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The severe combined immunodeficient (SCID) mouse model of human immunodeficiency virus encephalitis: Deficits in cognitive function

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The severe combined immunodeficient (SCID) mouse model of human immunodeficiency virus (HIV) encephalitis exhibits many of the histopathological and pathophysiological features of human HIV-associated dementia (HAD). Although deficits that may resemble HAD in humans have been reported for HIV-infected SCID mice, the cognitive deficit aspect of the model has very limited empirical support. Here, the authors report that HIV-infected SCID mice display cognitive deficits on a task requiring the animal to learn and remember the spatial relationship of cues in its environment in order to locate a submerged platform in a Morris water maze. The cognitive deficits manifest as longer latencies to locate the platform on the last day of the maze acquisition period and during a retention test 8 days later. Control experiments indicated that the poor performance by HIV-infected mice in comparison to controls was not due to impaired motor function or swimming ability, impaired visual acuity, or increased susceptibility to fatigue. Thus, the increased times required for HIV-infected mice to locate the submerged platform during the acquisition and memory tests likely reflect a cognitive deficit, rather than sensorimotor or emotional abnormalities. These behavioral deficits are associated with significant increases in astrogliosis and microgliosis in the HIV-infected mice. The results of this study strengthen the SCID mouse model of HIV encephalitis by definitively establishing cognitive deficits for the model in addition to its previously reported neuropathological features. *Journal of NeuroVirology* (2004) **10,** 109–115.

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Introduction

Human immunodeficiency virus (HIV)-associated dementia (HAD) occurs commonly in HIV-infected patients and is an acquired immunodeficiency syndrome (AIDS)-defining illness (McArthur, 1990; McArthur *et al*, 1993). Clinical features of HAD include psychomotor slowing, memory impairment, and visuoconstructional impairment (Navia *et al*, 1986; Tross *et al*, 1988). The advent of pharmacotherapeutic strategies, such as highly active antiretroviral therapy (HAART), have lowered the incidence of HAD by 40% to 50% (McArthur *et al*, 2003). These new therapies have also decreased the AIDS death rate and, presumably, increased the life span of HIV-positive individuals (Moore *et al*, 1998). Unfortunately, it is unclear how aging processes may affect the development of HAD in people treated with

HAART. Moreover, with increasing numbers of HIV-infected people worldwide, many of whom do not have access to the latest treatments, the number of HIV-infected people presenting to the clinic with HAD will likely increase. In fact, there is evidence that although the incidence of HAD has declined, the cumulative prevalence actually increased during the 1990s (McArthur *et al*, 2003).

Animal models have been developed to evaluate the underlying neurobiology of HIV encephalitis and HAD as well as potential therapies. These models provide experimental control and access to measures not available in human studies. The severe combined immunodeficient (SCID) mouse intracerebrally inoculated with HIV-infected human monocytes is one such model. In this model, pathology of mice inoculated with HIV-infected monocytes is compared with that developed by control mice inoculated with uninfected monocytes. HIV-associated neuropathology is evidenced by more severe pathology in mice inoculated with HIV-infected cells than observed for the control group of mice inoculated with uninfected monocytes. The SCID model is excellent for studying HIV-related neuropathology because the HIV-infected mice present neuropathology common to HIV patients, including the presence of astrogliosis, microglial nodules, HIV-infected mononuclear phagocytes, and apoptotic neurons throughout the frontal lobes of the brain (Tyor *et al*, 1993; Persidsky *et al*, 1996; Avgeropoulos *et al*, 1998). A preliminary report from our laboratory indicated that in addition to the neuropathological features of the SCID model, the mice were also cognitively impaired. This cognitive dysfunction was suggested by a deficit in the memory for a learned problem in a Morris water maze that required the utilization of spatial relationships among cues in the animal's environment to locate a submerged goal platform in the maze (Avgeropoulos *et al*, 1998). Further, recent *in vitro* experiments indicate that several indices of neuronal function are altered in hippocampal slice preparations from HIV-infected SCID mice, which, given the importance of the hippocampus in mediating learning and memory, is consistent with memory deficits noted in our study (Zink *et al*, 2002; Anderson *et al*, 2003). Taken together, these findings from the SCID mouse model of HIV encephalitis are consistent with the clinical and pathological features of HAD in AIDS patients.

Although the empirical support for the histopathology and the pathophysiology of HIV encephalitis in SCID mice is strong, the behavioral deficits associated with HIV encephalitis in the SCID mouse model are not firmly established. Aside from our earlier preliminary report of possible cognitive deficits in learning and memory of spatial relationships of environmental cues, there is only one other publication suggesting an HIV-related cognitive deficit in the SCID mouse model (Zink *et al*, 2002). The reported deficit in learning spatial relationships between environmental cues for HIV-infected mice in the latter study, however,

was only in comparison to unoperated control mice. The performance of HIV-infected mice did not differ from that of mice inoculated with uninfected monocytes. Thus, the two published studies do not provide unequivocal support for cognitive deficits in HIV-infected SCID mice.

As part of a series of experiments in our laboratory to investigate the effects of abused substances, as well as HAART, on the progression of HIV encephalitis, we further investigated the effects of HIV infection on cognitive function in SCID mice. SCID mice inoculated with HIV-infected monocytes were compared with control mice treated identically to the HIV-infected group, except that they were inoculated with uninfected monocytes. Cognition was assessed by determining the acquisition and recall of a learning problem in a Morris water maze. The learning problem involved utilization of the spatial relationship between cues in the animal's environment to reach a submerged goal platform as employed in our original publication (Avgeropoulos *et al*, 1998). The outcome of this experiment on a larger group of mice confirms our preliminary report of deficient cognitive performance by HIV-infected SCID mice on this task. Moreover, the deficit in the current study was obtained using fewer inoculations of HIV-infected monocytes across time than used in our previous report. The results establish cognitive deficits in HIV-infected SCID mice and support the suitability of this model in the study of HAD.

Results

Morris Maze

The assessment of basic visual and motor function using time required to locate the visible platform as described in Materials and Methods for the second phase of the procedure indicated no effect of HIV infection. Comparison of the four-trial average time required to locate the visible goal platform indicated no difference between the two groups (Figure 1, Visual/Motor; $t_{(22)} = -0.64, P > .5$). This result suggests that the basic motor and visual abilities required to perform the learning and memory task were not altered by the HIV infection.

The acquisition of the maze problem was retarded for HIV-infected mice compared to uninfected mice (Figure 1, day 1 *versus* day 6). Data reflect the time (s) required to locate the submerged goal platform averaged across the four trials between day 1 and day 6 of acquisition. Performance of uninfected mice improved across the six acquisition sessions, whereas that of HIV-infected mice did not. Bartlett's test on these data indicated heterogeneity of variance across the groups of data, hence they were $\log_{(e)}$ transformed prior to analysis. A 2 (infection) $\times$ 2 (session) analysis of variance (ANOVA) on the transformed data indicated significant effects of infection ($F(1, 22) = 4.4, P = .0468$) and session
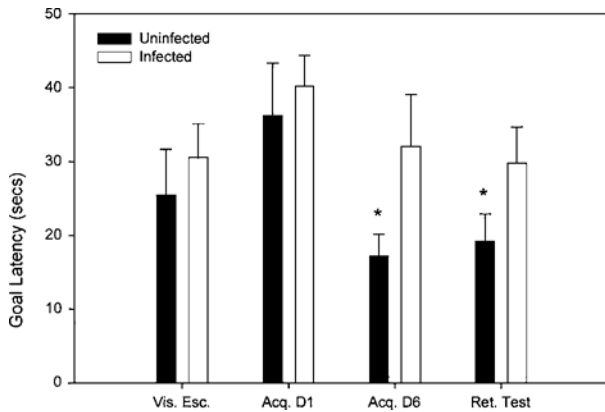
Figure 1 Results from the three phases of the Morris Maze procedure. Performance on the visible platform learning task was similar for the two groups. HIV-infected mice were deficient in the acquisition of the spatial learning task as indicated by longer latencies on day 6. HIV-infected mice were also deficient on the retention test conducted 8 days later. Values are means ±SEM (*$P < .05$).

$(F(1, 22) = 10.4, P = .0042)$, with no interaction between the two factors $(F(1, 22) < 1)$. Post hoc analysis confirmed that on day 6, the time required to locate the platform had declined for the uninfected group, but not in the HIV-infected group.

The retention test to assess long-term memory, conducted 8 days after the acquisition phase, indicated that HIV-infected mice were impaired (Figure 1; Retention Test). The time required to locate the submerged platform remained lower for uninfected than HIV-infected mice, indicating poorer performance for the latter $(t_{(22)} = -2.1, P = .0447)$.

HIV infection did not alter the impact of fatigue generated by a 2-min forced swim as assessed by a 15-min locomotor activity test. The activity data are summarized as total seconds of activity in 5-min bins across the 15-min session in Figure 2. A 2 (infection) $\times$ 3 (time) ANOVA on these data revealed
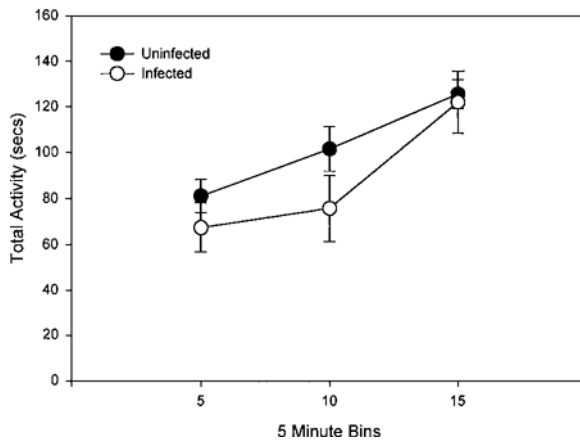

Figure 2 Locomotor activity at 5-min intervals following 2 min of forced swimming. Activity of both groups of mice increased over time to the same extent, suggesting the HIV infection did not impact recovery from fatigue.

only a significant effect of time $(F(2,71) = 29.4, P < .0001)$, with no effect of Infection $(F(1,22) = 1.2, P > .2)$ or of the interaction between the two factors $(F(2,22) = 1.4, P > .2)$. Thus, as indicated in Figure 2, motor activity increased across the test period to the same extent for both groups, indicating that HIV infection did not alter recovery from the fatiguing effects of the forced swim.

Histopathology
Histological analysis verified the presence of HIV-positive human monocytes in the frontal lobes of HIV-infected mice and uninfected monocytes in the control mice. Importantly, the numbers and distribution of macrophages was similar in both groups of mice, and multinucleated cells were elevated in the frontal lobe of the HIV-infected mice as observed in previous studies (Tyor *et al*, 1993; Persidsky *et al*, 1996; Avgeropoulos *et al*, 1998). The most severe pathology for both groups was confined to the anterior portion of the frontal cortex, extending medially and laterally across the entire hemisphere, and ventrally into the caudate putamen for most mice.

Figure 4 shows the mean astrogliosis and microgliosis ratings from HIV-infected and uninfected groups of mice derived from the rating scale illustrated in Figure 3. Mann-Whitney *U* tests comparing the two groups on assigned ratings indicated that both astrogliosis $(U = 20, P = .0009)$ and microgliosis $(U = 41, P = .0245)$ were greater for HIV-infected mice than for control mice inoculated with uninfected cells.

Discussion

The present study establishes that HIV-infected SCID mice were deficient in the acquisition and memory of a complex learning task requiring the utilization of the spatial relationship among environmental cues to locate a submerged goal platform in a Morris water maze. Although mice infused with HIV-infected monocytes were deficient on this complex learning task in comparison to control mice infused with uninfected cells, the two groups were comparable in the acqustion of a simple visual cued task in the water maze, and in recovery of motor activity from a forced swim challenge. Thus, the observed performance decrement for HIV-infected mice was likely due to a cognitive (learning/memory) deficit rather than to possible confounding effects of sensory/motor impairments, or greater susceptibility to fatigue. In addition to confirming cognitive deficits for the SCID mouse model of HIV encephalitis, the study establishes that the cognitive impairment can be observed after only two successive inoculations over 4 weeks with HIV-infected monocytes, as opposed to the four successive inoculations over 12 weeks reported in our earlier study (Avgeropoulos *et al*, 1998). As observed in our earlier studies using four inoculations
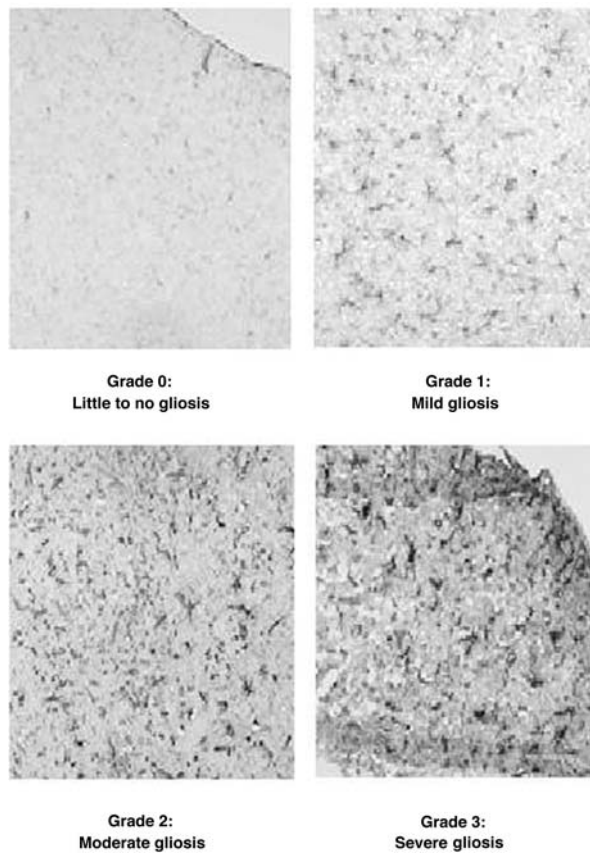
Figure 3 Grading scale for severity of astrogliosis. Increase in frequency and intensity of GFAP-stained cells reflects increased astrogliosis.

(Tyor *et al*, 1993; Persidsky *et al*, 1996; Avgeropoulos *et al*, 1998), mice inoculated twice with HIV-infected monocytes in the present study had significantly more astrogliosis and microgliosis than mice inoculated with uninfected monocytes. Thus, histological analysis indicates pathology due to HIV-infection beyond that produced by introducing uninfected monocytes into the frontal lobe.

Unique features of our SCID mouse model that likely contribute to the observed HIV-related cognitive deficits, as well as other effects of HIV exposure, include the length of exposure to the virus and the difficulty of the learning task. As noted above, in contrast to the four repeated exposures to the virus prior to assessing behavior in our previous study, mice in the current study received only two exposures prior to behavioral testing. In both studies, recall memory was impaired; in the present study, acquisition was also impaired. The timing of the reinoculations in these studies was based on postmortem histological analysis in other studies verifying the presence of the xenograft 4 to 6 weeks after inoculation (Tyor *et al*, 1993; Persidsky *et al*, 1996). The duration of exposure to HIV infection may be important because no difference between HIV-infected SCID

and uninfected controls was observed in the acquisition of a spatially cued learning task similar to ours, except that the mice were tested between 3 and 15 days after a single inoculation (Zink *et al*, 2002). Furthermore, HIV-related differences in electrophysiological measurements in that study were not seen until at least 8 days post inoculation (Zink *et al*, 2002). Thus, despite observing a cognitive dysfunction after two inoculations in the present study, rather than the four we previously used, a single inoculation may not be sufficient to produce the deficit in a similar learning paradigm. Further experiments in which the methodological features of the behavioral assessment are identical will be required to further examine the duration of HIV infection required to produce cognitive deficits in the SCID model.

Another feature of our model, which may have contributed to the detection of cognitive deficits in the HIV-infected mice, is the relative difficulty of learning the maze problem. For example, the tank surface area to platform surface area ratio has been reported to contribute to the difficulty of learning problems in the Morris water maze (Inman-Wood *et al*, 2000; Carman and Mactutus, 2001). This ratio was much greater in the present experiment (tank area/goal area = 160) than the ratio in the experiment in which HIV-infected mice did not differ from uninfected controls (tank area/goal area = 39; Zink *et al*, 2002), suggesting that the task used in the present study was more difficult. Thus, it is possible that more difficult learning problems may facilitate detection of cognitive dysfunction in HIV-infected mice. Additional experiments in which the HIV infection is held constant and task difficulty systematically manipulated are required to support this interpretation.

Water maze tasks have been used for many years to evaluate learning and memory (Morris, 1984; D'Hooge and De Deyn, 2001). The performance of hippocampal-lesioned rodents on hidden-platform problems in these mazes has established a dominant role for this structure on spatial learning and memory (Morris *et al*, 1982; Riedel *et al*, 1999). The performance of hippocampal-damaged humans on a virtual-reality maze task also established the importance of hippocampal function in human learning and memory (Astur *et al*, 2002). Furthermore, there is evidence that HIV infection alters hippocampal function in SCID mice, even though the inoculation site was several millimeters away (Zink *et al*, 2002; Anderson *et al*, 2003). Specifically, SCID mice inoculated with HIV-infected monocytes had deficits in fundamental properties of hippocampal neurons, such as long-term potentiation and paired-pulse facilitation, in comparison to mice infused with uninfected monocytes. These experiments also noted decreased expression of neurofilament and mitogen activated protein kinase (MAP)-2 proteins in subregions of the hippocampus, suggesting that the morphological integrity of hippocampal neurons was compromised by HIV infection (Zink *et al*, 2002;

Anderson *et al*, 2003). The results of these studies strongly suggest that the cognitive dysfunction of the HIV-infected SCID mice in the present study might well be related to hippocampal dysfunction. Moreover, hippocampal damage has been reported in AIDS patients with HAD. Masliah *et al* (1992) reported large decreases in parvalbumin immunoreactivity in the hippocampus of brains from HAD patients examined post mortem and the extent of these decreases correlated with HAD severity. Thus, the SCID model appears to be suitable for future studies on the hippocampally derived cognitive impairments found in AIDS patients and for future investigations of HAD.

In conclusion, this study conclusively established deficits in cognitive function for the SCID mouse model of HIV encephalitis. These deficits were not associated with impaired visual ability, impaired swimming ability, or differential susceptibility to fatigue, leaving cognition as the most likely deficit. Furthermore, this cognitive deficit was obtained with two inoculations of HIV-infected monocytes rather than four as in our previously reported experiment. Because the SCID mouse HIV encephalitis model links behavioral and neuropathological alterations due to HIV infection, this model will be valuable for future studies on the neurobiology of HAD.

Materials and methods

Animals
Male CB-17 SCID mice ($n = 24$) were obtained from Charles River Laboratory (Wilmington, MA) and singly housed in microisolator cages with free access to food and water. All cages, bedding, food, and water were autoclaved prior to use. The animals were maintained in isolation cubicles (BioSafety Level [BSL]-3 equivalent) within the colony room, which was maintained on a 12-h light cycle (lights on 0600). All procedures were conducted in AAALAC-approved facilities, were approved by the Institutional Animal Care and Use Committee, and were consistent with the guidelines of the National Institute of Health (NIH) *Guide for the Care and Use of Laboratory Animals* (NIH publication no. 80-23, revised 1996).

Experimental procedure
The experiment was conducted over a 9-week period beginning after 1 week of acclimatization to the colony room. HIV-infected mice were inoculated intracerebrally with HIV-infected human monocytes ($n = 12$) and control mice were inoculated with uninfected human monocytes ($n = 12$) during week 2 and again during week 5. Behavioral assessment in the Morris water maze began during week 6 and ended during week 9. All mice were sacrificed at the end of the study for histological verification of infection and neuropathology.

Cell infection and mouse inoculation
Cell culture and inoculation were carried out as previously described (Avgeropoulos *et al*, 1998). In brief, 10^8 purified primary human monocytes and HIV-1$_{ADA}$ virus were obtained from Dr. Howard Gendelman (Center for Neurovirology and Neurogenerative Disorders, University of Nebraska Medical Center). The cells were cultured at 37°C in 5% Dulbecco's modified Eagle medium that was supplemented with 10% human serum, glutamine supplement, and macrophage colony-stimulating factor (M-CSF) in Teflon coated flasks. After 7 days, the cells were infected at a multiplicity of infection (MOI) of 0.2 viral particles per cell for 1 h and then the virus was removed by centrifugation. A duplicate set of cells was left uninfected. Cells were collected 2 weeks later and resuspended in phosphate-buffered saline (PBS) for the inoculation procedure. HIV-infected mice were inoculated with 1×10^5 infected monocytes and control mice with uninfected monocytes while under ketamine (95 mg/kg) and xylazine (5 mg/kg) anesthesia. The cells were delivered into the right frontal lobe in a volume of 30 μl. Depth of injection was 4 mm from the top of the skull. While mice were anesthetized during the first inoculation procedure, ear punches were made to allow for positive identification of the groups.

Behavioral apparatus
Cognitive behavior was assessed via learning and memory for the spatial relationship of cues in the environment of a Morris water maze. The maze, a circular tank (57 cm diameter), was filled with water to a depth of 18 cm and shielded behind white cloth curtains. The water was made opaque with dark blue tempera paint and maintained at 25°C with a submersible electric heater. A camera suspended above the tank provided video input for a PolyTrack animal tracking system (San Diego Instruments, San Diego, CA). A moveable goal platform (4.5 cm diameter) was submerged 1 cm below the water surface for the assessment of spatial learning and memory, and was 1 cm above water surface for a control experiment to assess basic visual and motor function. During the latter experiment, the goal platform was placed in a Plexiglas track (15 cm wide, 57 cm length, extending 7 cm above the surface) that extended across the water tank. Three brightly colored extramazal cues were suspended from the curtain around the perimeter of the tank. The spatial relationship between these cues indicated the location of the submerged goal platform for the learning problem (i.e., the spatial learning task). In a final control experiment, the water tank was divided into four quadrants with Plexiglas (7 cm above the surface), which were used for the forced swim test described below.

Motor activity was assessed with an Infrared Activity Monitoring System, Version 2.10 (Colbourn Instruments, Allentown, PA), which uses body heat to detect and record movement. A lens array focuses

heat "images" on a heat sensor and the number of image transitions per unit time is recorded. The presence or absence of transitions defines activity, and amount of time engaged in activity is recorded.

Morris maze procedures
Tests in Morris water maze occurred over a 17-day period and consisted of four phases: habituation, sensory/motor evaluation, problem acquisition, and problem recall. Common features for each phase were that each day included four trials of 90 s each. Trials ended when the mice reached the platform or 90 s had elapsed. After 5 s on the platform, the mouse was removed and placed in holding cage under heat lamps for 3 to 5 min until its next trial. Mice not reaching the platform within 90 s were guided to it, and remained there for 5 s prior to being removed.

Habituation occurred on day 1, during which the mice were habituated to swimming in the tank for four trials without the submerged platform. Basic visual and motor functions were evaluated on day 2 by determining the time required to reach the visible platform in Plexiglas track described above. Problem acquisition began on day 3 and continued through day 8. During this phase, the mice learned to locate the submerged goal platform on the basis of the spatial relationship of cues located external to the maze as described above. The submerged platform was permanently located in the northwest quadrant of the tank. For each of the four daily trials during the acquisition phase, the mice were placed in the water at a different compass point along the edge of the tank. The distance and time required to locate the platform were recorded for each trail. Recall of the problem was assessed after an 8-day hiatus and the test was as described for the acquisition trials.

Locomotor activity following forced swim
To provide information about the possible impact of HIV pathology on endurance, the activity of the mice was evaluated after a forced swim. Immediately after the retention test in the Morris maze, the mice were returned to the water tank in groups of four for a 2-min forced swim. Upon completion of the forced swim, they were placed into the activity monitor for a 15-min assessment.

Histopathology
Histological analysis of the brains was conducted as previously described (Tyor *et al*, 1993; Persidsky *et al*, 1996; Avgeropoulos *et al*, 1998). In brief, the day after the Morris maze procedure was completed, mice were deeply anesthetized and sacrificed. Brains were extracted and snap frozen in embedding medium for pathological analyses. Five-micron coronal, serial brain sections were obtained for immunocytochemical staining. Starting at section number 100 (starting from the anterior tips of the frontal lobes), sets of 4 serial sections were obtained every 45 sections, fixed in

ethanol, and immunocytochemically stained for human macrophages (EBM/11; DAKO; diluted 1:50 in PBS), HIV p24 antigen (DAKO; diluted 1:50 in PBS), astrocytes (anti-GFAP; Chemicon; diluted 1:750 in PBS), and microglia (F4/80; Caltag; diluted 1:20 in PBS). These 11 sets of serial sections extended well beyond the injection site into the parietal/temporal lobes. The brain pathology of all mice was viewed using an Olympus microscope by one of the authors (WRT), who was blinded to the animal's prior condition (infected *versus* uninfected).

The sectioned brains were evaluated for the region of most severe pathology. This area was defined as a combination of the slides revealing the greatest number of human cells and highest grade of microgliosis and astrogliosis. For analysis of astrogliosis and microgliosis, all stained sections (total of 11 for each antibody) were graded according to a 4-point scale. The scale for rating astrogliosis is illustrated in Figure 3. The highest score for each mouse was used for the data summarized in Figure 4. Finally, a neuroanatomical atlas of the mouse was used to confirm the location of most severe pathology (Sidman *et al*, 1971).

Data analysis
Comparison of means of two groups was accomplished using Student's *t* test or the Mann-Whitney *U* test, as appropriate. Data from the spatial problem acquisition phase were subjected to ANOVA. Infection (infected *versus* uninfected) was a between-group factor and session was a repeated measure. The Newman-Keuls test was applied for post hoc analysis. In addition, Bartlett's chi square test was used to ascertain if the variance in the data set met the assumption of homogeneity across groups. Data sets with heterogenous variance were $\log_e$ transformed prior to analysis. Probabilities less than .05 ($P < .05$) were considered statistically significant for all analyses.
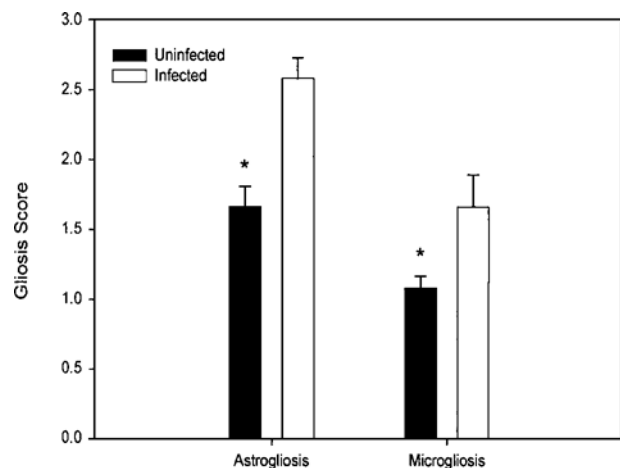

Figure 4 Mean astrogliosis and microgliosis ratings from frontal lobe sections of the mice. Astrogliosis and microgliosis ratings were greater in the HIV-infected mice. Values are means ±SEM ($^*P < .05$).

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