quadrant, so that the rats were unable to predict the exact location of the platform from trial to trial. In this test, the rats should be able to use the same memory for the general location of the platform that they established during training, but would need to learn an effective strategy to search in the NE quadrant for a "moving" platform (Choi et al., 2006). Thus, the rats were dropped into variable start locations around the maze and the latency to find the platform as well as time spent in each quadrant was measured.

The remaining phases were used to manipulate search strategy independent of place learning. Phase 2 resembled the second test performed at the end of Phase 1, only the platform location was reversed to the SW quadrant. As in Test 2, rats were dropped into the pool from various start locations, and were required to find a "moving" platform within the SW quadrant. Thus, the rats can use the same search strategy as in Test 2, but would need to learn the location of a new place (i.e., form a new memory for a new general location of the platform). This change is similar to the "reversal learning" procedures that have been used in previous studies. Video tapes of Phase 2 trials were scored using for time spent in each quadrant similar to the Probe Trials of Experiment 1 and at the end of Phase 1 of Experiment 2. At the end of Phase 2, the cues were rotated 90°, but the platform location remained in the SW quadrant and varied from trial to trial, just as in Phase 2. Rotation of the cues, but not the target quadrant should result in the rats searching in a different quadrant if they are relying on the cues for a place learning strategy. This test helped determine how dependent upon the cues the rats were and how well the cues lend themselves to the use of a place learning strategy, similar to the cue removal in Experiment 1. Rats that are more dependent upon the cues may have a more difficult time adjusting to the cue rotation. After six days of Phase 2 with the SW target quadrant, Phase 3 began.

Phase 3 was designed to force a change in the search strategy and make place learning irrelevant. In phase 3 the rats were given five days of strategy reversal learning, where the platform was located in variable quadrants, but always along the outside edge of the pool. The rats must switch strategies from general quadrant search, to a thigmotaxic strategy in order to find the platform. The rats were dropped into the pool

from variable start locations and the location of the platform was varied from trial to trial, but always 10 cm from the pool wall. Latency to find the platform was measured as before. Video tapes were also scored for time spent in each quadrant as in Test 2 and Phase 2. This final phase was especially difficult since rats are required to change to a completely new swim strategy and abandon the use of place information.

Data Analysis

Training, Test 1, and Test 2. For Phase 1 latencies, a 3 x 5 x 4 three factor (group x day x trial) mixed ANOVA was performed with strain (HIV Tg/ F Tg/ F) as a between subjects factor and days and trials as within subjects factors. Pairwise comparisons were preformed using LSD post-hoc analyses to determine where significance found in the ANOVAs lies between groups, days, and trials in all analyses when necessary. For latencies on the test of behavioral flexibility immediately after Phase 1, a 3 x 4 two factor (group x trial) mixed ANOVA was performed. Latencies on the behavioral flexibility test day was also compared with the last day of Phase 1 using a three factor 3 x 2 x 4 (group x day x trial) mixed ANOVA. Similarly, Test 2 was analyzed separately using a two factor 3 x 4 (group x trials) mixed ANOVA. Test 2 was also analyzed compared to the last day of Phase 1 using a 3 x 2 x 4 three factor (group x day x trial) mixed ANOVA. If the HIV Tg rats had a more difficult time with the switch from a specific place strategy to a general place strategy, significant interactions with group and day or trial would emerge in this analysis. The data from the remaining phases was analyzed separately with similar 3-way ANOVAs, followed by additional analyses when necessary. In addition, for each transition between stages the last day of the previous phase was compared with the first day of the subsequent phase with a 3-way ANOVA. If the HIV

Tg group experienced greater difficulty with the transitions from one phase to the next, the group factor should interact significantly with days and/or trials.

Probe Trials. Probe trials were scored as time spent in each quadrant during the entire 60 second duration. The water maze was divided into 8 quadrant locations as in Experiment 1 and the pilot study: inner and outer NE, NW, SE, and SW. Also as in the previous two experiments, video tapes were scored for time spent in each quadrant with a template of the quadrant designations marked on a television screen using the program Etholog (Ottoni, 2000). New quadrant location entries were determined as when the head of any rat passes through the designated quadrant boundaries. Time spent in each quadrant (inner and outer) for the probe data was analyzed using a 3 x 8 two factor mixed ANOVA (group x quadrant) with strain (HIV Tg/F Tg/F) as the between subjects factor and time spent in each quadrant as the within subjects factor. Further two factor ANOVAs were performed to analyze general time spent in each quadrant (collapse across inner and outer) and general time spend in the inner and outer annuli of the pool (collapse across quadrants). A 3 x 4 two factor (group x quadrant) mixed ANOVA was used to analyze general time spent in each quadrant. A 3 x 2 two factor (group x area) mixed ANOVA was used to analyze time spent in the inner and outer areas of the pool.

Swim Paths. Swim paths were traced and transformed into digital format. Comparisons were then made across days and trials to determine the number swim paths that met 3 categorical differentiations. The first category was perimeter search, where the rats had to show the majority (more than 50%) of their path length in the outer area of the pool. The second category was looping or mixed strategy. If the rats did not consistently show a perimeter search pattern, their overall category for the 4 traced trials fell into this

category. The third category was unknown strategy. Any swim paths that did not meet the criteria of the other two categories were classified as unknown strategies. Percentage of perimeter search patterns were calculated as well as the overall category for each individual rat and number of rats displaying which categorical swim paths were determined.

All data from all experiments and all phases was analyzed using SPSS for Windows version 10.0. Data for both experiments is presented as the mean± standard error. Significance was accepted at $p < 0.05$.

Results

Phase 1 (Training, Probe, Test 1, and Test 2). Data for training days 1-5 of Phase 1 are shown in Figure 9. Data points are averaged values of all four trials for each day. As is seen in Figure 9, rats in all three groups significantly decreased latencies over days, $F(4, 144) = 68.22$, $p = .000$.

There was also a significant day x trial interaction, $F(12, 432) = 1.823$, $p = .042$, reflecting a decrease in the difference between trials 1 and 4 over days (data not shown). That is, on days early in training latencies decreased over trials, but by the last day of training the latencies were similar across trials. This data shows that all of the rats were able to use the available non visual cues to solve the modified MWM. There was a significant between subjects effect of group, $F(2, 36) = 3.840$, $p = .031$, however,

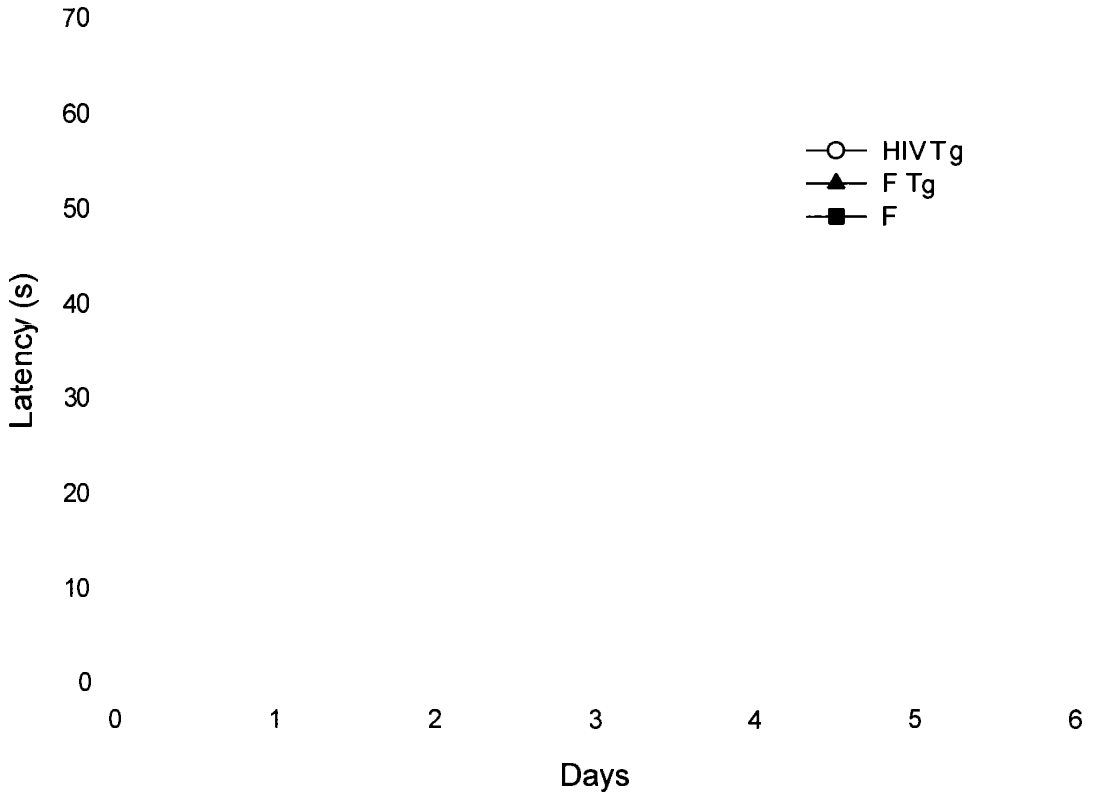


Figure 9. Training latencies for all three groups, HIV-1 Tg, F Tg, and F 344 during Phase 1 of experiment 2. During this phase, the platform location and the start location were fixed for all trials on all days.

indicating that as in Experiment 1, the HIV Tg rats were overall slower to find the platform than the two control groups.

On the final training day of Phase 1 (day 5), a single 60 second probe trial was performed. The probe trial was scored for percent time spent in each quadrant, shown in Figure 10.

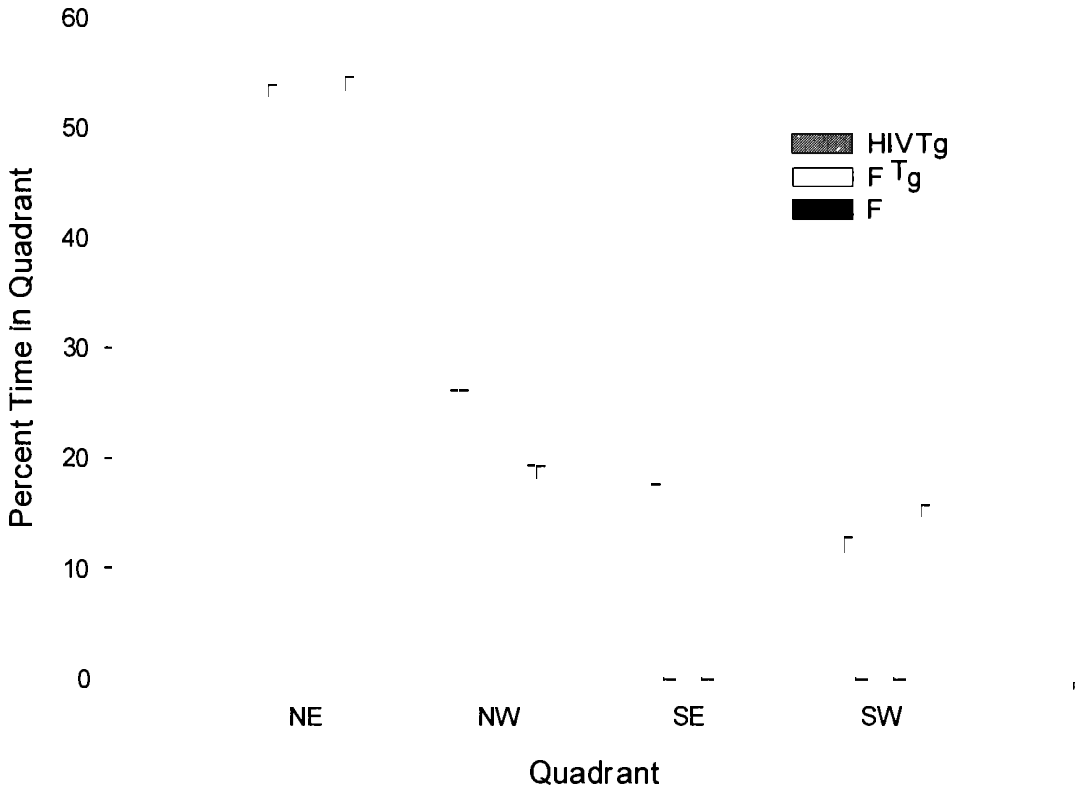


Figure 10. Probe data in which the platform is removed and the rats allowed to swim for 60 seconds. The probe trial was performed after the last trial of the last day (5) of phase 1. Probe data is presented in percent time spent in each of the four quadrants.

Rats in all three groups spent significantly more time (about 50% of time) in the NE or target quadrant, $F(3, 108) = 85.856$, $p = .000$. This indicates that all three groups learned the location of the platform during training in Phase 1. However, an analysis of the time spent in the inner and outer areas of the NE quadrant revealed a significant group x area (inner or outer) interaction, $F(2, 36) = 7.396$, $p = .002$.

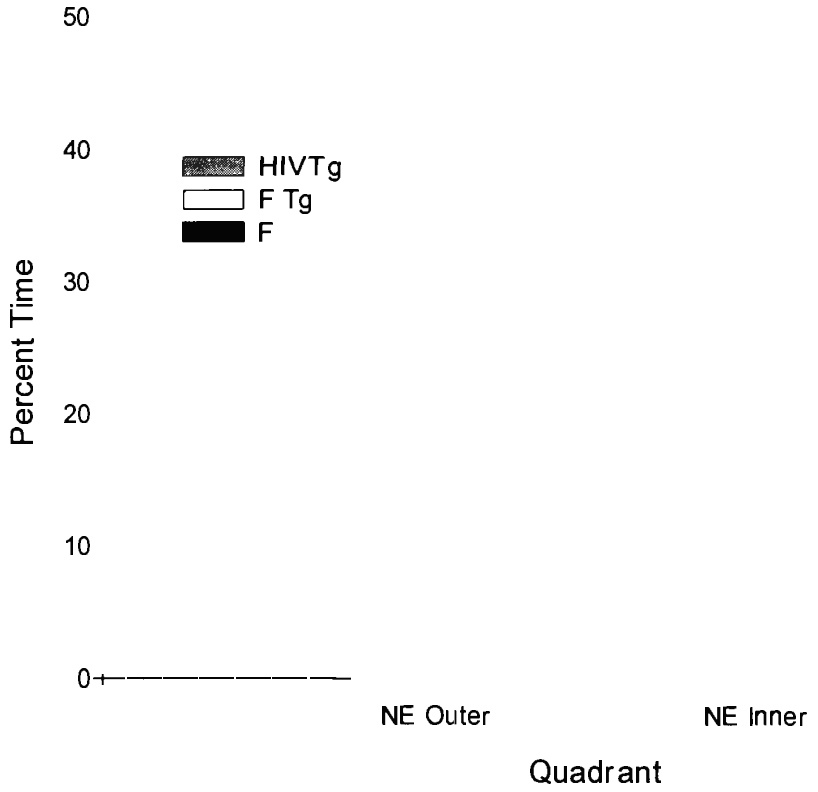


Figure 11. Probe data broken down across inner and outer areas of the NE quadrant.

The source of this interaction can be seen in Figure 11. While the HIV Tg rats spent significantly more time in the NE quadrant, just as did the controls, these rats displayed a different behavioral pattern. The platform was always fixed in the inner annulus of the NE quadrant in Phase 1. The F Tg and F rats spent more time in the inner NE area versus

the HIV Tg rats, $F(2, 36) = 61.548$, $p = .000$, indicating that these rats may have learned the platform location more precisely. The HIV Tg rats, in comparison, spent equal amounts of time in the NE inner and outer areas. There may be several reasons for the behavior of the HIV Tg rats in the probe trial which will be explored further later in this paper.

The trial-by-trial latencies of Test 1, when the rats were started from randomized locations, can be seen in Figure 12. The latencies from the last training day of Phase 1 (Day 5) are included for comparison. To determine if groups responded differently to this change in the procedure the latencies from both days were analyzed with a groups (3) x day (2) x trials (4) mixed ANOVA.

Figure 13 shows latencies for all four trials on Test 2, when the platform was moved between trials within the NE quadrant. The latencies from the last training day of Phase 1 (day 5) are included for comparison.

Test 2 was compared with the final day of Phase 1 using a 3 (groups) x 2 (days) x 4 (trials) mixed ANOVA. A significant day x trial interaction here, $F(3, 108) = 3.213$, $p = .026$, indicated that the difference between trials was affected by the movement of the platform. This was due to the fact that the rats could not reliably go to one single location within the quadrant in Test 2 as they had during Phase 1, therefore the trial-by-trial mean latencies varied for all three groups during Test 2. Although the group differences no longer appear apparent during Test 2, the expected groups x days interaction was not significant, $F(2, 36) = 2.45$, $p = .10$. The overall decrease in group differences on Test 2, however, was sufficient to result in a nonsignificant main effect of groups, $F(2, 36) = 2.19$, $p = .13$.

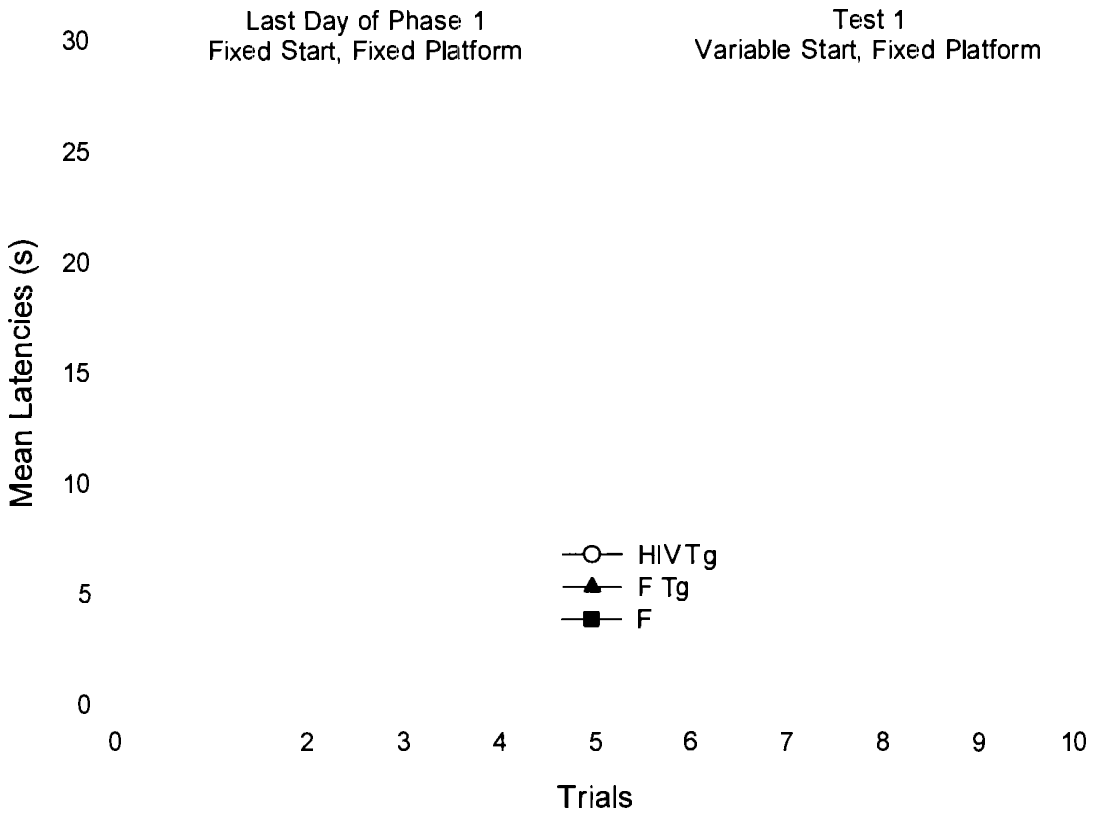


Figure 12. Latencies for all trials on the last day of phase 1 (day 5) and on Test 1 which took place the next day. Test 1 consisted of randomized start locations with the same fixed platform location as in phase 1.

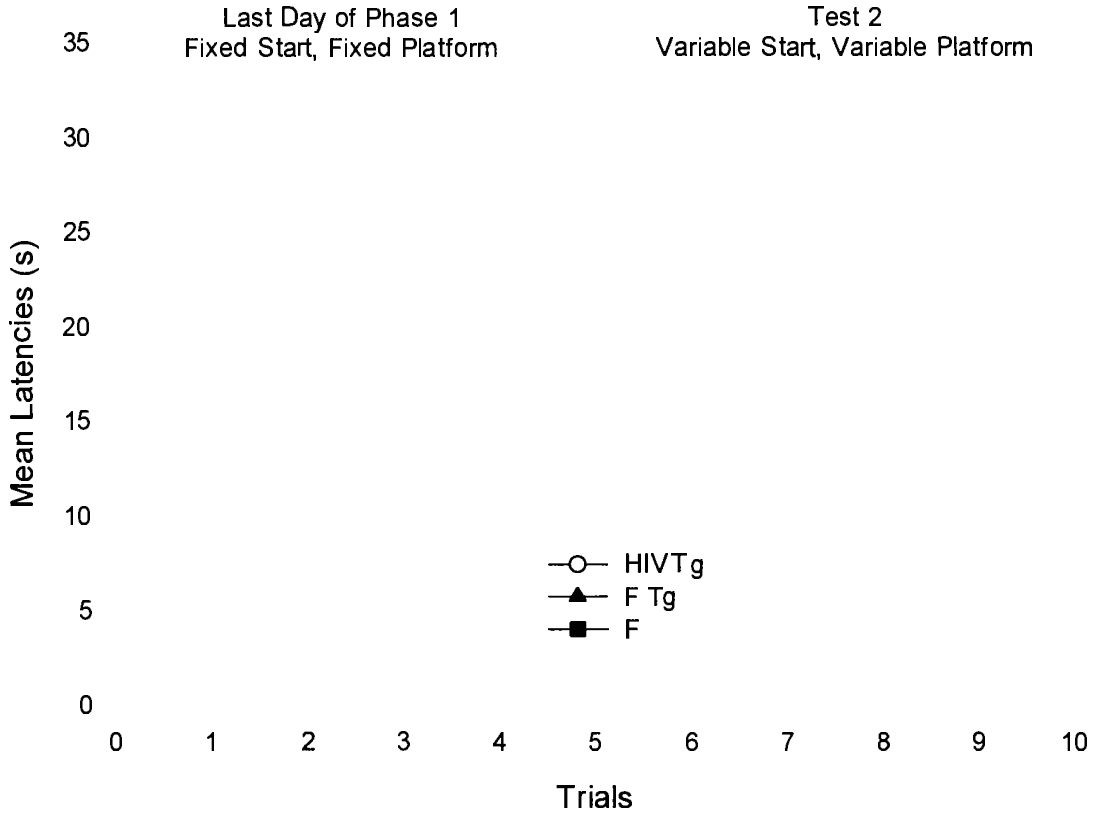


Figure 13. Latencies for all trials on the last day of Phase 1 (day 5) and on Test 2 which took place two days later. Test 2 consisted of randomized start locations and randomized platform locations within the NE quadrant, which was the same location as during Phase 1 training.

Phase 2. Phase 2 reflected the implementation of both a general quadrant search strategy and a reversal learning of what quadrant to search in. Results of Phase 2 are shown in Figure 14. Phase 2 revealed a significant day x trial interaction, $F(12, 432) = 3.829$, $p = .000$, as well as a between subjects main effect of group, $F(2, 36) = 20.301$, $p = .000$. Post-hoc analyses revealed the between subjects effect of group to lie between the HIV Tg group and the 2 control groups ($p < .05$). The F Tg and F groups did not differ from each other. The HIV Tg rats clearly showed increased latencies throughout Phase 2 over days. The day x trial interaction here indicates a similar change as that seen in Phase 1, where the difference between trials 1 and 4 decreased over days, indicating the decreased time it took the rats to locate the platform on the first trial as training went on.

Phase 2 data was then scored for percent time spent in NE quadrant (the platform location in Phase 1) and in the SW quadrant (the platform location in Phase 2) and analyzed using separate 3 (group) x 6 (days) x 4 (trials) mixed ANOVAs. Figure 15 (top) reveals that all three groups learned to spend less time in the NE quadrant over days of Phase 2, $F(5, 175) = 74.54$, $p = .000$. The HIV Tg rats, however, spent significantly more time in the NE quadrant than controls during all days of Phase 2, $F(2, 35) = 10.97$, $p = .000$. Post-hoc pairwise comparisons indicated that the F Tg and F groups did not differ from each other, but that both groups were significantly different from the HIV Tg group ($p < .05$). The reverse pattern was seen in the SW quadrant where the platform was located (Figure 15, bottom). All groups learned to spend

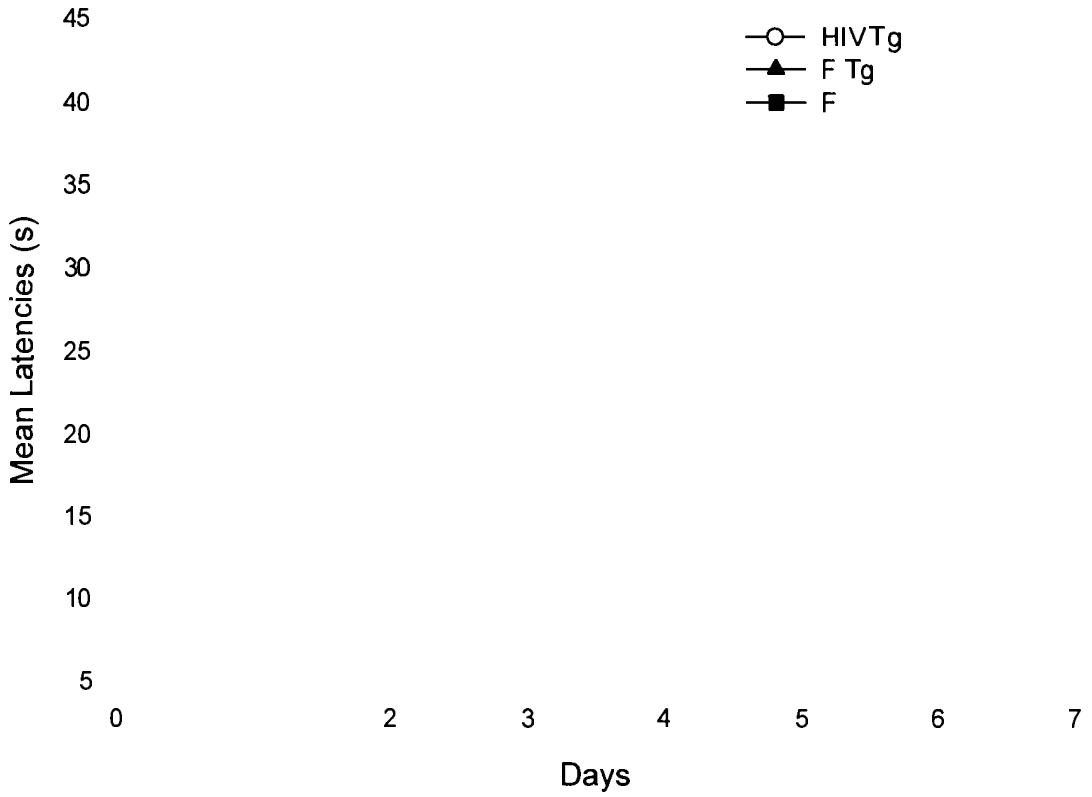


Figure 14. Escapelatencies during phase 2. The platform location was reversed to the SW quadrant, and platform location was randomized for each trial. Start locations were also random.

more time in SW quadrant over days, $F(5, 175) = 35.04$, $p = .000$, but the HIV Tg rats consistently spent less time in this area than the other control groups , $F(2, 35) = 15.404$ $p = .000$. Post-hoc pairwise comparisons revealed this difference to lie between the HIV Tg rats and the two control groups only ($p < .05$). Both percent time spent in NE quadrant and SW quadrant revealed significant day x trial interactions [NE: $F(15, 525) = 45.969$, $p = .000$; SW: $F(15, 525) = 25.708$, $p = .000$]. The day x trial interactions indicate that rats in all three groups are learning the new platform location over days as well as over trials, yet the HIV Tg rats may have found the reversal more difficult. Analysis of the percent time spent in the SE quadrant also showed significant between subjects effect of group, $F(2, 35) = 7.809$, $p = .002$, (data not shown). Post-hoc paired comparisons indicated that the HIV Tg rats spent more time than the controls groups in this quadrant ($p < .05$), which is located between the NE and the SW quadrants. Significant time spent in the SE quadrant by the HIV Tg rats may help reveal the nature of the HIV Tg rats' deficit.

Percent time spent in the inner annulus during Phase 2 likewise revealed significant day x trial interactions, $F(15, 525) = 8.903$, $p = .000$, and an effect of group, $F(2, 35) = 8.285$, $p = .001$. Figure 16 shows the percent time spent in the inner annulus of the pool during Phase 2. The HIV Tg rats clearly spent more time in the outer area of the pool and consequently, less time in the inner area compared to controls. The divergence of time spent in the inner and outer areas between groups when the platform could not reliably be found in either area, indicates that these rats may have been using different strategies to solve the maze, or one group in particular may have been having a more

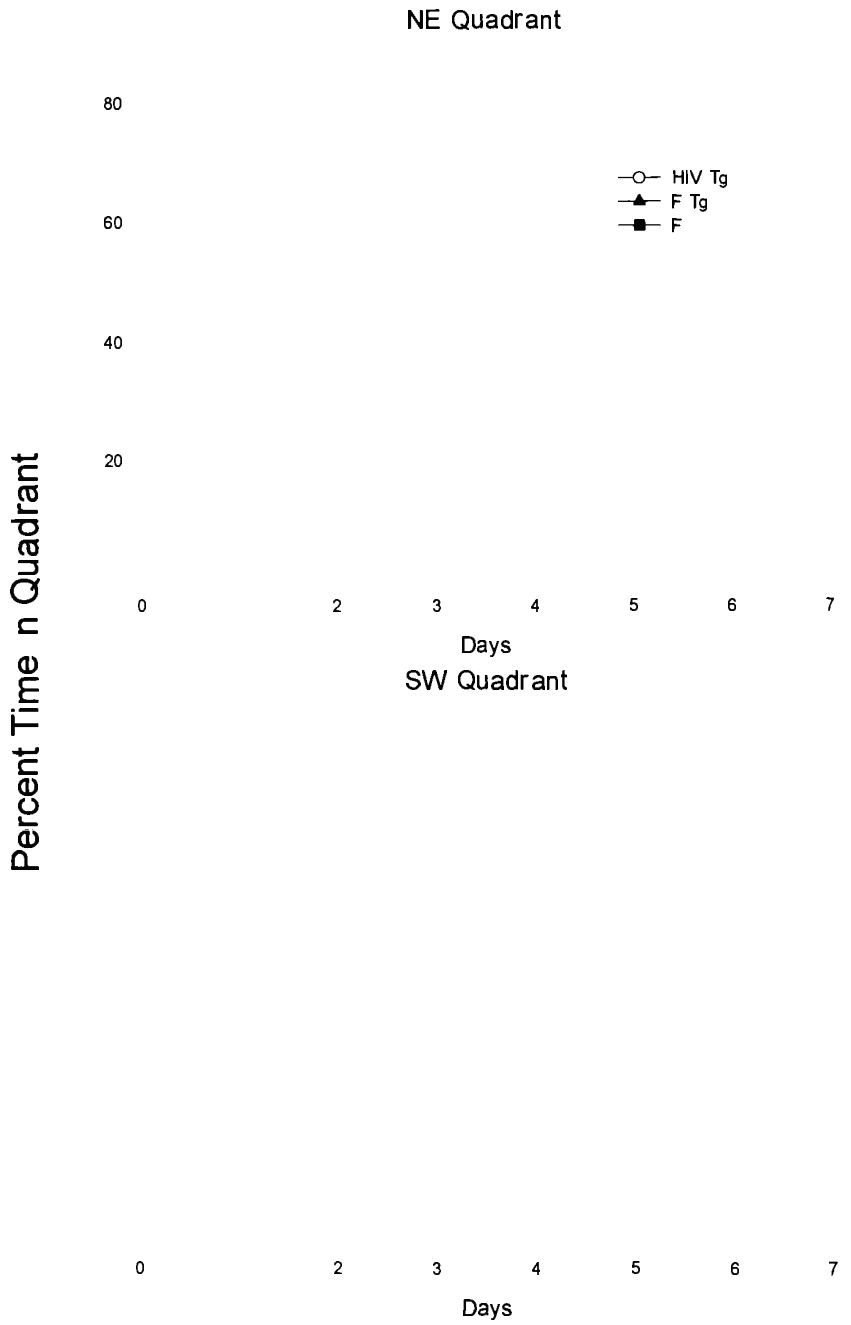


Figure 15. Percent time spent in the NE quadrant (Top) and SW Quadrant (bottom) during the six training days of phase 2. The NW quadrant was the location of the escape platform in the previous phase (Phase 1) and the SW quadrant was the location of the escape platform during Phase 2.

difficult time learning the randomized search strategy. Post-hoc comparisons revealed differences between the HIV Tg group and the F Tg and F groups ($p < .05$), but not between the F Tg and F groups.

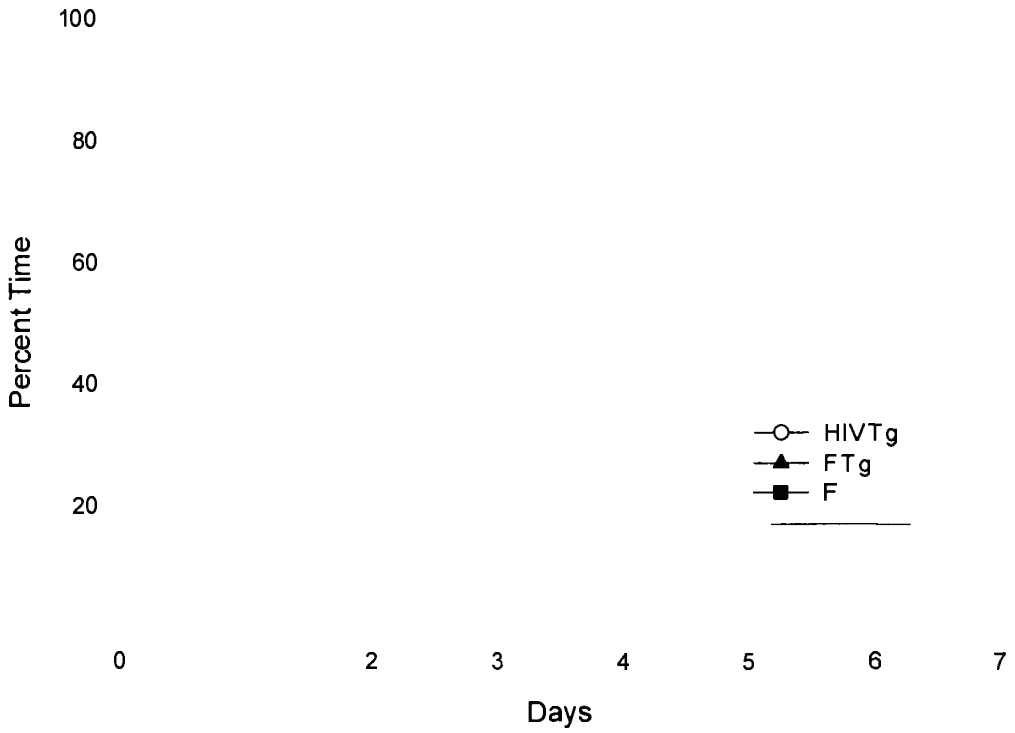


Figure 16. Percent time spent swimming in the inner annulus during Phase 2. The escape platform varied from trial to trial in the SW quadrant, but was always in the inner annulus.

Cue Rotation. The cue rotation data on the day after the end of Phase 2 is shown in Figure 17. The latencies from the last day of Phase 2 are also provided for comparison. Cue rotation was analyzed using a 3 (groups) x 2 (days) x 4 (trials) mixed ANOVA.

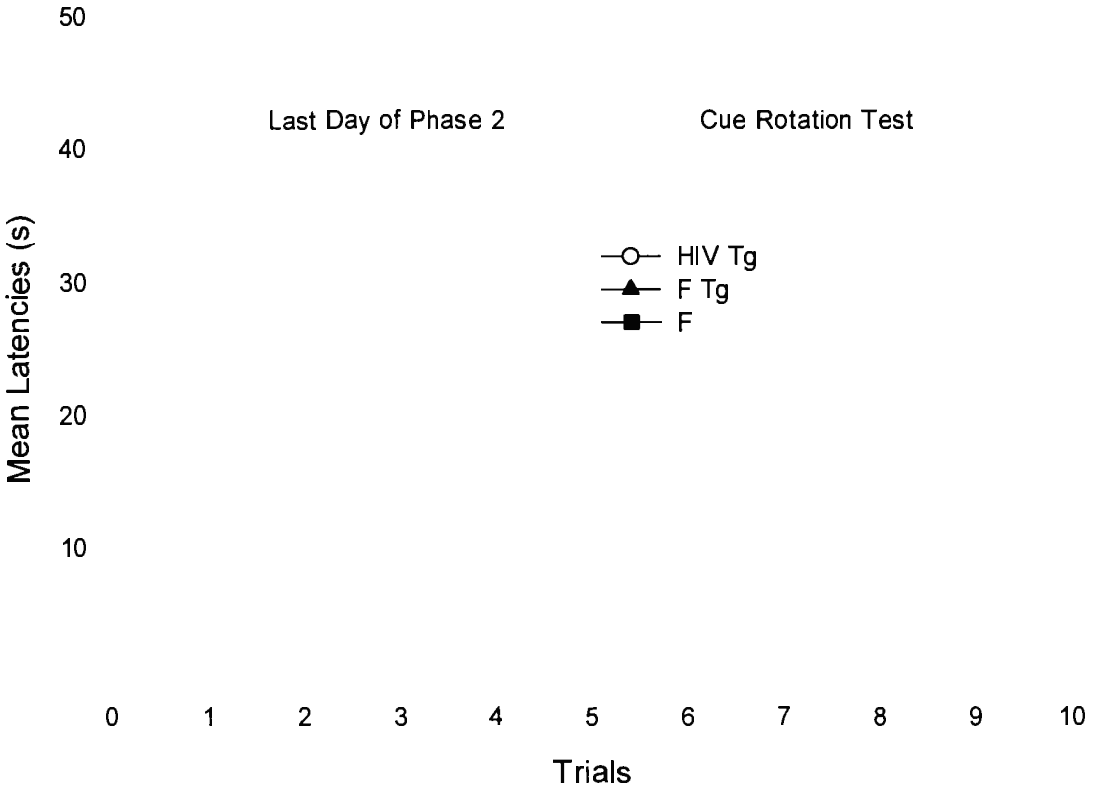


Figure 17. Results of the cue rotation that took place on the day immediately after the end of phase 2. The cues (olfactory, auditory, and tactile) were rotated 90° and the rats were placed in the pool at random start locations.

Statistical analysis of this data revealed significant group x day, $F(2, 36) = 4.129$, $p = .024$, and day x trial, $F(3, 108) = 3.848$, $p = .012$, interactions, and a between subjects effect of group, $F(2, 36) = 9.204$, $p = .001$. The group x day interaction indicates that the three groups responded differently to the rotation of the cues. This interaction reflects a decrease in latencies seen only in the HIV Tg rats on the cue reversal day when compared to performance on the last day of Phase 2. For some reason, the HIV Tg rats actually seemed to benefit from the cue rotation, while the F Tg and Frats did not. The day x trial

interaction reflects the increase in latencies in the final trial of the cue reversal day, which occurred for unknown reasons (possibly an interaction with the start and platform location with the cue rotations).

Phase 3. Figure 18 shows the mean latencies to reach the hidden platform for all five days of Phase 3 when the platform was randomly placed along the wall of the pool. Analysis of the Phase 3 escape latencies revealed a day x trial interaction, $F(12, 432) = 16.935$, $p = .000$, and a between subjects effect of group, $F(2, 36) = 14.427$, $p = .000$. Again, the day x trial interaction is showing that the rats in all three groups showed increasing latencies between trials and between days, and that this difference between trials decreased over days. This effect indicates a learning curve evident in all three groups. However, the group effect indicates that the HIV Tg rats showed a slower rate of learning than the two control groups. Post-hoc pairwise comparisons revealed significant differences between the HIV Tg rats and the F Tg and F controls but not between the control groups themselves ($p < .05$). A groups (3) x phase (2) x trials (4) mixed ANOVA was performed to compare Phase 2 (last day) to Phase 3 (first day). This statistical analysis revealed a marginally significant phase x group x trial interaction, $F(6, 108) = 2.1$, $p = .059$, implying that the HIV Tg rats may have had a more difficult time transitioning between the two phases.

Phase 3 data was scored for percent time spent in each quadrant in the same manner as the probe test in Phase 1. There were no significant differences between

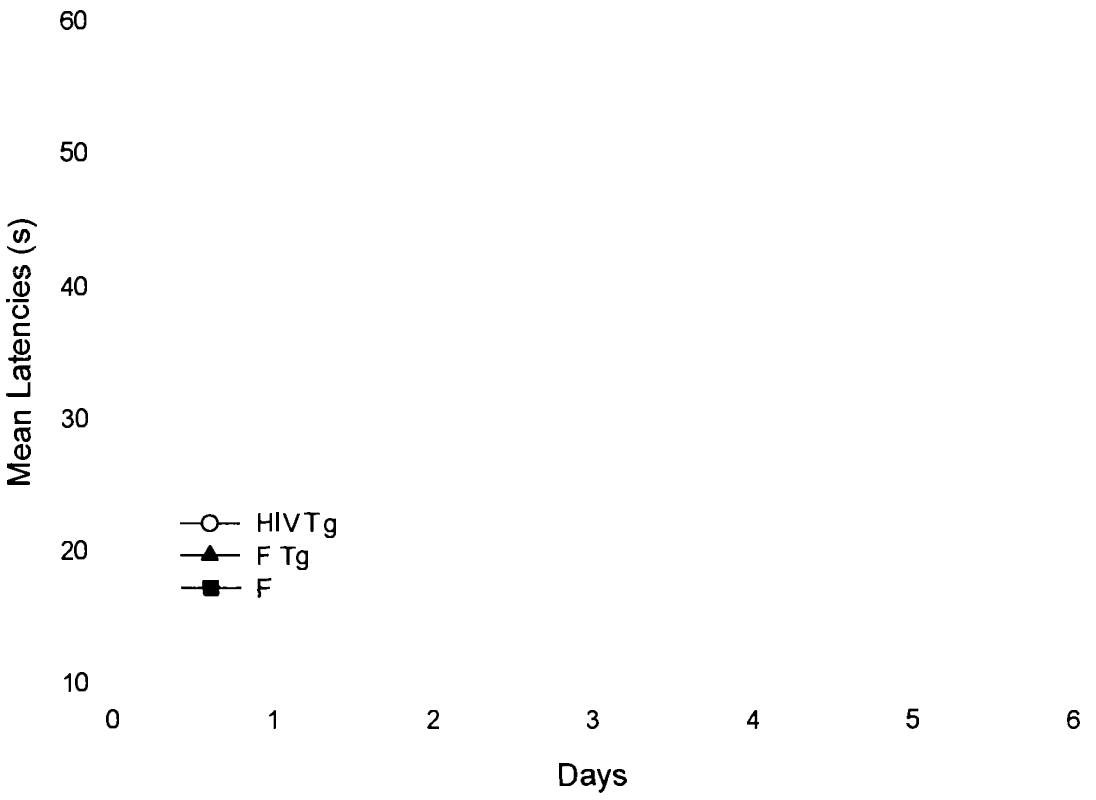


Figure 18. Phase 3 latencies for rats in all three groups. Phase 3 consisted of randomized start locations and randomized platform location along the perimeter of the pool.

groups in time spent in any quadrants during Phase 3. This result reflects that the platform could not reliably be found in one particular quadrant, but instead was found randomly along the outer edge of the pool. The better strategy, therefore, would be to swim in the perimeter of the pool.

Figure 19 shows the percent time spent in the outer annulus for all three groups over days. While the HIV Tg rats spent significantly more time in the outer area of the

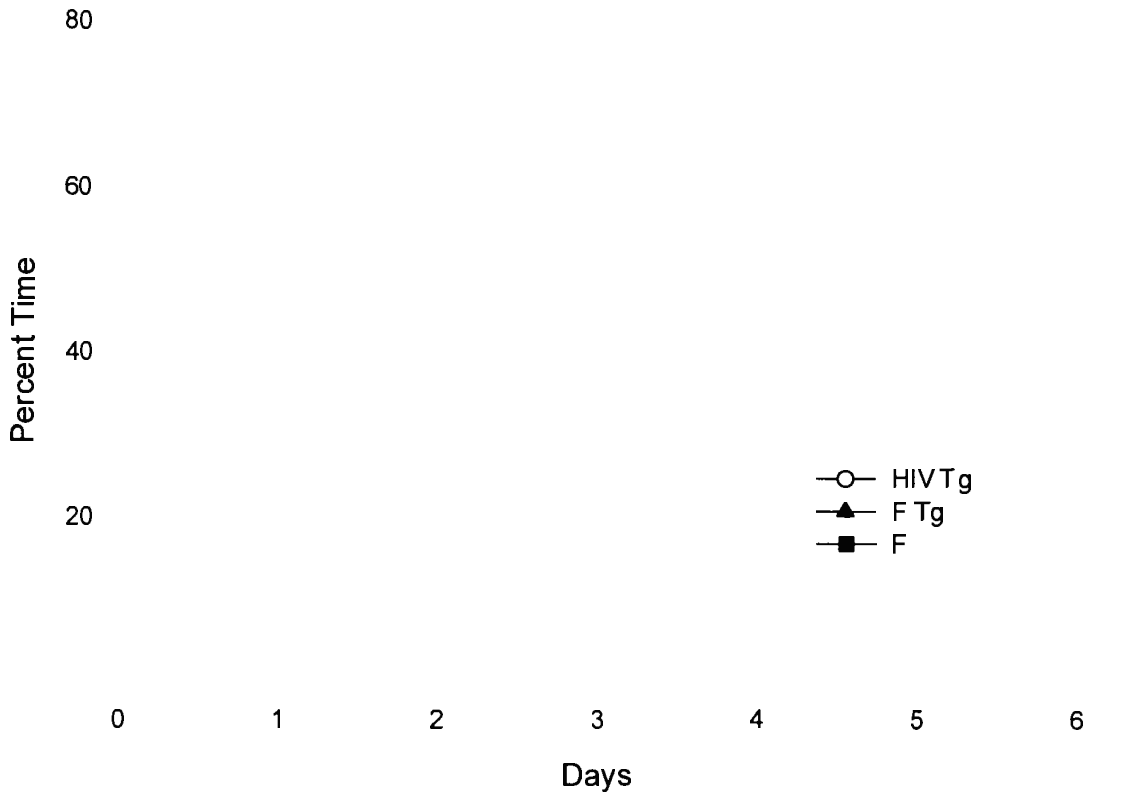


Figure 19. Percent time in the outer annulus during phase 3. The platform was located in the outer annulus for all days of phase 3.

pool during Phases 1 and 2 compared to the control groups, this difference was no longer evident in Phase3. Instead, the HIV Tg rats showed a trend in Phase 3 toward spending less time in the outer annulus when compared to the controls. A significant group x days interaction, $F(8, 144) = 2.135$, $p = .043$, revealed that this group difference emerged on day 5 of Phase 3. Additional post hoc analyses indicated that the Frats spent significantly more time in the outer annulus compared to the HIV Tg rats on this day, but did not differ from the F Tg rats ($p < .05$). The F Tg rats were showing a similar trend toward spending more time in the outer area, thus a more prominent difference between the HIV Tg rats and the controls may have occurred in Phase 3 if training had continued for another day or two. Anecdotal observation of the animals suggested that some rats began to form a circular swim pattern in the outer area of the pool, but did not meet the criteria of being in the outer annulus. For this reason, swim paths for Phase 3 were traced to determine the specific strategy used by rats in all three groups, particularly during the last 2 days when the group difference emerged in the time spent in the outer annulus. Swim paths were traced for trials 1 and 4 on days 4 and 5. The traced swim paths were analyzed and grouped into three distinct categories: unknown/inconsistent, looping/consistent, and perimeter/consistent. Swim path inclusion criteria for the unknown category were random search with an indistinguishable or inconsistent pattern (Figure 20a). Swim paths in the looping/consistent category included paths that contained at least one loop consistently in three out of the four scored trials (Figure 20b). Perimeter/consistent swim paths were those with the majority of the path in the outer annulus of the pool, approximately 30 centimeters from the edge, in at least three out of the four scored trials (Figure 20c).

The number of rats in each group that displayed one of three different swim patterns is presented in Table 1. The results indicate that the F Tg and Frats were more advanced than the HIV Tg rats in their acquisition of perimeter and looping search strategies required in Phase 3, Chi Square: $\chi^2 = 8.82$, $p = .03$. This search strategy deficit in the HIV Tg rats does not reflect perseveration as the rats did not spend significantly more time in the SW quadrant (data not shown). This phase of the experiment was particularly difficult since even the control animals did not show 100% acquisition of the correct strategy. Since the HIV Tg rats showed increased latencies from the beginning of Experiment 2, when the task was relatively easy, it follows that these impairments would carry over into other, more difficult tests. Even though they are not using the required strategy, however, the rats in all three groups are locating the platform on nearly every trial during this phase. The use of a sweeping, overlapping loop strategy and an arc-like pattern in the water maze seemed to precede the use of a true perimeter search strategy. The F and F Tg groups displayed greater percentage of both

Figure 20. Representative swim paths for the three categorical divisions. The left most column represents unknown/inconsistent patterns, the middle column represents looping/consistent patterns, and the right most column represents perimeter/consistent patterns. These categories are represented by rats in the HIV Tg group (left column), the F Tg group (middle column), and the F group (right column) respectively.

search patterns compared to the HIV Tg rats that seemed to be dwelling in areas near the pool wall, rather than searching along it, or searching randomly throughout the maze.

Table 1
Number of Rats by Group Showing One of Three Categories of Swim Patterns in Phase 3

Swim Pattern	HIV-1Tg	F Tg	F 344
Perimeter/Consistent	3 (23)	5 (38)	6 (46)
Loop/Consistent	4 (31)	6 (46)	7 (54)
Unknown/Inconsistent	6 (46)	2 (15)	0 (0)

Note. Number in parenthesis are percentages
$\chi^2 = 8.82$, $p = .03$

Discussion

In Phase 1 of Experiment 2, significant effects over days and trials indicate that rats in all three groups improved performance over days. That is, that rats in all groups learned the location of the platform and were able to find it relatively quickly and efficiently. This result, once again replicates what has been found in other studies that animals are capable of learning and solving a water maze in the absence of visual cues. This study also replicated the results of Experiment 1 and the pilot study, showing the provided non-visual cues were sufficient for finding a hidden platform in a particular location. Phase 1 also replicated Experiment 1 with the overall group effect showing the HIV Tg rats performed worse than both control groups overall. This is surprising result as Phase 1 of Experiment 2 was thought to be an easier task than training in Experiment 1. In Experiment 2, the platform and the start point both remained fixed, so the animals

were not actually required to form a spatial representation of the platform location to solve the maze. Instead, the rats simply had to swim in a particular direction to reach the platform once they were placed in the water (e.g. swim to the right, a little away from the wall). In spite of the easier nature of the task, the HIV Tg rats continued to perform poorer than controls as in Experiment 1. This is interesting, as the typical progression of strategies in the water maze is from thigmotaxic to place to response (de Bruin et al., 1997). Since place learning is not necessary during this phase, it is typically assumed that rats are using a response strategy to locate the platform (de Bruin et al., 1997; Da Cunha et al., 2006). However, rats often use multiple strategies simultaneously, and prefer a place strategy, switching to a response strategy only after a place strategy proves unsuccessful (de Bruin et al., 1997). It is possible that the rats in all three groups were using a place strategy to solve Phase 1, even though it was not necessary. If this is the case, then Phase 1 of Experiment 2 replicated the significant effects found during training in Experiment 1. Thus, even though this task was set up to be easier than training during Experiment 1, it actually seems to be easier for the rats to use their default place strategy than to use a response strategy. Similar results have been found with the radial arm maze which also tests spatial learning and memory. In the radial arm maze, rats are placed in the center of an apparatus with radiating arms (typically at least 6, but as many as 17). The ends of the arms are baited with food and the animals are allowed to explore the maze and access food within the various arms. Once the food is collected, however, the arms are not re-baited. Thus, it is beneficial for the rat to remember which arms were previously visited as to avoid wasting time and energy searching in a previously visited location. One may expect that in this paradigm rats might search the arms serially,

visiting one arm and then neighboring arms sequentially, but this is not the case. Normal rats will randomly select an arm on each visit and simply remember, based on distal visual cues, the previously visited locations. Even with a 17 arm maze, rats and mice perform exceedingly well in this task, rarely revisiting arms (Olton et al., 1977). When the maze is placed on the floor so that animals may deviate from the arm paths and simply walk on the outer edge of the maze, a strategy with even greater efficiency, the rats still choose to use a random search strategy and rely on their spatial memory to avoid re-visitations (Hoffman et al., 1999). In addition, animals rarely deviate from the arm paths when alternate routes are available (i.e. when the maze is on the floor), suggesting the strength of initial strategies, even when more efficient strategies can be used (Hoffman et al., 1999).

The results of Test 1 further enhance the finding that the rats are using a place strategy to solve the water maze, even though there are no visual cues available. Since the rats were using a place strategy already, the switch to randomized start locations in Test 1 did not significantly impact the performance of the rats in any of the groups, although the HIV Tg rats still failed to match the performance of the controls. This demonstrates the flexibility of behavior necessary for place learning, and implies that this is the primary method of solving the water maze by HIV Tg rats and littermate and normal controls. HIV Tg rats, however, show a slight deficit in place learning in Phase 1 as indicated by the significant group effect reflecting the poorer performance of these rats.

The probe trial immediately following trial 4 on day 5 of Phase 1 also demonstrates that the rats in all three groups were using a place strategy to solve the

maze. Rats in all three groups spent significantly more time in the quadrant where the platform was located. This indicates that all the rats learned the spatial location of the platform in the NE quadrant. The HIV Tg rats, however, were spending more time in the outer areas of the pool compared to controls. Even when the HIV Tg rats were searching in the target NE quadrant they were spending equal amounts of time in the inner and outer areas, indicating that they did not discriminate between these two areas relative to the platform location. This excessive swimming in the outer areas of the pool may be the result of a failure to suppress thigmotaxis in the HIV Tg rats compared to controls, or a failure to recognize that the platform was a certain distance away from the pool wall (and thus in the inner area). It is also possible that the HIV Tg rats may have had a greater reliance on the olfactory cues which were located in the outer area of the pool. A mild olfactory impairment, which has been reported in HIV patients (Razani et al., 1995), may have been present in the HIV Tg rats due to the presence of the virus and associated proteins, and therefore the rats may have needed to be closer to the edge of the pool to use these cues effectively. This possibility is currently under investigation. It is interesting to note that olfactory deficits are linked with cognitive deficits in HIV positive individuals (Razani et al., 1995).

In Test 2 of Phase 1 the rats were required to switch from a specific location search to a general search strategy within a target quadrant. Again, because the rats were using a place strategy during Phase 1 training, it was relatively easy for the rats to switch to a general search strategy. The rats could simply swim to the previously learned location and dwell in the general area for some time. Since this is similar to a probe trial which the rats had previously experienced on the last training day of Phase 1, the rats

quickly learned to search in the NE quadrant to find the platform. Interestingly the HIV rats and the two control groups performed similarly even though it was the first day where the platform varied from trial to trial.

The group difference appeared again after the reversal of the platform location in Phase 2. In this second phase, the platform location was reversed to the SW quadrant, but the general strategy to search within a target quadrant, which was required in Test 2, was still necessary in Phase 2, as the platform was not in a fixed location from trial to trail. All three groups were able to learn the new strategy and were able to find the platform with increasing efficiency, but the HIV Tg rats performed worse than both control groups throughout phase 2. The group effect here suggests that the HIV Tg rats had a more difficult time with the reversal.

The HIV Tg rats spent a greater percentage of time in the NE quadrant throughout Phase 2 when compared to controls (Figure 15 top). Since the NE quadrant was the target quadrant from the previous Phase, this result suggests a tendency for perseveration in the HIV Tg rats. In addition to spending more time in the NE quadrant, the HIV Tg rats also spent less time in the SW quadrant when compared to controls, further indicating that it was the reversal manipulation that resulted in the significant effects evident in Phase 2. The HIV Tg rats also tended to spend more time in the SE quadrant, particularly on day 4. Since the SE quadrant is in between the NE and SW quadrants, it is possible that the increased time spent in the SE quadrant in the middle of Phase 2 reflects an approach-avoidance conflict in the rats. The SE quadrant is the point where the rats must decide to swim toward the previous target quadrant and the new target quadrant. Reversal learning reflects two components of learning, (1) forgetting or

suppression of previously learned information and (2) the learning of new information (Lattal et al., 2003). Thus, for successful reversal learning, animals must re-learn information about stimuli already experienced (Lattal et al., 2003). For example, in Phase 1, the animals in this experiment learned that the configuration of cues available in the MWM predicted the location of a platform in the NE quadrant. In Phase 2, the animals had to learn that those same cues in the same configuration predict the location of a platform in the SW quadrant. This information is conflicting, and may result in uncertainty manifested in behavior as increased latencies. Since the peak difference in performance between groups is on day 4, it is probable that this reflects a difficulty in the HIV Tg rats in re-learning the relationship between the available cues and the new platform location. It is also possible that this difference is reflecting the general place learning deficit in the HIV Tg rats. This possibility is unlikely, as the rats spent approximately 50% of their time in the target quadrant during the probe trial in phase 1, indicating that they are capable of place learning, but their ability is not as precise as controls. While increased time in the SE quadrant may indicate imprecise place searching, percent time would not peak on a single day if this was the case. Also, during the probe trial, the performance of the HIV Tg rats was still precise enough to be in the correct quadrant, albeit more distributed within that quadrant. Taken together, these data indicate that the HIV Tg rats displayed increased difficulty in learning a reversal task in theMWM.

Following Phase 2, the cues were rotated 90° and the rats given 4 trials with the platform location varied in the SW quadrant. The cue rotation improved performance in the HIV Tg group to levels comparable to both control groups, the latter groups of which

were not affected. It is possible that a greater reliance on the cues by the HIV Tg group contributed to this improvement in escape latencies. If the HIV Tg rats were using the cues for navigation, the original orientation of the cues may have been confusing to the HIV Tg rats because, as posited above, it resulted in conflicting responses, one to approach the NE quadrant, and one to approach the SW quadrant. By rotating the cues (but not changing the platform location), the HIV Tg rats may have shifted their search accordingly, and thus spent more time in the SE quadrant since this quadrant now had the cue configuration that previously marked the NE quadrant. The SE quadrant is closer to the SW quadrant, and thus may have resulted in more general search in that area, and lower latencies to find the platform. It is also likely, however, that the cue reversal test day served as just another training day. That is, since the cue rotation occurred immediately after the last day of Phase 2 for all rats , it is possible that the cue rotation had no effect and that the comparable performance between all groups reflected the continuation of a learning curve such that HIV Tg groups caught up to the performance of the control groups. The lack of improvement seen in the F Tg and F groups may not mean that the cue rotation had no effect on them, but rather that latencies for this group reached a floor effect, and the rats could not improve further.

Phase 3 explicitly tested the ability of the rats to switch from a general search strategy within a target quadrant to a perimeter search strategy. The non-visual cues remained available, but did not reliably predict the location of the platform. The platform could not consistently be found in a single quadrant, but instead was reliably found 10 cm away from the pool wall. In this phase, a place strategy, the default strategy typically used by rats, is an unsuccessful strategy to use. In this way, the rats are forced to learn

and use a new strategy independent of the place learning that took place in Phases 1 and 2. The rats in all three groups were able to learn the new strategy and were able to find the platform with increasing efficiency over days as well as within trials, but the HIV Tg rats performed substantially worse than both control groups over all 5 days of Phase 3. There was no evidence of perseveration in the previous target quadrants by the HIV Tg rats. Further analyses revealed that the HIV Tg group was spending less time than the F group in the outer annulus by the last day of Phase 3. Increasing time spent in the outer area is consistent with the acquisition of a perimeter search strategy, but it was still unclear from the percent time data whether this difference reflected strategy learning or some non-specific effect. Evaluation of the swim paths during the last 2 days of Phase 3 revealed that the majority of the F Tg and Frats developed a circular search strategy around the outer area of the pool, whereas most of the HIV Tg rats did not develop such a strategy. These results indicate that the HIV-1 Tg rats may have been impaired in the learning of the perimeter search strategy, rather than failure to inhibit the previously learned strategy, as there was no evidence of perseveration in the previous target quadrants.

General Discussion

Both normal rats and rats showing evidence of performance impairment are able to solve a modified Morris water maze with the aid of olfactory cues, an auditory cue, and a tactile cue. The HIV Tg rats performed worse than controls in all phases of both Experiment 1 and Experiment 2. Results from Experiment 1 may reflect impairments in place learning, although the probe trial in this experiment did not indicate an impairment in reference memory. In Experiment 2, the HIV Tg rats showed impairments in place learning, reversal learning, and strategy learning. Even the probe trial in Experiment 2 suggested a mild impairment in specificity of reference memory. These results indicate a general overall effect in the brain, although several brain areas may play key roles in the specific observed deficits.

The hippocampus is associated with place learning largely because it is the home of certain pyramid cells known as place cells (Hollup, Molden, Donner, Moser, & Moser, 2001; Lattal et al., 2003; Best, White, & Minai, 2001). These cells reflect the space surrounding an individual by responding to particular place fields (Best, White, & Minai, 2001). Place fields have been shown to accumulate around the location of the platform in the MWM, suggesting that these cells may also carry information about a particular goal within the environment (Hollup, Molden, Donnet, Moser, Moser, 2001). The first evidence for the role of the hippocampus in place learning, however, came from running rats in the MWM with hippocampal lesions (Morris et al., 1982). These rats were shown to improve performance, but to never reach the levels accomplished by the sham lesion and no surgery controls (Morris et al., 1982). This suggests that hippocampal lesioned rats still maintain some ability to learn the location of a hidden platform through the use

of non-spatial strategies (Pouzet, et al., 2002). The use of alternate strategies allows the rats to compensate for any spatial memory impairments due to the lesion (Pouzet, et al., 2002). It is possible, if not likely, that the HIV Tg rats suffered some damage to the neurons of the hippocampus, and through the direct role this brain area plays in place learning these rats showed longer latencies and impaired performance. However, the subtle results seen in the above experiments may be the result of either a mild impairment or the ability of these rats to compensate through the use of non-spatial strategies. These non-spatial strategies also explain the development of the looping strategy seen in both the HIV Tg rats and controls during the last two days in phase 3.

The precise nature of the place learning deficit in these animals, however, is unclear. The probe data in both Experiment 1 and Experiment 2 suggests that the learning deficit may not be in learning where the platform is located, since the three groups did not differ in the percentage of time searching in the target quadrant. In Experiment 1, the rats reliably spent more time in the target quadrant as early as day 3 when the first probe was given. Previous studies have shown that rats with hippocampal lesions will swim in the vicinity of the platform during probe trials, but also have longer swim latencies with a greater number of errors (Whishaw et al., 1995). Gradually, these animals develop an overlapping loop pattern search strategy that has been interpreted as a problem with how to reach the target location, rather than a problem of knowing where to search (Cain et al., 2006; Whishaw et al., 1995).

It is also possible that damage to the hippocampus in these HIV Tg rats is causing a different problem in solving the MWM. A growing body of evidence has linked the hippocampus with increased pliancy or flexibility of behavior (Day et al., 1999;

McNaughton & Wickens, 2003). Many drug or lesion induced impairments in spatial navigation can actually be attributed to deficits in non-spatial components of navigation (Day et al., 1999). For example, habituation to the maze and the experimenter, handling procedures, swimming experience, and testing procedures can all affect the final results of a test such as the MWM (Day et al., 1999). It is possible that the hippocampus is not necessary for forming place responses; rather other deficits combined with hippocampal lesions make it difficult to form a place response without contributions from that brain area (Day et al., 1999). In this way, hippocampal damage may reflect a deficit in the ability to efficiently alter or switch between response strategies (Day et al., 1999). Some studies have found that rats with hippocampal lesions will continue to use initial thigmotaxic strategies throughout training and area slow to switch to a new, more efficient strategy (Day et al., 1999). It is possible that the increased times spent in the outer area of the pool by the HIV Tg rats during the probe test in Experiment 2 and as measured by percent time in the outer annulus in Phase 2 of Experiment 2 are reflecting a failure to completely overcome thigmotaxis potentially due to hippocampal damage. The olfactory deficit hypothesis mentioned previously is also a possibility. Olfactory deficits typically accompany cognitive deficits in HIV-1 infected patients (Ranzi et al., 1995). Thus, if these rats do show an olfactory deficit, it may be that they are also experiencing cognitive impairments.

The prefrontal cortex (medial prefrontal cortex in rats) has also been associated with behavioral flexibility and task switching (Granon & Poucet, 1995; de Bruin et al., 1997). Damage to the medial prefrontal cortex has been shown to result in the use of inefficient navigational strategies in the MWM (Kolb et al., 1983). It is likely that such

inefficient searching reflects inflexibility of behavior, and an inability to plan maze routes (Granon & Poucet, 1995). The frontal lobe is also associated with attention, and switching attentional focus in particular (Granon & Poucet, 1995). It is possible that rats with damage to this area have difficulty simultaneously attending to their current location and to the platform location (Granon & Poucet, 1995). Hand in hand with its role in attention, the frontal cortex is also linked with working memory, a system thought to be necessary for planning complex sequences of behavior, such as those required of the rats in MWM tasks (Granon & Poucet, 1995).

The striatum is an area known to be atrophied in patients with advanced HIV-1 infection (Paul et al., 2002). Evidence has implicated the striatum in response learning rather than place learning in the water maze test (Packard & McGaugh, 1992; Da Cunha et al., 2006). Neurologically intact rats may use a combination of place learning and response learning to negotiate the MWM task and may switch between the two methods depending upon the demands placed upon them (de Bruin et al., 1997). Damage to the striatum, however, may result in impaired response learning or the ability to switch between strategies dependent on either place or response learning (Da Cunha et al., 2006). These deficits are especially prevalent during acquisition of the task where procedural information regarding the nature of escape from the maze is learned (Baldi et al., 2003). Since this is where the HIV Tg rats showed deficits, both in Experiment 1 and in the training days of Phase 1 in Experiment 2, it is possible that damage to the striatum is contributing to the poor performance seen in these studies. In Phase 1 of Experiment 2, the start location was fixed and the platform location was fixed. Thus, this task did not require the use of spatial navigation strategies but rather supported a stimulus-response

learning strategy based on the fixed location of the cues relative to the platform. This kind of training is expected to encourage rats to associate the extramaze cues with a particular swim path to the refuge of the platform (Da Cunha et al., 2006). Our results, however, indicated that the rats were using a place strategy during this phase, even though it was not required. Since this phase replicated the results of acquisition in Experiment 1, it is likely that results from both of these studies reflect hippocampal dependent place learning rather than response or strategy learning. When the start points were randomized in Test 1 of Experiment 2, the HIV Tg rats continued to perform worse than controls, further indicating that these rats were using a place strategy even though it was not required. Thus, response learning was not explicitly tested in this study and impairments in this form of learning can not be ruled out. Further research is needed to explore this possibility by more explicitly manipulating the use of response strategies.

The HIV Tg rats also performed worse in Phase 2 of Experiment 2 indicating that the rats are impaired in learning a reversal of platform location and in learning a general search strategy to find the platform. It is unclear whether this effect is a result of the reversal or if the rats are deficient in strategy learning in general. Since there were no significant differences between groups in Test 2 where the rats were tested for the first time with the platform located in varying positions in the NE quadrant (same as Phase 2 training), this may suggest that the effect here is an impairment in the reversal, rather than the search strategy. This may be further evidence for the role of the hippocampus and prefrontal cortex in pliancy and the ability to switch between goal locations. The perseveration seen in the HIV Tg rats during Phase 2 of Experiment 2 may also have been the result of medial prefrontal damage (de Bruin et al., 1997). A diffuse dementia in

the HIV Tg rats may be affecting both the medial prefrontal cortex and the hippocampus, or one area may be affected more than the others and is primarily responsible for the observed deficits. It is unclear without direct knowledge of where the viral load accumulates in the brains of these rats. Further research needs to be done to characterize the distribution of viral proteins and cytokine induction in the CNS of these HIV-1 Tg rats.

Research linking the MWM with traditional Pavlovian classical conditioning and associative learning has been on the rise in recent years after declining with the spatial map theory (Lattal et al., 2003; Mackintosh, 2002). Associative learning phenomenona such as blocking and overshadowing have been demonstrated in the spatial domain using the MWM (Rodrigo et al., 1997; Sanchez-Moreno et al., 1999). It would be interesting to expand the learning and memory tests performed on the HIV Tg rats to include associative learning mechanisms. Given the difficulty with the reversal seen in Experiment 2, it would be interesting to see if the HIV Tg rats would show similar impairments in extinction during a traditionally associative task such as fear conditioning. Since the hippocampus is an overlapping structure with contextual learning and the MWM, it would be interesting to see the effect of temporarily blocking the activity of this area during either task. Infusions of NMDA antagonists or local anesthetics directly into the hippocampus before or after learning may help illuminate the nature of the deficit in the HIV Tg rats more precisely in terms of brain areas involved. Altering the context within the MWM may also help to further characterize the integrity of the hippocampus in these rats. Other tests that may specifically tax the medial prefrontal cortex such as a working memory version of the MWM, or a different non-aversive working memory

task, may likewise aid in understanding the role of this brain area in the MWM as well as the specific deficit of the HIV Tg rats. Since stress and motor effects are potential confounds when using the MWM, it may be beneficial to test these rats in an appetitive paradigm (Sparkman et al., 2005; Shaw et al., 2001).

The HIV Tg rats showed an overall deficit in several aspects of MWM navigation. The rats seemed to have an especially difficult time on the first trial of each day. This may indicate a specific memory deficit in the HIV Tg rats in addition to the learning deficits demonstrated here. However, since the rats were able to improve performance across trials within a single day, no working memory impairment is suggested. Nevertheless, it may be of interest to manipulate the inter-trial interval to better access working memory. Since memory was not explicitly tested in this study, further behavioral analysis of these rats needs to be done to characterize the memory abilities of these rats (e.g. manipulate time between training sessions, which was fixed at 24 hrs in the present experiments).

Overall, the HIV Tg rats show deficits in place learning, response learning, and strategy learning in the modified MWM. Thus, the recently developed noninfectious HIV transgenic rat model and the ability to introduce procedural variations in the modified MWM and related paradigms to further tease apart the nature of these deficits makes it possible to experimentally evaluate the effects of the chronic presence of HIV-1 and associated proteins on cognition and learning.

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