

Spatial Learning and Memory in HIV-1 Transgenic Rats

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Abstract HIV-1 infection of the central nervous system impairs neural, cognitive, and behavioral functioning in patients despite antiretroviral therapy. However, studying mechanisms underlying HIV-1-related neurological and cognitive dysfunction has been limited without an adequate animal model. A novel, noninfectious HIV-1 transgenic (HIV-1Tg) rat model was recently created that expresses an HIV-1 provirus with a deletion of functional *gag* and *pol* genes. This HIV-1Tg rat reportedly develops clinical manifestations of human HIV disease and thus appears to mimic the persistent infection that results from the presence of HIV viral proteins in the host. We evaluated the HIV-1Tg rat model using the Morris water maze, a popular paradigm for testing learning and memory deficits in rodents. Because of congenital cataracts in HIV-1Tg rats, however, the traditional use of visual navigational cues in this paradigm were precluded. We first designed a modified Morris water maze and demonstrated that neurologically intact rats can effectively learn the water maze in the absence of visual cues and in the presence of non-visual navigation cues. We then tested HIV-1Tg rats in this modified Morris water maze. These HIV-1Tg rats showed a deficit in learning how to swim to the location of the hidden platform but did not show a deficit in their memory

of the general location of the hidden platform. These results suggest that the noninfectious HIV-1Tg rat can be a valid model for the behavioral studies of HIV-related neurological dysfunction.

Keywords HIV-1 · animal model · brain · neuroaids · spatial learning

Introduction

Infection with human immunodeficiency virus type 1 (HIV-1) is associated with a variety of neurological deficits, including cognitive and motor impairments (Barak et al. 2002; Gonzalez-Scarano and Martin-Garcia 2005; Lawrence and Major 2002; Navia and Rostasy 2005; Nottet and Gendelman 1995). These neurological impairments result from the presence of the virus, which acts through both direct and indirect mechanisms (Gonzalez-Scarano and Martin-Garcia 2005). Viral proteins, such as the coat glycoprotein, gp120, the transcriptional transactivator protein (Tat), and viral protein R, are shed or secreted by an infected cell, and can consequently damage neurons and other nervous system cells (Gonzalez-Scarano and Martin-Garcia 2005; King et al. 2006; Lawrence and Major 2002). Tat has also been linked with disruption of long term potentiation (LTP), an essential element in some forms of memory (Morris 1989), in hippocampal area CA1 (Behnisch et al. 2004). Transcriptional changes in Tat in the hippocampus are associated with spatial learning and memory impairment (Burger et al. 2007). Gp-120 has likewise been linked with damage to this particular area of the hippocampus. For example, transgenic mice expressing gp120 showed alterations in both short and long-term potentiation in CA1 (Krucker et al. 1998).

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Neurons can also be affected by inflammation caused by the secretion of pro-inflammatory cytokines by non-infected activated macrophages (Barak et al. 2002; Gonzalez-Scarano and Martin-Garcia 2005). In addition, these cytokines can further stimulate macrophages as well as microglia to produce cellular toxins (Barak et al. 2002; Lawrence and Major 2002). Conditions associated with the peripheral induction of cytokines have also been shown to result in increased cytokine levels in the hippocampus (Barrientos et al. 2002). This suggests that HIV-1, through cytokine induction, can potentially interfere with hippocampal LTP. Thus, peripheral immune activation, including cytokine induction, may interfere selectively with learning and memory, which are dependent on the LTP of the hippocampus (Barrientos et al. 2002).

The Morris water maze (MWM) has been used extensively to examine learned performance on a hippocampal-dependent task (Morris 1989; Morris et al. 1982). In the standard MWM procedure, rats are placed in a pool of water with a hidden escape platform in a target location and with distal visual cues (landmarks) placed around the pool to serve as navigational cues to the target. Learning is demonstrated by decreased latencies to the platform and shortened swim path lengths. Successful performance in the MWM requires procedural learning (e.g., learning that there is a platform, that there is no alternative escape route, and that thigmotaxis is an ineffective search strategy) as well as the encoding of a memory for the location of the escape platform. Memory of the platform location is confirmed with probe trials whereby the platform is removed and the time spent in the target location is measured. Hippocampal lesions and impairment disrupt performance in a MWM when navigational cues are required to solve the task, but not when the platform is visible or marked with a visual “beacon” (Devan et al. 1996; Morris et al. 1982; Whishaw and Kolb 1984). These results suggest that a functioning hippocampus is necessary for spatial memory (i.e., the use of navigational cues or landmarks to locate a goal), although the precise characteristics of the spatial memory is still debated (Hollup et al. 2001; Mackintosh 2002).

Because rodents cannot be infected with HIV-1 an approach that has been utilized to examine the neuro-behavioral effects of HIV-1 infection in rodents is to inject products of HIV-1 infection, such as pro-inflammatory cytokines (Pugh et al. 1999) and/or viral proteins (Giunta et al. 2006). For example, intracerebral ventricular injections of gp 120 results in impaired acquisition (Galicía et al. 2000) and recall (Pugh et al. 2000) of fear conditioning (Pugh et al. 2000), as well as deficits in spatial learning in the water maze (Glowa et al. 1992) and Barnes maze (Sanchez-Alavez et al. 2000). The deficits in spatial learning are consistent with the disruption in hippocampal neuronal functioning seen in rodents treated with gp 120

(Sanchez-Alavez et al. 2000) and in transgenic mice overexpressing this viral protein (Krucker et al. 1998).

The development of severe combined immunodeficiency (SCID) mice has proven useful in the investigation of the neurodegenerative effects of HIV in mice. SCID mice lack functional B and T cells; thus, they can accept xenografts without rejection. Human HIV-infected cells can survive for months in the brains of these mice and produce many of the histological features of human HIV encephalitis. Moreover, HIV-infected SCID mice display learning deficits in the MWM (Griffin et al. 2004; Zink et al. 2002) as well as hippocampal synaptic dysfunction (Zink et al. 2002) that is attenuated with NMDA antagonists (Anderson et al. 2003). Although the SCID mouse model of HIV is a valuable model for the study of HIV neuroaids, the biosafety requirements and complex technical procedures make it impractical for most laboratories.

Another approach is to develop noninfectious rodent models with an HIV-1 transgene. Transgenic mice models produce some of the pathology seen in human HIV patients, but the highest viral expression is primarily in skin or in atypical tissue (Dickie et al. 1991; Santoro et al. 1994). In a more recently developed transgenic rat model, however, HIV-1 gene expression occurs in tissues consistent with human HIV-1 patients including lymph nodes, spleen, liver, thymus, and circulating blood (Reid et al. 2001; Mazzucchelli et al. 2004). The HIV-1 transgenic rat (HIV-1Tg) carries a gag-pol deleted HIV-1 provirus derived from the pNL4-3 plasmid that is regulated by the viral long term repeat, and expresses seven of the nine HIV-1 genes (Reid et al. 2001, 2004). The presence of viral transcripts in this rat model indicates functional Tat, which is not present in the transgenic mouse (Garber et al. 1998). Although viral proteins are expressed in the HIV-1Tg rat, the functional deletion of the gag-pol gene prevents viral replication. Nevertheless, HIV-1Tg rats develop pathologies similar to humans infected with the HIV-1 virus, including immune-response alterations, T-cell abnormalities, kidney failure, cardiac irregularities, and brain pathology in rats displaying some motor deficits (Reid et al. 2001). Indeed, the HIV-1Tg rat model appears to mimic the conditions of patients undergoing highly active anti-retroviral therapy (HAART). The antiretroviral drugs in HAART consist of inhibitors that target HIV-1 viral entry, reverse transcriptase, and viral protease to control viral replication and restore immunity. Despite controlled viral replication under HAART, the continued presence of viral proteins (Agbottah et al. 2006; Vigano et al. 2006) results in a persistent HIV-1 infection that is associated with mild to moderate cognitive deficits and slow progressive neurodegeneration.

Reid et al. (2001) reported that disease progression in the HIV-1Tg rat resembles the progression toward AIDS in humans including neurological deficits manifested as

circling behavior and hind limb paralysis by 9 months of age. Learning and memory deficits in the HIV-1Tg rat, however, have not been evaluated. Because cachexia or wasting is also associated with progressive HIV or AIDS, testing for learning deficits at advanced stages of disease progression is problematic. Any observed behavioral deficit may be a result of motor and motivational deficiencies rather than the result of a learning impairment. At 5 months of age, however, HIV-1Tg rats do not show the neurological signs of disease progression (Reid et al. 2001). We have also found that although the HIV-1Tg rats may weigh less than age-matched controls at 5 to 6 months, there is no sign of anorexia and the HIV-1Tg rats gain weight at the same rate as controls (unpublished observations). Thus, testing presymptomatic HIV-1Tg rats in the water maze test at 5 months of age makes it possible to study the direct effects of HIV-1 on learning and memory without gross motor and motivational deficits. Moreover, presymptomatic HIV-1Tg rats, having viral proteins and no viral replication, resemble the controlled viral replication and persistent HIV-1 infection seen in HAART patients more closely than any other rodent HIV model.

Another consequence of the gag-pol deleted HIV-1 genome is the development of cataracts. In the commercially available HIV-1Tg rat, the cataracts are very opaque and congenital, which make the use of visual navigational cues in MWM test challenging. In the present study, a modified MWM was developed to examine potential learning and memory deficits in HIV-1Tg rats. Because control animals would have a visual advantage over the HIV-1Tg rats, thereby confounding the results, the animals were tested under dim red-light illumination. In the absence of visual cues, we provided auditory and olfactory cues that can potentially be used by the rats as navigation cues, but not act as target beacons. We first demonstrated that the performance of normal Sprague-Dawley rats in the modified MWM is comparable to their performance in the standard MWM. We then compared HIV-1Tg rats with controls in the modified procedure and added an additional nonvisual cue (a tactile cue) to encourage the use of our nonvisual navigational cues. The HIV-1Tg rats showed a deficit in learning how to swim to the location of the hidden platform, but did not show a deficit in their memory of the general location of the hidden platform.

Materials and methods

Animals

Eighteen male Sprague-Dawley rats were used to compare the standard and modified MWM. These rats were 5 months of age when testing began and were housed in pairs. Eleven HIV-1Tg rats, nine Fischer 344 transgenic (F-Tg) littermate

controls, and ten Fischer 344 (F) controls were tested in the modified MWM. These rats were single-housed and approximately 5 months of age at the start of the experiment. All rats were maintained on a 12-h light/dark schedule and tested in the light phase. Food and water was provided ad libitum during the course of the experiment. All procedures were in accordance with the Seton Hall University Institutional Animal Care and Use Committee.

Apparatus

The apparatus was a black round pool 130 cm in diameter and 52.5 cm in depth, and was located in a room that was 3.7×3.7 m. A white plastic curtain hid a rack with hanging stainless steel wire cages on the west side of the pool. The pool was filled with water to a depth of 30 cm. The platform was 15.2 cm² and 28 cm in height, leaving 2 cm of water above the platform. The surface of the water was covered with packaging peanuts to hide the location of the platform (Cain et al. 1993). The four quadrants of the pool were identified as northeast (NE), northwest (NW), southeast (SE), and southwest (SW). Although the room contained several fixed items that the rats could use as visual navigation cues (e.g., the curtain, a door, and a large sink), additional visual cues (two posters of vertical or horizontal black and white lines and a picture of Edward Tolman) were affixed to the north and east walls. To provide olfactory cues, pipe cleaners (12-in. chenille stems, Westrim Crafts, Van Nuys, CA) were dipped in a scented liquid and draped over the rim of the pool so that one pipe cleaner hung from the center of each inner quadrant wall perpendicular to the water surface. The pipe cleaners in the two northern quadrants were dipped in mint scented mouthwash (Pathmark™ Spring Mist), and the pipe cleaners in the two southern quadrants were dipped in vanilla extract (McCormick™ Imitation Vanilla). A metronome (Seiko Quartz Metronome SQ50) set at 96 beats per min, located 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. For available tactile navigation cues, four plastic poles (17 cm apart) lay parallel across the top of the two western quadrants of the pool. Lengths of monofilament fishing line (25 lb test) were tied to the poles spaced 10 cm apart so that the fishing line brushed the surface of the water and across the rat's head as it swam in the western half of the pool, providing a tactile cue. Overhead fluorescent lighting was left on for rats tested in the visual navigation cue condition (Light). To eliminate the visual navigation cues, the rats were tested under dim red-light illumination (referred to as "Dark"). A single experimenter stood about 55 cm from the outer wall of the SW quadrant with a stopwatch to measure escape latencies. A camcorder with infrared nightshot (SONY

TRV460), mounted on the ceiling directly above the pool, was used to record the rat's swimming behavior on a video tape for swim path analysis.

Procedures

The rats were run in squads of six for 8 days. At the start of each training day, the rats were placed in individual wire mesh holding cages behind the white curtain. On day 1, the rats were given a 60-s pre-training trial immediately before the first trial. During the pre-training trial, the rats were placed on the platform for 60 s, then removed and placed back into the holding cage until trial 1 began. The platform location was always fixed in the NE quadrant, 22 cm from the pool wall. For trial 1, the rats were placed, one by one, into the maze at randomized start locations but always facing the maze wall. Each day consisted of four trials where each rat's latency to find the platform was recorded with a stopwatch. The time was recorded when the rat's full body was on the platform and remained there at least 3 s. If the rat did not find the platform within 90 s, the trial was terminated, and the rat was placed on the platform. The rats were allowed to remain on the platform for 15 s before being placed back in the holding cages between trials. Training took place for 7 days. On day 8, the navigation cues available during training were removed to evaluate the impact on the rat's ability to find the location of the escape platform.

Probe trials were included after the fourth trial on days 3, 5, and 7 of training. During the probe trials, the platform was removed, and the rats were allowed to swim freely in the maze for 90 s. Video recordings of the probe trials were used to examine behavior during the probes.

Sprague-Dawley rats The Sprague Dawley rats were divided into three groups ($n=6$ per group): Light, Dark, and Dark+Cues. The rats in the Light group were tested in the MWM with the overhead lights on and, therefore, with the visual navigation cues available to them. The rats in the Dark group were tested under red light illumination only, and were not provided any visual or non-visual cues. The rats in the Dark+Cues group were tested under red light illumination as well, but they were also provided with the olfactory and auditory cues. On day 8, the navigation cue availability was altered for each group. The Light group was tested under red light illumination, thereby removing the visual navigational cues. The Dark group was tested with the overhead lights on for the first and only time. The Dark+Cues group was tested under red light illumination as before, but with the auditory and olfactory cues removed. Before testing on day 8, the walls of the pool were washed with a diluted bleach solution to eliminate any lingering odor cues.

HIV-transgenic rats and controls HIV-1Tg rats, F-Tg controls, and the F controls were all tested in the dark, with the olfactory and auditory cues in place, as well as the tactile navigation cues. On day 8, the navigation cues were removed, and all rats were tested again in the dark. The rats were then placed, one by one, into the pool at randomized start locations. The platform location remained in the NE quadrant of the pool. Cue removal was used to determine the extent to which the animal's pool navigation was dependent upon the available cues.

Path lengths and swim speed

Video tapes of the training sessions were used to measure path lengths of the HIV-1Tg and control rats. The rat's swim path was traced onto tracing paper that covered the screen of a television monitor. Digital pictures were taken of the path tracings and analyzed using ImageJ software (ImageJ, Rasband, WS; US National Institutes of Health, Bethesda, MD, USA; <http://rsb.info.nih.gov/ij/>, 1997–2006) to provide total distance swam per rat. This distance was divided by the escape latency to determine the swim speed.

Statistical analysis

All data are presented as the mean±standard error. All results were analyzed using SPSS for Windows version 10.0. Behavioral data were analyzed using three- or two-factor ANOVA with group (Light, Dark, Dark+Cues) or strain (HIV-1Tg, F-Tg, F) as between subjects factor and all other factors as within subjects. Significant three-way and two-way interactions were analyzed with additional ANOVAs and *t* tests. Significance was accepted at $p<0.05$.

Results

Sprague-Dawley rats

Training The mean escape latencies (four trials/day) of the Sprague-Dawley rats in the Light, Dark, and Dark+Cues groups during the 7 days of training are shown in Fig. 1. All groups were able to learn the task as indicated by decreasing latencies to find the escape platform over days [$F(6, 90)=22.82, p=0.000$]. The Light group showed faster latencies than the Dark group during days 5 to 7, and the Dark+Cues group showed intermediate latencies. However, the main effect of groups [$F(2, 15)<1.00$] and the groups × days interaction failed to be significant [$F(12, 90)<1.00$]. Latencies were higher on the first trial, and tended to decrease on subsequent trials in all groups [$F(3,45)=9.21, p=0.000$] (data not shown).

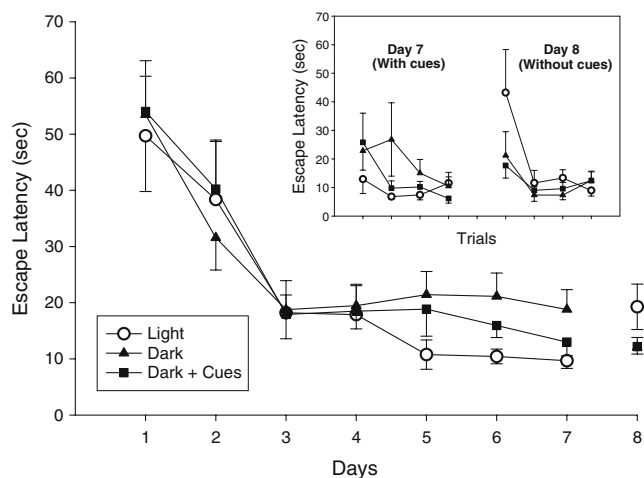


Fig. 1. Escape latency (sec) during the seven training days for all three groups of Sprague-Dawley rats. Data points on day 8 indicate the mean latencies following cue removal. The inset graph shows the trial-by-trial changes in escape latencies on the last day the cues were available (day 7), and on the day that the cues were removed (day 8). The data represent means±SEM.

Cue removal On day 8, the visual cue was removed for the Light group by testing them in the dark, and the nonvisual cues were removed for the Dark+Cues group. The Dark group was tested with the lights on. The inset in Fig. 1 shows the group latencies for each trial on day 8 as well as day 7 (the last day of training with cues present). The latencies were analyzed with a groups (3) × days (2) × trials (4) mixed ANOVA. 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 [three-way interaction, $F(6,45)=2.45, p=0.04$].

Probe trials To analyze the probe trials the pool was divided into equal-sized quadrants by two lines that crossed in the middle of the video image of the pool. In addition, the pool was defined in terms of an inner and outer annulus (i.e., circular region). The escape platform was in the inner annulus and in the NE quadrant (see Fig. 2). The probe trials were scored for percentage of time spent in each defined area of the pool to evaluate if the rats had learned the location of the escape platform and if the groups were using different search patterns.

Analysis of the percentage of time spent in the training quadrant (NE) during the three probe tests (Fig. 2, top) yielded a significant groups × probes interaction [$F(2,15)=3.42, p=0.02$]. 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 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 various divisions of the pool during the probe tests indicated that the cues affected the development of search strategies differentially. The percentage of the time spent in the inner annulus is shown in Fig. 2 (bottom). The animals with the cues added in the dark spent 60% or more of their time searching in the inner annulus on all three probe tests, whereas the groups searching in the light or dark spent significantly less time in the inner annulus early in training (probe 3) before approaching the percentage of the Dark+Cues group in the later probes [groups × probes, $F(4, 30)=3.04, p=0.03$]. 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 × probes, $F(4, 30)=3.11, p=0.03$]. Figure 3 shows that the Light group (top panel) tended to avoid the outer annulus of the NW quadrant (open square), and spent more time in the NE quadrant (open circle) on all probe days. The Dark+Cues group (Fig. 3, bottom panel), however, did not show this pattern until day 7. The animals tested in the dark (Fig. 3, middle panel) showed a similar pattern to the

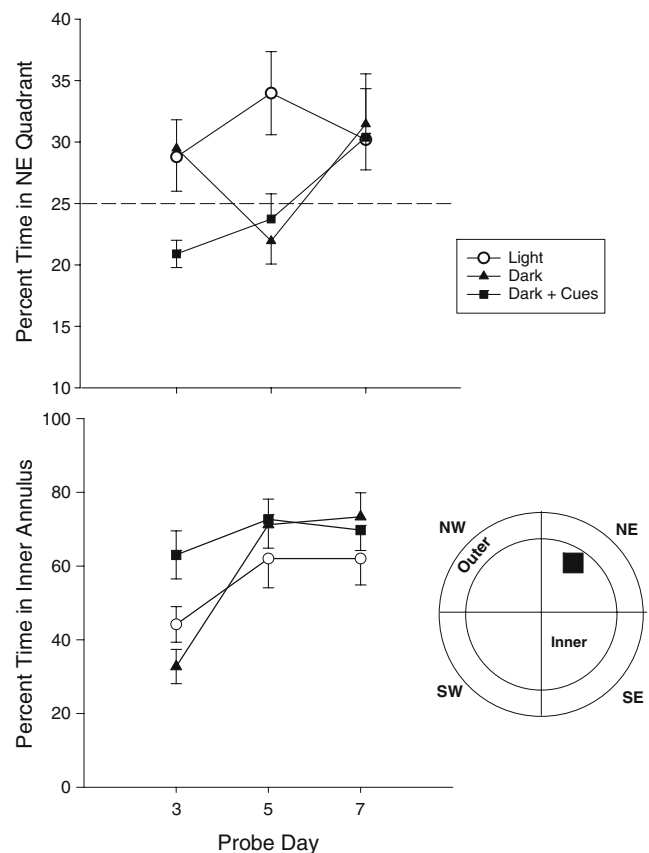


Fig. 2. Percent time that the three groups of Sprague-Dawley rats spent in the NE quadrant (top) and in the inner annulus (bottom) of the water maze during the three probe tests. The diagram in the bottom panel shows the location of the escape platform in the inner annulus of the NE quadrant. The data represent means±SEM.

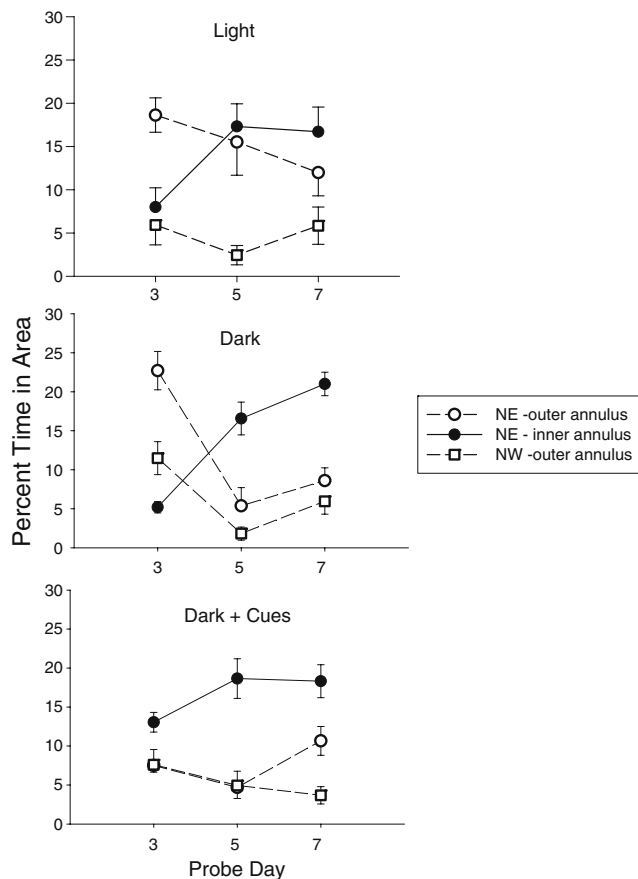


Fig. 3. Percent time that the Light (*top*), Dark (*middle*), and Dark+Cues (*bottom*) groups searched in selected areas. The *dashed lines* allow comparison of percent time in the two northern quadrants (NE and NW) of the outer annulus; the *circles* allow comparison of the inner (solid) and outer (open) annulus of the NE quadrants. The data represent means±SEM.

Light group. The groups also differed in the development of searching patterns while in the NE quadrant [groups × probes, $F(4, 30)=5.70$, $p=0.001$]. The Dark group (Fig. 3, middle panel) spent more time in the outer annulus (open circle) than in the inner annulus (solid circle) of the NE quadrant on probe day 3, but by the next probe, the pattern reversed. The Light group (Fig. 3, top panel) 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. The Dark+Cues group (Fig. 3, bottom panel) spent more time in the inner quadrant on all probe days.

HIV-1Tg rats and controls

Training As shown in Fig. 4, the HIV-1Tg group and two control groups (F-Tg and F) showed decreasing latencies to find the platform over training days [$F(6,162)=61.97$, $p=0.000$]. 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 × trials: $F(18,486)=2.90$, $p=0.000$]. A significant main effect of group [$F(2,27)=4.07$, $p=0.03$] indicated that the HIV-1Tg group took significantly longer to escape to the platform. The group factor failed to interact significantly with any of the other factors.

Cue Removal On day 8, the auditory, olfactory, and tactile cues were removed prior to testing. The inset in Fig. 4 shows the group latencies for each trial on day 8 as well as day 7 (the last day of training with cues present). The latencies were analyzed with a groups (3) × day (2) × trial (4) mixed ANOVA. The effect of test day [$F(1, 27)=3.48$, $p=0.07$] and trial [$F(3, 81)=1.00$, $p=0.40$], as well as all of the interaction terms, were not significant. However, a significant effect of groups indicated that the HIV-1Tg rats displayed higher escape latencies compared to the other two control groups [$F(2, 27)=4.92$, $p=0.02$].

Probe trials Analysis of the percentage of time spent in the training quadrant (NE) during the three probe tests yielded no groups differences [groups: $F(2, 27)<1.00$; groups × probes [$F(4, 54)=1.87$, $p=0.13$] and no effect of probe tests [$F(2, 54)<1.00$]. The groups also did not differ in the percentage of time spent in the inner annulus across probe days [$F(4, 54)<1.00$], averaging 69.0, 65.2, and 64.4% across probe days for the HIV-1Tg, F-Tg, and F groups, respectively. Because probe days did not interact with any other factor, the percentage of time in the four quadrants was averaged over the three probe days. All three groups searched more in the NE training quadrant compared to the other three quadrants [$F(3, 81)=56.66$, $p=0.000$], and the groups did not differ [$F(6, 81)<1.00$] (Fig. 5).

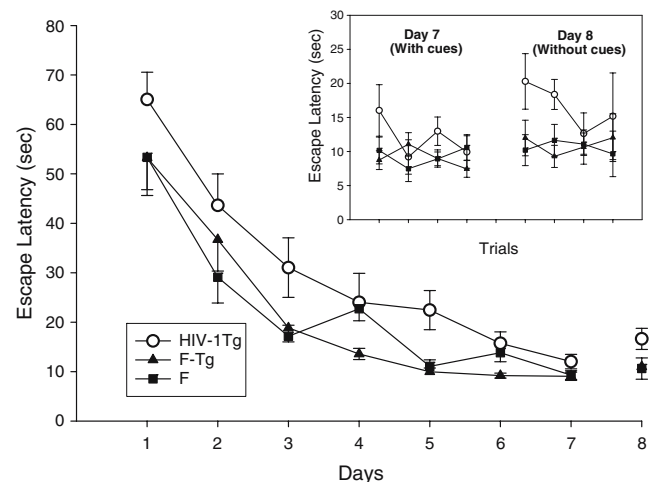


Fig. 4. Escape latencies (sec) during the seven training days for HIV-1Tg, F-Tg, and F rats. Data points on day 8 indicate the mean latencies following cue removal. The inset graph shows the trial-by-trial changes in escape latencies on the last day that the cues were available (day 7), and on the day that the cues were removed (day 8). The data represent the means±SEM.

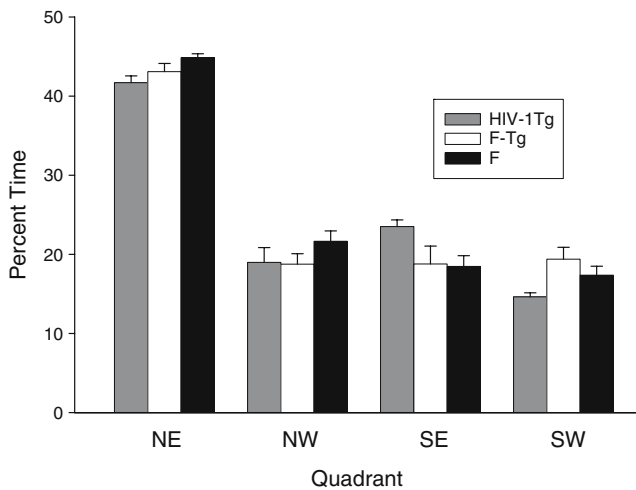


Fig. 5. Percent time that the HIV-1Tg, F-Tg, and F rats searched in the four quadrants during the probe tests. The escape platform was located in the NE quadrant. The data represent the means±SEM of all probe days.

Path lengths and swim speed To determine if the group differences in escape latencies could be explained by poorer swimming ability of the HIV-1Tg rats, the mean path lengths (cm) were measured, and swim speeds (cm/s) were calculated on days 3, 4, and 5 (see Fig. 6), when the group differences were most obvious, and analyzed with separate groups (3) × days (3) × trials (3) mixed ANOVAs. All groups swam decreasing distances across trials [$F(3, 81)=5.27, p=0.002$] and over days [$F(2, 54)=5.71, p=0.006$], but the HIV-1Tg group swam significantly longer paths than the two control groups, which did not differ [$F(2, 27)=5.20, p=0.01$]. A marginally significant three-way interaction [$F(12, 162)=1.78, p=0.055$] was due to unusually poor performance of the F controls on the first trial of day 4; the reason for this poor performance is unknown.

The swim speed did not change over days [$F(2, 54)=1.06, p=0.35$], although it tended to decrease by the last trial on each day [$F(3, 81)=3.19, p=0.02$]. A significant effect of groups [$F(2, 27)=3.43, p=0.047$] and a three-way interaction [$F(12, 162)=1.82, p=0.048$] suggested some differences in swim speed between groups. Post-hoc analysis indicated that the HIV-1Tg group generally swam slower than the F-Tg controls during the first three trials, but not on the fourth trial, when the F-Tg animals may have been showing some fatigue. The F controls showed a similar pattern as the F-Tg controls; however, with the exception of one trial (day 4, trial 3), they did not differ significantly from the HIV-1Tg group.

Discussion

These results from the training, probe trials, and cue removal of the Sprague-Dawley rats (Figs. 1, 2 and 3)

confirm the findings of previous studies that rats can “solve” a Morris water maze in the absence of visual cues (Lindner et al. 1997; Prusky et al. 2000; Rossier et al. 2000). The addition of either the visual or non-visual navigation cues did not reliably improve performance of the Sprague-Dawley rats in training. However, the probe tests showed that the rats that were provided with additional non-visual cues (Dark+Cues group) were using those cues, at least early in training, since their pattern of searching differed from the Light and Dark groups. Interestingly, the Dark group probe data were more similar to the Light group than to the Dark+Cues group, suggesting that the Light group may not have been using the visual navigation cues that were provided. This is unlikely, however, since the removal of the visual cues on day 8 dramatically disrupted the performance of the Light group on the first trial. The improved 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. Another possible explanation for the disruptive effects on Trial 1 may have been a nonspecific distraction effect, since the rats were trained in the light and then abruptly tested in the dark. However, this nonspecific effect seems unlikely since the Dark group also experienced a sudden change in the environment, being trained in the dark and abruptly tested in the light on day 8, but they did not show disruption in performance.

Removal of the non-visual cues for the Dark+Cues group and for the HIV-1Tg rats and controls did not disrupt performance in the MWM (Fig. 4). This suggests that the added cues may influence the initial strategies learned to find the location of the platform, but may not be necessary for continued performance in well-trained animals (Rossier et al. 2000). For example, during training, the swimming rats may pick up additional cues unknown to the experi-

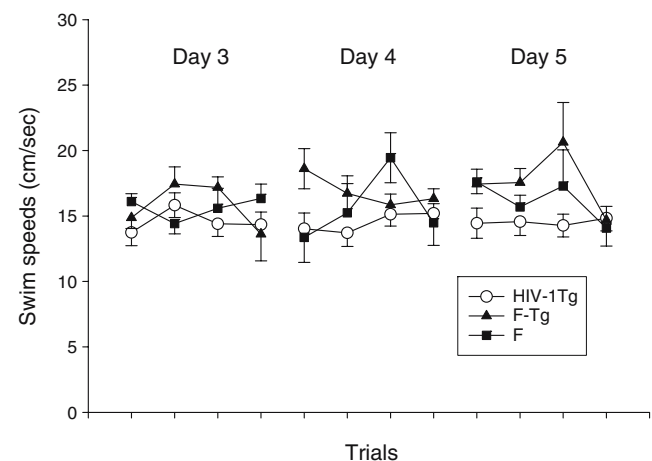


Fig. 6. Mean swim speeds (cm/sec) of the HIV-1Tg, F-Tg, and F groups on days 3, 4, and 5 of training. The data represent the means±SEM.

menters (e.g., changes in the echo of the splashing sound as they approach a wall), or they may develop a searching strategy that continues to be effective even when the original cues are removed. Potential behavioral strategies include combinations of suppression of thigmotaxis, circular swim patterns in the center of the maze, looping strategies, and a focal search pattern in the target quadrant.

The probe data showed that the rats not only learned how to find the platform, but also learned where the platform was located (Fig. 5). The addition of the third tactile navigation cue appears to have facilitated the place learning component of the MWM. If rats randomly searched all four quadrants, they would be expected to spend 25% of the time in any one quadrant. The Sprague-Dawley rats, with two nonvisual cues, spent an average of 30.7% of the time in the target quadrant by day 7, but the rats tested with the added tactile cue (HIV-1Tg, F-Tg, and F) spent 43.7% of the time in the target quadrant on the first probe trial on day 3.

The standard MWM task is used with the availability of visual landmark cues, and successful performance in this task is typically interpreted as reflecting the formation of a cognitive map (i.e., a mental representation of multiple visual landmarks); however, empirical evidence for formation of a cognitive map is rarely provided to confirm this interpretation (Mackintosh 2002). The present results confirm previous studies that visual cues are not necessary to learn the water maze, and behavior is sufficiently flexible to adapt to changes in the available cues (Lindner et al. 1997; Prusky et al. 2000; Rossier et al. 2000).

Despite the ease of learning the task, the HIV-1Tg rats showed an overall longer latency to the escape platform than the two control groups. The longer path lengths of the HIV-1Tg rats indicate that the poorer performance was not due to a general motor deficit, but a result of longer search times. The HIV-1Tg rats swam slightly slower than controls in the daily sessions that were analyzed, but this effect was due to the control groups swimming faster early in the trials and slowing down by the last trial to swim speeds equivalent to the HIV-1Tg animals (Fig. 6). The HIV-1Tg rats, however, were very consistent in their swim speed across all trials, and showed no evidence of fatigue. The poorer performance of the HIV-1Tg rats, therefore, suggests a learning deficiency rather than a reduced ability to swim.

The presence of the HIV transgene in the HIV-1Tg rats may cause neuronal damage to the hippocampus as a result of the presence of viral proteins, including Tat and gp120, which are both active in this animal model (Reid et al. 2001), or it may cause neuronal damage indirectly through the release of cytokines. Viral loads in human HIV patients are greater in the hippocampus and basal ganglia than in any other brain areas (Berger and Arendt 2000). Viral proteins injected into the brains of normal rodents (Sanchez-Alavez

et al. 2000) and HIV-infected human cells injected into the brains of SCID mice (Zink et al. 2002) have been shown to cause dysregulated hippocampal synaptic function and learning deficits in the MWM. Although symptomatic HIV-1Tg rats display a loss of blood-brain barrier integrity, gliosis, and neuronal cell loss (Reid et al. 2001), more precise pathological brain changes have not been determined. Histopathology of presymptomatic HIV-1Tg rats also awaits further study and is encouraged by the learning deficits observed in the present study.

Since the hippocampus plays an important role in spatial learning, damage to this area in presymptomatic HIV-1Tg rats may be responsible for the observed deficits (D'Hooge and De Deyn 2001). However, the precise nature of the learning deficit in the HIV-1Tg rats is unclear. The probe data suggest that the deficit may not be with learning where the platform is located, since the three groups did not differ in the percentage of time searching in the target quadrant as early as day 3 when the first probe was given. Studies have shown that rats with hippocampal lesions not only 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 get there, rather than a problem of where to go (Cain et al. 2006; Whishaw et al. 1995).

Damage to the striatum including the caudate-putamen, an area known to be damaged by HIV-1 (Berger and Arendt 2000; Wang et al. 2004), may also contribute to the behavioral deficits seen in the HIV-1Tg rats in the present study. Several studies have implicated the striatum in procedural or stimulus-response (S-R) learning in general (Yin and Knowlton 2006; Packard and Knowlton 2002), and in the water maze test in particular (Packard and McGaugh 1992). S-R learning is a form of habit-like learning that has the property of slow acquisition, but does not involve the cognitive factors (e.g., memory and expectancy) that are presumed to occur during place learning (Kirsch et al. 2004). Neurologically intact rats may use a combination of place learning and S-R learning to “solve” the MWM task and may switch between the two methods depending upon the demands placed upon them. Damage to the striatum, however, may result in impaired S-R learning that competes with place learning, or there may be an impaired ability to switch between the two modes of learning. These deficits are especially prevalent during acquisition of the task (Baldi et al. 2003). It was during acquisition that the learning deficits were observed in the present study.

Despite advances in antiretroviral treatment for prolonging the lives of HIV-1 infected individuals, neurodegenerative processes and cognitive dysfunction continue to be observed (Navia and Rostasy 2005). The present study is

the first to suggest that the noninfectious HIV-1 transgenic rat model can have promising utility for investigating the mechanisms underlying HIV-related neuropathogenesis, and for screening potential therapeutic compounds.

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