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Further characterization of the spatial learning deficit in the human immunodeficiency virus-1 transgenic rat

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Human immunodeficiency virus (HIV)-associated dementia (HAD) encompasses a spectrum of cognitive and motor deficits resulting from the progression of central nervous system abnormalities caused by the HIV-1 virus. With the advent of highly active antiretroviral therapy (HAART), these deficits have become milder, but more prevalent as the population of HIV-positive individuals ages. Mild impairment in cognition has also been identified in asymptomatic HIV-1 patients. The noninfectious HIV-1 transgenic (Tg) rat recently developed to study the pathogenesis of acquired immunodeficiency syndrome (AIDS) may also be useful for the study of the early and chronic effects of HIV-1 on learning and cognition. In a previous study, we demonstrated that HIV-1Tg rats show a deficit in learning how to swim to a hidden platform in a modified water maze task compared to normal and transgenic controls. In the present study, we replicate this result and demonstrate that HIV-1Tg rats also show a significant deficit in reversal learning and new strategy learning. These results indicate that the HIV-1Tg rat is a promising model in which to study the neuropathogenic mechanisms that can cause cognitive deficits in patients with HAD as well as asymptomatic HIV-positive individuals. *Journal of NeuroVirology* (2009) **15,** 14–24.

Keywords: behavior; HIV-1Tg rat; learning

Introduction

Human immunodeficiency virus-1 (HIV-1) can have profound effects on the brain of infected individuals, leading to behavioral and cognitive deficits (Paul *et al*, 2002). These deficits, which include impairments in processing speed and attention as well as psychomotor slowing (Kelly *et al,* 1996; Grant *et al*, 2005), are characterized as subcortical dementia because of the associated pathological changes in the basal ganglia and related brain areas (Paul *et al*, 2002), and because of the absence of impairments in language, problem solving, and reasoning that are characteristic of Alzheimer's disease and cerebral cortex damage (Cummings and Benson, 1984). The presence of HIV-1 in the brain is believed to cause persistent cognitive deficits through the chronic release of cytokines by both infected and uninfected microglia, which leads to neuronal damage (Goodkin and Asthana, 1997; Gonzalez-Scarano and Martin-Garcia, 2005). In addition, loss of the regulatory actions of astrocytes leads to excess extracellular glutamate, and thus, activation of apoptotic pathways (Nottet and Gendelman, 1995; Gonzalez-Scarano and Martin-Garcia, 2005). These mechanisms cause neuronal damage in the striatum of the basal ganglia (Navia *et al*, 1986), but research suggests that they also result in degeneration of the cerebral cortex and the hippocampus (Wiley *et al*, 1991). Dopaminergic circuitry extending throughout subcortical and cortical structures may be particularly vulnerable to HIV (Ferris *et al*, 2008). Nevertheless, the neuropsychological impairments in HIV-associated dementia (HAD) patients are more similar to the

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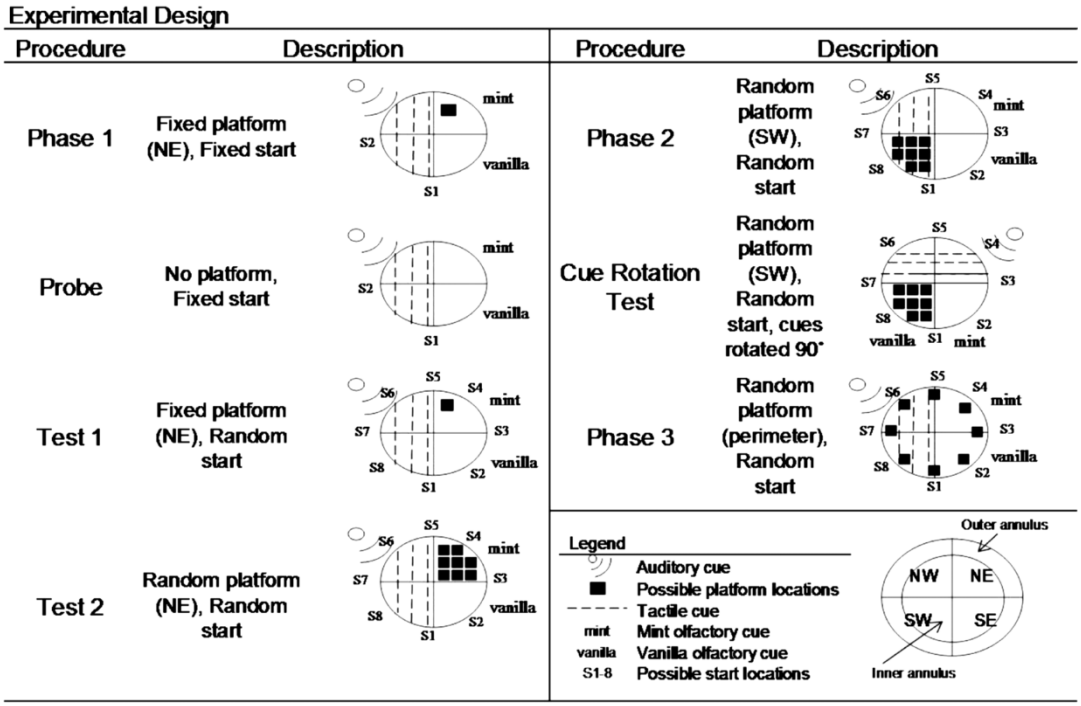

Figure 1 Procedures for all phases and tests. Phase 1 training and the probe test evaluated response and place learning. Test 1 assessed behavioral flexibility whereas Test 2 assessed the ability to conduct a general search for a moving platform in a target quadrant. Phase 2 examined reversal learning by changing the location of the target quadrant. The dependence on the provided olfactory/auditory/tactile cues was assessed with a cue rotation test. Phase 3 tested for the ability to change to a new (perimeter) search strategy.

behavioral-cognitive deficits observed in the subcortical dementias than in the cortical dementias (Peavy *et al*, 1994).

The development of a new transgenic (Tg) rat with an integrated HIV-1 genome allows the opportunity to study behavioral-cognitive deficits in an animal model of HIV-1 (Reid *et al*, 2001). The HIV-1Tg rat expresses the HIV-1 genome minus the *gag* and *pol* genes necessary for viral replication (Reid *et al*, 2001). This rat model expresses functional gp120 and Tat, both of which are distributed throughout many organs, including the brain (Reid *et al*, 2001), and displays dysregulation of at least two parameters of immune function in response to an immune challenge (Chang *et al*, 2007). The HIV-1Tg rat could be a promising rodent model of HIV-induced neurocognitive deficits because (1) it is more readily available to laboratories not equipped to work with infectious rodent models, and (2) this model may better reflect the condition of human HIV patients undergoing highly active antiretroviral therapy (HAART) (i.e., limited viral replication, but persistent HIV infection) (Chang *et al*, 2007).

Studies using transgenic mice expressing specific HIV-associated proteins have shown that these neurotoxic products cause deficits in learning and memory as demonstrated by fear conditioning and in the Morris water maze (MWM) test (Oitzl *et al*, 1993; Pugh *et al*, 2000; Gibertini *et al*, 1995). In addition, disruptions are noted in hippocampal long-term potentiation (LTP), the long-lasting synaptic changes that are believed to underlie some aspects of learning and memory (Krucker *et al*, 1998; Bjugstad *et al*, 2004; Li *et al*, 2004). We previously reported that the HIV-1Tg rat shows a deficit in the acquisition of a MWM task relative to control animals (Vigorito *et a*l, 2007). In the typical water maze procedure, rats are placed in a pool and are required to swim and locate a hidden platform to escape the water. Although the water maze is one of the most often used behavioral neuroscience tools to identify hippocampal-dependent spatial memory, water maze performance is complex, involving several forms of learning and different brain structures (D'Hoodge and De Deyn, 2001). Whereas place learning in the MWM (which involves the formation of a memory for the location of the escape platform) is dependent on hippocampal LTP (Moser *et al*, 1998), strategy and reversal learning appear to be independent of hippocampal LTP (Hoh *et al*, 1999) and require the striatum (Packard and White, 1991). In addition, the inhibition of inappropriate responses that is critical for successful reversal learning has been linked to a basal ganglia-frontoparietal circuit (Ances *et al*, 2006; Thompson *et al*, 2005).

Our previous work with the HIV-1Tg rat indicated that the rats do not show deficits in learning the general location of the platform, but are impaired in

learning how to effectively reach the platform. The current study was designed to further characterize the nature of the learning deficit in the HIV-1Tg rat by examining place learning, reversal learning, and new strategy learning.

Results

Phase 1

The data from training days 1 to 5 of Phase 1 are shown in Figure 2. Rats in all three groups significantly decreased latencies over days ($F(4, 144) = 68.22$, $P = .000$). There was also a significant day × trial interaction ($F(12, 432) = 1.823$, $P = .042$), reflecting the typical pattern of acquisition in this task. On days early in training, latencies decreased over trials, but by the last day of training, the latencies were similar across trials (data not shown). Moreover, there was a significant main effect of group ($F(2, 36) = 3.840$, $P = .031$), with the HIV-1Tg group showing longer escape latencies than the two control groups.

On the final training day of Phase 1 (day 5), a single 60-s probe trial was performed. The probe trial was scored for percent time spent in each quadrant (Figure 3). Rats in all three groups spent significantly more time (about 50% of time) in the NE or target quadrant ($F(3, 108) = 85.856$, $P = .000$). However, an analysis of the time spent in the inner and outer areas of the NE quadrant revealed a significant group × area (inner or outer) interaction ($F(2, 36) = 7.396$, $P = .002$). As the platform was always fixed in the inner annulus of the NE quadrant in Phase 1, the Tg and F344 control rats spent more time in this area compared to the HIV-1Tg rats ($F(2, 36) = 61.548$, $P = .000$). The HIV-1Tg rats, in comparison, spent an equal amount of time in the NE inner and outer areas (data not shown).

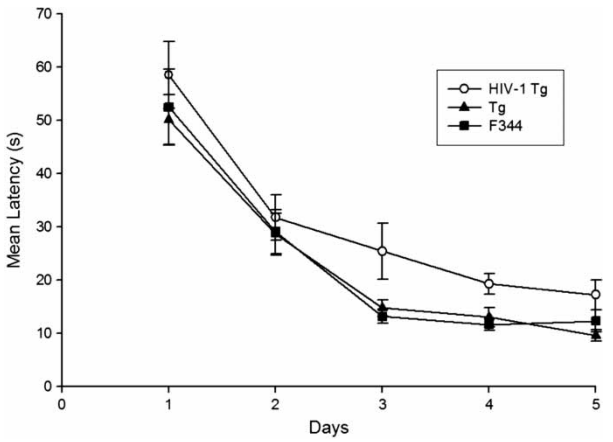

Figure 2 Training latencies for HIV-1Tg, Tg control, and F344 control groups during Phase 1. During this phase, the platform location and the start location were fixed for all trials on all days. Values represent the means ± SEM.

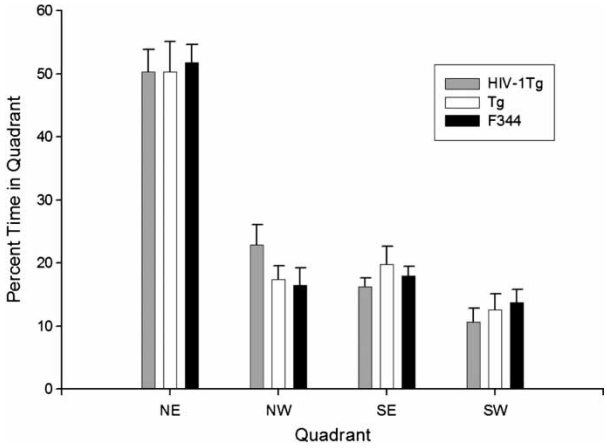

Figure 3 Probe data in which the platform is removed and the rats allowed to swim for 60 s. The probe trial was performed after the last trial of the last day (day 5) of Phase 1. Probe data are presented in percent time spent in each of the four quadrants. Values represent the means ± SEM.

The trial-by-trial latencies of Tests 1 and 2, when the rats were started from randomized locations, are shown in Figure 4; the latencies from the last training day of Phase 1 (day 5) are included for comparison. To determine if groups responded differently to the change in procedure on Test 1, the latencies from Phase 1 (day 5) and Test 1 were analyzed with a groups (3) × days (2) × trials (4) mixed analysis of variance (ANOVA). A significant main effect of trials ($F(3, 108) = 3.27$, $P = .02$) showed that the rats in all three groups had lower latencies across the four trials of each day. There

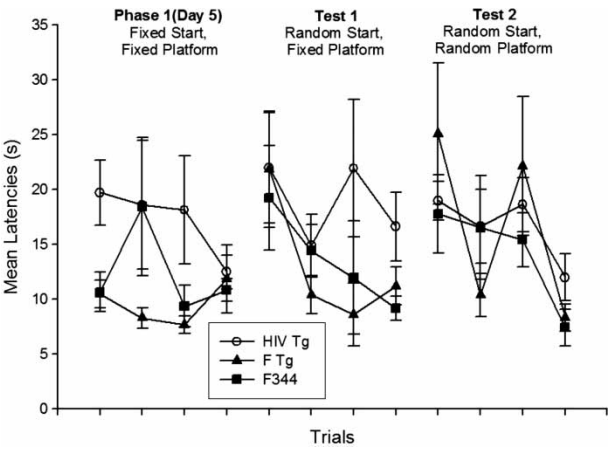

Figure 4 Latencies for all trials on the last day of Phase 1 (day 5) and in Test 1 and Test 2, which took place 1 and 2 days later, respectively. Test 1 consisted of randomized start locations with the same fixed platform location as in phase 1. Test 2 consisted of randomized start locations and randomized platform locations within the NE quadrant, which was the same location as during Phase 1 training. Values represent the means ± SEM.

was a lack of a significant day × trial interaction ($F(3, 108) = 2.29$, $P = .02$); however, once again there was a significant main effect of group ($F(2, 36) = 4.19$, $P = .02$). The latencies for all four trials in Test 2, where the platform was moved within the NE quadrant between trials, are also shown in Figure 4. Test 2 was compared with the final day of Phase 1 using a 3 (groups) × 2 (days) × 4 (trials) mixed ANOVA. There was a significant day × trial interaction ($F(3, 108) = 3.213$, $P = .026$). The trial-by-trial mean latencies varied for all three groups during Test 2, but group differences were no longer evident ($F(2, 36) = 2.45$, $P = 0.10$).

Phase 2

Phase 2 implemented reversal learning by changing the location of the escape platform to the SW quadrant. There was a significant day × trial interaction ($F(12, 432) = 3.829$, $P = .000$) as well as a main effect of group ($F(2, 36) = 20.301$, $P = .000$) (Figure 5). Post hoc analyses revealed that the effect of group was between the HIV-1Tg rats and the two control groups ($P < .05$). The Tg control and F344 control groups did not differ from each other.

The Phase 2 data were then scored for percent time spent in the 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) × 6 (days) × 4 (trials) mixed ANOVAs. Figure 6A shows that all three groups learned to spend less time in the NE quadrant over days in Phase 2 ($F(5, 175) = 74.54$, $P = .000$). The HIV-1Tg rats, however, spent significantly more time in the NE quadrant than the controls during all days of Phase 2 ($F(2, 35) = 10.97$, $P = .000$). Post hoc pairwise comparisons showed that the Tg and F344 control groups did not differ from each other, but that both groups were significantly different from the HIV-1Tg group

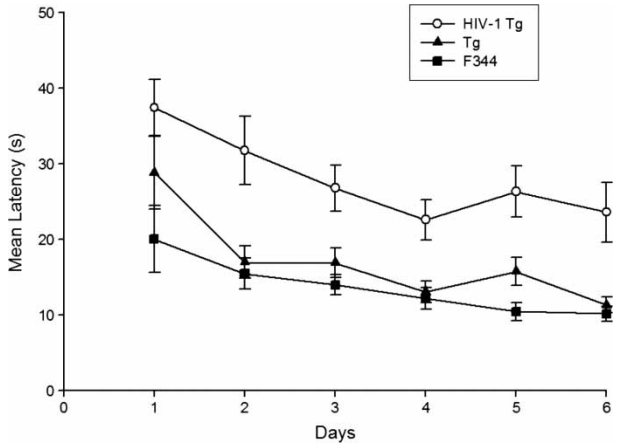

Figure 5 Escape latencies during Phase 2. The platform location was reversed to the SW quadrant, and platform location was randomized for each trial. Start locations were also random. Values represent the means ± SEM.

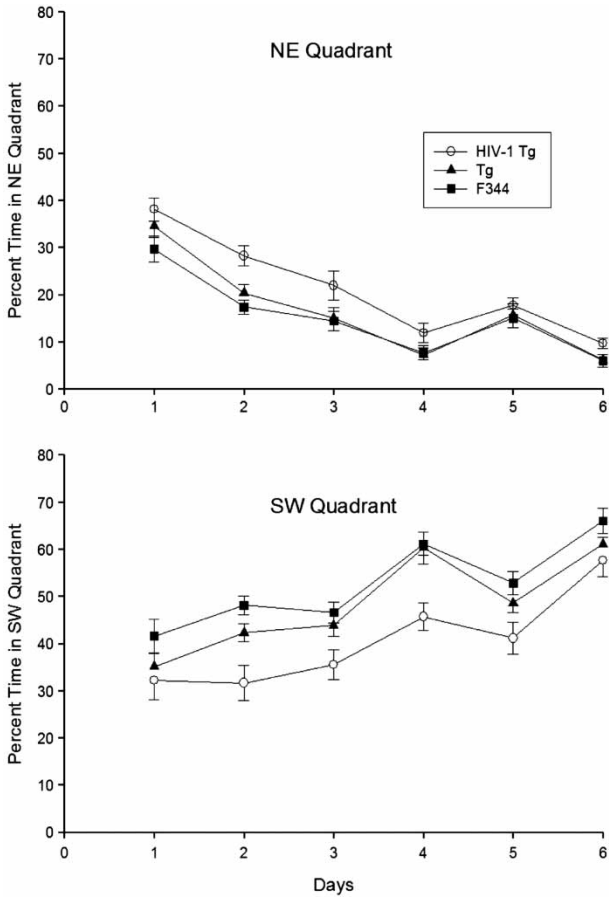

Figure 6 Percent time spent in the NE quadrant (*top*) and SW quadrant (*bottom*) during the 6 training days of Phase 2. The NW quadrant was the location of the escape platform in the previous phase (Phase 1), and the SW quadrant was the location of the escape platform during Phase 2. Values represent the means ± SEM.

($P < .05$). The reverse pattern was seen when the platform was located in the SW quadrant in this experimental phase (Figure 6B). All groups learned to spend more time in the SW quadrant over days ($F(5, 175) = 35.04$, $P = .000$), but the HIV-1Tg rats consistently spent less time in this area than the control groups ($F(2, 35) = 15.404$, $P = .000$). Post hoc pairwise comparisons showed this difference to lie between the HIV-1Tg rats and the two control groups only ($P < .05$).

Analysis of the percent time spent in the SE quadrant also showed a significant main effect of group ($F(2, 35) = 7.809$, $P = .002$) (data not shown). Post hoc paired comparisons revealed that the HIV-1Tg rats spent more time than the control groups in that quadrant ($P < .05$), which is located between the NE and the SW quadrants. Percent time spent in the inner annulus during Phase 2 likewise showed significant day × trial interactions ($F(15, 525) = 8.903$, $P = .000$) and an effect of group ($F(2, 35) = 8.285$, $P = .001$) (data not shown). The HIV-1Tg rats

spent more time in the outer area of the pool and, consequently, less time in the inner area compared to controls. Post hoc comparisons showed differences between the HIV-1Tg rats and the Tg and F344 control groups ($P < .05$), but not between the Tg and F344 control groups.

The Cue Rotation immediately followed the last day of Phase 2. Data from this day are shown in Figure 7, with the latencies from the last day of Phase 2 provided for comparison. The data from the Cue Rotation test were analyzed using a 3 (groups) $\times$ 2 (days) $\times$ 4 (trials) mixed ANOVA. The results showed significant day $\times$ trial ($F(3, 108) = 3.848$, $P = .012$) and group $\times$ day ($F(2, 36) = 4.129$, $P = .024$) interactions, the latter indicating that the performance deficit in the HIV-1Tg rats was no longer evident on the cue rotation test day.

Phase 3

Figure 8 shows the mean latencies to reach the hidden platform for all 5 days of Phase 3, when the platform was randomly placed along the wall of the pool. Analysis of the Phase 3 escape latencies showed a day $\times$ trial interaction ($F(12, 432) = 16.935$, $P = .000$) and a between-subject effect of group ($F(2, 36) = 14.427$, $P = .000$). Post hoc pairwise comparisons showed significant differences between the HIV-1Tg rats and the Tg and F344 controls ($P < .05$), but not between the control groups themselves.

The Phase 3 data were scored for percent time spent in each quadrant in the same manner as the probe test in Phase 1 (data not shown). There were no significant differences between groups in time spent in any quadrants during Phase 3. Although the HIV-1Tg rats spent significantly more time in the outer area of the pool during Phases 1 and 2 compared to the control groups, this difference was no longer evident in Phase 3. In contrast, in Phase 3, the HIV-1Tg rats showed a trend toward spending less time in the outer annulus when compared to the controls. A significant group $\times$ days interaction ($F(8, 144) = 2.135$, $P = .043$) showed that this group difference emerged on day 5 of Phase 3. Additional post hoc analysis showed that the F344 control rats spent significantly more time in the outer annulus compared to the HIV-1Tg rats on this day ($P < .05$), but did not differ from the Tg control rats ($P > .05$). The Tg control rats showed a similar trend toward spending more time in the outer area.

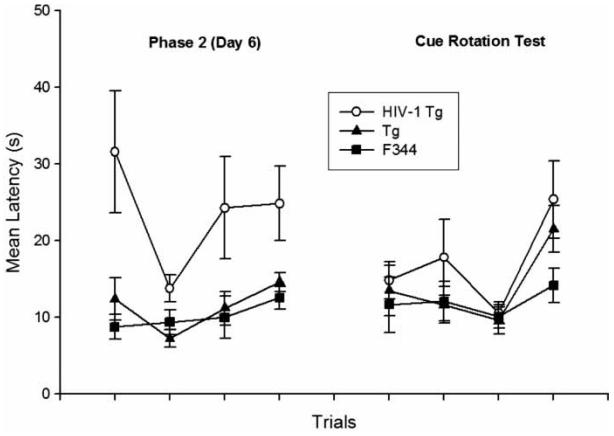

Figure 7 Cue Rotation that took place on the day immediately after the end of Phase 2. The cues (olfactory, auditory, and tactile) were rotated 90°, and the rats were placed in the pool at random start locations. Values represent the means $\pm$ SEM.

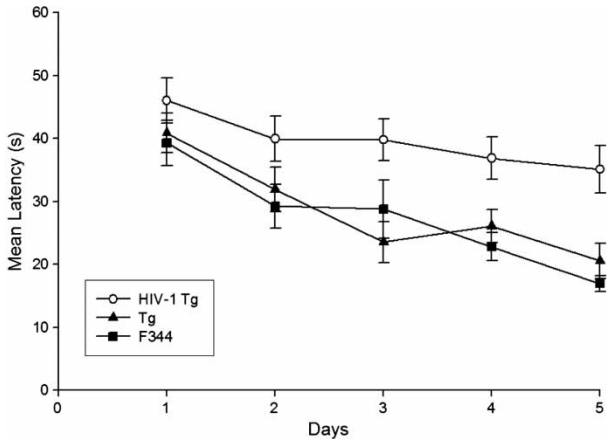

Figure 8 Phase 3 latencies for rats in all three groups. Phase 3 consisted of randomized start locations and randomized platform locations along the perimeter of the pool. Values represent the means $\pm$ SEM.

Some of the rats displayed a circular swim pattern that caused them to occasionally enter the outer annulus of the pool, but this pattern did not meet the criteria of searching in the perimeter. For this reason, swim paths for Phase 3 were traced to determine the specific strategy used by the rats in all three groups during the last 2 days of Phase 3 when the group differences were most pronounced. Swim paths were traced for Trials 1 and 4 on both days. 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 9A). 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 9B). Perimeter/consistent swim paths were those with the majority of the path in the outer annulus of the pool, approximately 30 cm from the edge, in at least three out of the four scored trials (Figure 9C). The number of rats in each group that displayed one of the three different swim patterns is presented in Table 1. More HIV-1Tg rats showed unknown/inconsistent swim patterns than perimeter or mixed/loop swim patterns, whereas the

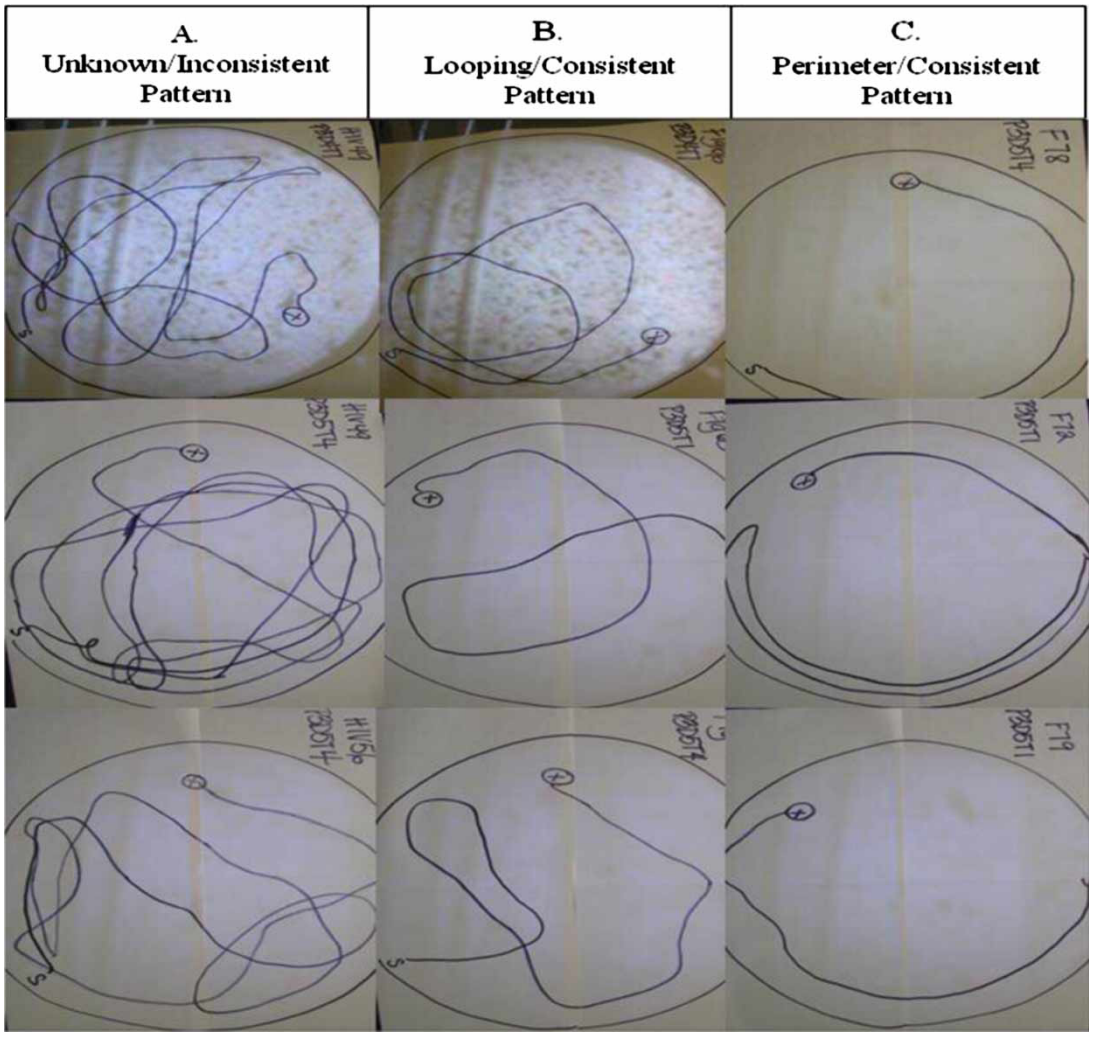

Figure 9 Representative swim paths for the three categories of swim patterns: A, unknown/inconsistent; B, looping/consistent; C, perimeter/consistent.

opposite was true for both control groups (chi-square: $\chi^2 = 8.82$, $P = .03$).

Discussion

The Phase 1 results in the present study confirmed the findings from our previous work with HIV-1Tg rats in the modified Morris water maze (Vigorito *et al*, 2007). Rats in all three groups, HIV-1Tg, Tg control, and F344 control, were able to navigate and solve a modified MWM with no visual cues. Although place information was not necessary in this phase because the start and platform locations were always fixed, the probe trial on the last day of Phase 1 training indicated that all three groups were using place information in their swim strategy, at least by day 5. Place learning is the dominant strategy used by rats when such information is available, even when more efficient methods of locating the escape platform are present (de Bruin *et al*, 1997). Although the HIV-1Tg rats did not have difficulty learning the general location of the escape platform, analysis of swim time in the inner and outer areas of each quadrant showed the less precise nature of the HIV-1Tg rat's search in the target area, which may explain, in part, the increased Phase 1 latencies relative to controls. Test 1 confirmed that all groups could use place information to locate the platform even when placed in novel start locations, although the HIV-1Tg group performance was again

Table 1 Number of rats by group showing one of three categories of swim patterns in phase 3

Swim pattern	HIV-1Tg	Tg	F344
Perimeter	3 (23)	5 (38)	6 (46)
Mixed/loop	4 (31)	6 (46)	7 (54)
Inconsistent	6 (46)	2 (15)	0 (0)

Note. Numbers in parentheses are percentages.
$\chi^2 = 8.82$, $P = .03$.

less efficient. In Test 2, when the platform location was now varied within the target quadrant, the two control groups no longer had a significant advantage over the HIV-1Tg group in locating the platform.

Phase 2 results showed an unequivocal deficit in the HIV-1Tg rats compared to the Tg and F344 control groups, as indicated by the increased latencies on all days of training. Because Phase 2 involved both a new place (location reversal) and a new strategy (a switch from swimming towards a specific location in a target quadrant to a general search in the target quadrant), it is uncertain which manipulation is responsible for the increased latencies seen in the HIV-1Tg rats. The analysis of percent time that the rats spent in the inner and outer sections of the quadrant suggest that the difference is most likely due to the reversal component of this phase. Moreover, in Test 2 (the first day that the general quadrant search was introduced), the HIV-1Tg rats were performing comparable to the control groups, indicating that the HIV-1Tg rats do not have a deficit in general quadrant search, but rather a difficulty suppressing the tendency to approach a previous, now irrelevant, target quadrant. Further evidence for this perseveration effect is found in the increased time spent in the SE quadrant, which lies between the NE and SW quadrants (the previous and current targets, respectively). Time spent in this area may reflect the approach-avoidance conflict in the HIV-1Tg rats that led to increased time in the NE quadrant and decreased time in the SW quadrant relative to controls. It is also possible that the HIV-1Tg rats employed a less effective, unknown strategy, and that this is responsible for the group differences in Phases 1 and 2.

The Cue Rotation data showed enhanced performance in the HIV-1Tg rats compared to their performance on the last day of Phase 2. This improvement may have been due to an increased reliance on the available cues by the HIV-1Tg rats. If the HIV-1Tg rats were more dependent upon the cues than the control groups, then they may have been guided by the new configuration to the SE quadrant (the configuration of cues that previously indicated the NE quadrant), which is slightly closer to the SW quadrant, and thus, the escape platform. An alternative explanation is that improved performance on this day simply reflected a continuation of the Phase 2 learning curve, with the HIV-1Tg group catching up to the performance of the control groups.

Phase 3 results may reflect a difficulty in the transition from place strategy to perimeter search strategy as well as the implementation of the perimeter search strategy itself. This new search strategy deficit in the HIV-1Tg rats does not reflect perseveration because the rats did not spend significantly more time in the quadrant that contained the platform in the previous phase (SW). It is noteworthy that switching to a perimeter search strategy was difficult for all groups, because less than 50% of the animals in the control groups showed the more efficient perimeter search swim pattern. Nevertheless, the rats in all three groups located the platform on nearly every trial during this phase. The use of a sweeping, overlapping loop strategy and an arc-like pattern in the MWM preceded the use of a true perimeter search strategy. The F344 and Tg control groups displayed a greater percentage of both looping/consistent and perimeter/consistent search patterns compared to the HIV-1Tg rats, which displayed a greater percentage of unknown/inconsistent patterns than the other two categories. These results also suggest that the HIV-1Tg rats may be deficient in strategy learning and use.

Stress or other nonspecific sensory or motor effects should also be considered as alternative explanations for the results seen in all phases of the present experiment. HIV-1–positive patients show an abnormal regulation of the hypothalamic-pituitary-adrenal (HPA) axis, and thus, show abnormal stress responses (Kumar *et al*, 2003). It is possible that the HIV-1Tg rats likewise have an altered stress response that is activated by the aversive water maze task. The MWM produces a strong stress response in rodents, although it is gradually attenuated with further training (Aguilar-Vallesa *et al*, 2005). The water maze task used in this and our previous study (Vigorito *et al,* 2007) was modified to eliminate visual navigation cues because the presence of pronounced cataracts in the HIV-1Tg rats introduces an obvious confound. The navigation cues that were introduced to replace visual cues were auditory, tactile, and olfactory cues. Although there are no studies that have compared sensory capacities of HIV-1Tg rats with controls, the HIV-1Tg rats did not appear to display any other obvious sensory deficits. To test the possibility that the HIV-1Tg rats are anosmic, at the conclusion of water maze testing, the rats from the present study were trained to find mint- and vanilla-scented Cheerios buried in ¼-inch corn-cob bedding in an open field arena ($60 \times 60 \times 60$ cm). The mean latency to find the buried Cheerios did not differ between the groups (unpublished observations), indicating that the HIV-1Tg rats are not significantly impaired in their use of olfactory cues. Sickness behavior, as seen in animals injected with lipopolysaccharide (LPS) to induce chronic immune system activation, has been shown to interfere with performance in the MWM (Sparkman *et al*, 2005). Sickness behavior is a nonspecific motivational effect resulting from chronic cytokine induction that does not involve learning, and is associated with slower performance. However, our

experience with the HIV-1Tg rats has revealed no evidence of sickness behavior or anhedonia as indicated by body weight gain, food intake, and acceptance of sucrose solutions (unpublished observations). Moreover, it is unlikely that a decline in gross motor performance resulted in the observed deficits in the HIV-1Tg rats because swim paths clearly indicated ineffective search patterns and swim speeds analyzed in a previous study showed no significant decrease in swimming ability (Vigorito *et al*, 2007).

It is unclear to what extent the cognitive deficits in HIV-infected humans and animal models of HIV are a result of the inflammation processes in the brain, neuronal injury, and neuronal apoptosis. In at least one study using a severely compromised immunodeficiency (SCID) mouse model, HAART effectively reduced neuroimmune responses in the brain, but failed to reverse the deficits in the retention of a spatial water maze task and the concomitant reduction in mitogen-activated protein (MAP)-2 expression (Cook *et al,* 2007). These results are consistent with studies suggesting that neuronal dysfunction is a prominent cause of HAD.

The neurotoxic effects of HIV proteins is known to damage the dopaminergic system of HIV-infected humans (Nath *et al*, 2000), resulting in the psychomotor slowing characteristic of subcortical dementia and Parkinsonism. Disruptions of the dopamine system may also result in cognitive deficits that contribute to the poorer learning of the HIV-1Tg rats in the water maze task. Lack of dopamine prevents dopamine-deficient mice from performing the water maze task efficiently, but does not prevent learning (Denenberg *et al*, 2004). Lesions of the mesohippocampal dopaminergic tract has been shown to impair the performance of rats in the Morris water maze by disrupting place learning (Gasbarri *et al*, 1996). Given the ability of the HIV-1Tg rats to solve the maze, but with impairments in specificity and efficiency of where to search, it is possible that dopamine disruption is an underlying factor. D1 receptor antagonism has been shown to have detrimental effects on swim speed while leaving the ability of animals to solve a water maze intact; however D2 receptor antagonism has been shown to result in prolonged escape latencies without decreased swim speed (Stuchlik *et al*, 2007). At least in rodent models, D2 antagonism may translate better to subcortical dementias in humans than D1 antagonism. Given the learning deficit seen in the HIV-1Tg rats in the absence of gross motor deficits, it seems likely that these effects would be mediated by the D2 dopamine pathway of the basal ganglia, hippocampus, and frontal cortex.

Although there is no evidence for a decrease in D2 receptors in HIV-infected humans with HAD, evidence suggests that there is a functional change in the dopamine transporter protein (Wang *et al*, 2004). Interestingly, the vulnerability of dopaminergic terminals to damage appears to emerge early in HIV-infected humans (Ferris *et al,* 2008) and in simian immunodeficiency virus (SIV)-infected monkeys (Jenuwein *et al*, 2004), and may be a major cause of the minor cognitive deficits seen in infected, but asymptomatic, individuals.

The learning deficits of the HIV-1Tg rats throughout all phases of this experiment may have implications for the human population suffering from cognitive effects of HIV infection. Little is known about the spatial learning ability of HIV-1–positive individuals. However, in a recent study, asymptomatic HIV patients were found to display significantly impaired performance in a visuospatial mental rotation task (Olesen *et al*, 2007). Thus, at least some aspects of cognitive spatial ability appear to be impaired in asymptomatic HIV-infected humans (Olesen *et al*, 2007) and asymptomatic HIV-1 transgenic rats (present study).

It is unclear if the deficits in the HIV-1Tg rats are directly attributable to the effects of the viral proteins present in these rats. Little is known regarding the presence of cytokines or viral proteins in specific brain areas of these rats. Although the present study demonstrates the potential use of the HIV-1Tg rat as a model for cognitive effects of HIV-1, much work still needs to be done to determine the neuropathogenic mechanisms of these deficits to better model what is known regarding the human condition.

Materials and methods

Animals

Thirteen HIV-1 transgenic (HIV-1Tg) rats, 13 transgenic controls (Tg), and 13 Fischer 344/NHsd (F344) control background rats (all approximately 5 months of age at the start of the study) were purchased from Harlan (Indianapolis, IN). The animals were double or triple housed in standard shoebox cages, and provided free access to food and water for the duration of the study. The rats were maintained on a 12-h light/dark schedule, and were tested in the light portion of the cycle. All experimental procedures were approved by the Seton Hall University Institutional Animal Use and Care Committee.

Apparatus

The pool used for the Morris water maze (MWM) in this study was a black round tub 130 cm in diameter and 52.5 cm in depth. The tub was filled with water, and a platform was placed in the NE quadrant. The top surface of the platform was 2 cm below the water surface. HIV-1Tg rats are born with very opaque cataracts limiting visual acuity; therefore, the MWM

was modified to replace visual navigation cues with a combination of olfactory, auditory, and tactile cues. The animals were trained under dim, red-light illumination, and provided with two different olfactory cues (mint in the northern quadrants, vanilla in the southern quadrants), a tactile cue in the western quadrant (strings of fishing line hung over the pool that brushed the surface of the water), and a single auditory cue (a metronome) in the NW quadrant (see Vigorito *et al*, 2007, for more details). The surface of the water was covered with packing peanuts to hide the location of the platform when the room lights were on (Cain *et al*, 1993). Although the visual navigation cue condition was not included in the present study, the packing peanuts were retained in the procedure.

Procedures

For all phases of the study, the rats were trained in groups of five or six rats with four trials per day. A trial began by placing the rat in the water facing the wall of the pool, and ended when the rat climbed the platform to escape the water. A stop watch was used to record the escape latency, and an infrared video camera mounted above the pool recorded the behavior of the rats for further analysis of swim paths. If a rat failed to find the platform in 90 s, the experimenter lifted the rat out of the water and placed it on the platform. After about 10 s on the platform, the rat was returned to a stainless steel wire-mesh waiting cage. After all the rats in the group finished a trial, the rats were retested for the next trial. This procedure continued until all four trials were completed. The rats were then dried with a towel and returned to their home cages.

The experimental procedures consisted of three phases, and are summarized in Figure 1. Phase 1 was designed to test response learning and behavioral flexibility. In Phase 1, all the rats were started from a fixed start location, with half the rats from each group starting from the south (S1 in Figure 1), and the other half starting from the west (S2 in Figure 1). The rats were required to swim to an escape platform fixed in a location in the NE quadrant. Under these training conditions, the rats have the opportunity to learn an invariant motor response (e.g., turn left and swim in a straight line), or the location of the platform as a goal target (e.g., the platform is away from the sound and strings, and towards the mint smell). To look for evidence that the rats learned the location (place) of the platform, a single 60-s probe trial was performed following the last trial of Phase 1 (day 5). For the probe trial, the platform was removed, and the rats started from the same start location that they experienced throughout their training. Test 1 was another measure of place learning, and took place on a single day immediately following Phase 1 training. In Test 1, the rats were started from one of eight start locations randomly selected on each trial, but the platform location remained fixed in the NE quadrant as in Phase 1. If the rats learned the location of the platform by using the available navigation cues, rather than by developing an invariant motor response towards the platform, then they should be able to efficiently locate the platform from any novel start location. Test 1 was immediately followed by a single day of Test 2, where the rats were started from random locations again, but the platform location was moved from trial to trial within the NE quadrant. In this test, the rats were required to use a general quadrant search strategy. This procedure essentially eliminated the need for separate probe trials to obtain evidence of place learning (Choi *et al*, 2006).

Phases 2 and 3 were adapted from the procedures used in Choi *et al* (2006), and were designed to test place reversal learning and strategy reversal learning. Phase 2 reversed the target quadrant (SW instead of NE), but maintained the general quadrant search strategy introduced in Test 2. Phase 2 was followed by a single day of Cue Rotation, where the cues were rotated 90°. The Cue Rotation day was included to determine reliance on the navigation cues in the three groups. Phase 3 began after the Cue Rotation, and consisted of 5 days of strategy reversal learning. The platform was located at random locations around the perimeter of the pool (10 cm from the pool's edge) rather than in a location closer to the center of the pool, as in previous phases. This change required that the rats dramatically change their search strategy from a centrally located search to a perimeter search.

The primary dependent variable in the study was the escape latency. In addition, the videos of swimming rats were traced to determine swim patterns and scored to determine the percent time spent searching in the quadrants. For a more detailed analysis, the pool was divided virtually into an inner and outer annulus (see Figure 1 legend) so that the percent time spent in the inner and outer quadrants could also be evaluated.

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