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Translational spatial task and its relationship to HIV-associated neurocognitive disorders and apolipoprotein E in HIV-seropositive women

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Abstract HIV-associated neurocognitive disorders (HAND) continue to be a neurological complication of HIV infection in the era of combined antiretroviral therapy. Hippocampal neurodegeneration and dysfunction occurs as a result of HIV infection, but few studies to date have assesses spatial learning and memory function in patients with HAND. We used the Memory Island (MI) test to study the effects of HIV infection, *apolipoprotein E* (*ApoE*) allele status, and cerebral spinal fluid (CSF) ApoE protein levels on spatial learning and memory in our cohort of Hispanic women. The MI test is a virtual reality-based computer program that tests spatial learning and memory and was designed to resemble the Morris Water Maze test of hippocampal function widely used in rodent studies. In the current study, HIV-seropositive women (n=20) and controls (n=16) were evaluated with neuropsychological (NP) tests, the MI test, *ApoE*, and CSF ApoE assays. On the MI, the HIV-seropositive group showed significant reduced learning and delayed memory performance compared with HIV-seronegative controls. When stratified by cognitive performance on NP tests, the HIV-seropositive, cognitively impaired group performed worse than HIV-seronegative controls in ability to learn and in the delayed memory trial. Interestingly, differences were observed in the results obtained by the NP tests and the MI test for *ε4* carriers and noncarriers: NP tests showed effects of the *ε4* allele in HIV-seronegative women but not HIV-seropositive ones, whereas the converse was true for the MI test. Our findings suggest that the MI test is sensitive in detecting spatial deficits in HIV-seropositive women and that these deficits may arise relatively early in the course of HAND.

Keywords Spatial memory · Apolipoprotein E · Cognitive impairment · HIV · Women · HAND

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Introduction

The prevalence of HIV-associated neurocognitive disorders (HAND) has increased owing to the widespread use of

combined antiretroviral treatment (cART) and resulting longer survival of HIV-infected patients (McArthur 2004; Sacktor et al. 2002; Selnes 2005). In the USA, HAND's prevalence among HIV-infected individuals on cART is as high as 50 %, and HAND continues to be a significant cause of morbidity in chronically infected patients (Heaton et al. 2010). The majority of these patients suffers from the milder forms of HAND (Antinori et al. 2007; Heaton et al. 2010), which can develop and progress despite adequate viremia control and good cART adherence (Cohen and Gongvatana 2010; McArthur and Brew 2010; McArthur et al. 2010; Ragin et al. 2004). HAND is characterized by cognitive impairments and motor dysfunction (Antinori et al. 2007; McArthur and Brew 2010; Rackstraw 2011), all of which can persist even with cART treatment (Al-Khindi et al. 2011; McArthur et al. 2010; Rackstraw 2011). Cognitive deficits in HAND can include alterations in visuospatial learning and memory.

The hippocampus is necessary for the acquisition and retrieval of spatial memory (Burgess 2008; D'Hooge and De Deyn 2001; Mishkin and Appenzeller 1987; Zola et al. 2000). Hippocampal dysfunction in HAND may include atrophy, neuronal cell loss, and functional magnetic resonance imaging abnormalities (Archibald et al. 2004; Castelo et al. 2006; Maki and Martin-Thormeyer 2009; Sa et al. 2004; Wiley et al. 1998). Studies in rodent models also suggest that HIV infection has multiple deleterious effects on the hippocampus, including synaptic loss and cell death (Kim et al. 2008), impaired neurogenesis (Lee et al. 2011), and disruption of NMDA receptor trafficking (Xu et al. 2011). Despite considerable evidence that HAND affects the hippocampus, only a handful of studies in HIV-infected patients have incorporated tests of spatial memory into their batteries of neurocognitive assessments (Maki and Martin-Thormeyer 2009; Schouten et al. 2011).

Some studies conducted on HIV-seropositive cohorts (where >70 % of all subjects are male) suggest that HIV infection leads to deficits in spatial attention (Covert Orientation of Visual Attention Task; Maruff et al. 1995; Rohit et al. 2007) and spatial navigation (Money Road Map Test; Olesen et al. 2007). However, other researchers who used the Rey–Osterrieth Complex Figure Test or Visual Design tests (Valcour et al. 2004b; Krikorian et al. 1990) did not find evidence of impaired visual spatial functioning in subjects with HAND. The variability in these results suggests the need for a more reliable and sensitive tool for testing visuospatial function in HIV-infected patients.

The Morris Water Maze (MWM) has been widely used in rodent models as an instrument with particular sensitivity to the effects of hippocampal lesions (Morris 1984); reviewed in (D'Hooge and De Deyn 2001). Table 1 summarizes some of the evidence that spatial learning and memory impairments occur as a consequence of expression of HIV-1 and its related proteins in rodent models using the MWM (D'Hooge et al. 1999; Glowa et al. 1992; Griffin et al. 2004; Tang et al. 2009; Zink et al. 2002). Rodent HIV models also tend to show impairment in the Barnes maze (Sanchez-Alavez et al. 2000), radial arm maze (Li et al. 2004), and radial arm water maze (Keblesh et al. 2009) (Table 1). However, it has been questioned how well the MWM translates to learning and memory tests typically used in in humans. Therefore, we sought to determine if there are spatial learning and/or memory impairments in HIV-seropositive individuals using a task that may more faithfully bridge animal and human studies, the "Memory Island" (MI) test. The MI, described previously in Rizk-Jackson et al. (2006), is a virtual reality-based spatial learning and memory task developed for used in humans based on the MWM. The MI is a virtual island on which participants are asked to navigate to four different targets marked with visible indicators, each of which is located in a different quadrant of the island (visible trial). Then, in subsequent trials, they return to the island and find the targets again, this time without visible indicators (hidden trials). MI has been successfully used in a wide range of age groups (6–99 years) and shows sensitivity to gender, age, and genetic factors (such as *ApoE* genotype) similar to that seen with the MWM in animal (Acevedo et al. 2010; Raber 2008; Raber et al. 2000; Rizk-Jackson et al. 2006).

A second question that we addressed using the MI concerned the impact of *Apolipoprotein E* (*ApoE*) allele status on cognitive performance in HIV-infected individuals. ApoE proteins play a central role in the metabolism and transport of lipoproteins and cholesterol and also appear to be important modulators of neuronal development, plasticity, and recovery from injury (Herz and Beffert 2000; Vance and Hayashi 2010). Distinct alleles (*ε2*, *ε3*, and *ε4*) encode human isoforms and have been studied as risk factors for many different conditions. For example, compared with *ε2* and *ε3*, the *ε4* allele confers increased risk of age-related cognitive impairment and the development of Alzheimer's disease (AD), especially in women (Bu 2009; Raber 2008). The *ε4* allele has also been linked to worse cognitive outcomes in various conditions that cause brain injury, such as cardiac bypass surgery (Newman et al. 1995) and multiple sclerosis (Shi et al. 2008). The *ε4* allele may also contribute to the development of HAND. Possession of the *ε4* allele has been linked to accelerated progression of HIV infection and a higher incidence of HIV-associated dementia (Corder et al. 1998; Dunlop et al. 1997; Valcour et al. 2004b). In addition, a recent study in HIV-seropositive men showed that higher levels of cerebral spinal fluid (CSF) ApoE protein were correlated with better cognitive performance in *ε2* or *ε3* carriers, but worse cognitive performance in *ε4* carriers (Andres et al. 2011). Thus, *ε4* allele may interact with HIV infection to increase risk of HAND, particularly in women. We therefore examined the potential associations

Table 1 HIV-1-related proteins effects on spatial learning and memory in rodents

Model	Effect	References
HIVE/SCID mice	The cognitive deficits manifest as longer latencies to locate the visible platform on the last day of the MWM acquisition period and during a retention test (probe trial) 8 days later. Evidence of impairments in spatial memory in radial water arm maze	Griffin et al. 2004; Keblesh et al. 2009; Zink et al. 2002
Spargue–Dawley rats (HIV-1Tg)	Deficits in modified MWM in latency to platform in hidden trials, but did not show a deficit in their memory of the general location of the hidden platform. Deficits in reversal learning and new strategy learning in modified MWM	Lashomb et al. 2009; Vigorito et al. 2007
Transgenic mice expressing gp120	MWM learning (visible trials) impairments in 3-month-old. Spatial memory (probe trial) impairments in 12-month-old	D'Hooge et al. 1999
Wistar rats with ICV infusion of gp120	Results showed that while both HIV- and FIV-derived gp120 impaired the performance in the Barnes maze, only HIV-derived gp120 impaired the induction and maintenance of LTP	Sanchez-Alavez et al. 2000
Doxycycline-inducible transgenic mice expressing HIV-1 Tat_{1-86} under GFAP	Significantly longer latency and traveled longer distances to find the platform than Tat− mice. No differences in swim speed were observed. The Tat-induced disruption in spatial learning coincided with suppression of LTP in hippocampal	Fitting et al. 2008, personal communication
Sprague–Dawley rats ICV infusion of gp120	The gp120 treated rats are deficit in learning (visible trials) and spatial memory (hidden trials) in MWM	Glowa et al. 1992; Tang et al. 2009
Wister rats ICV infusion of Tat	Attenuation of learning in radial arm maze	Li et al. 2004

gp120 glycoprotein 120 in HIV-1, *Tat* trans-activator of transcription in HIV-1, *HIVE* HIV-1 encephalitis, *MDM* monocyte-derived macrophages, *SCID* Severe combined immunodeficient, *MWM* Morris water maze, *LTP* long-term potentiation, *HIV-1Tg* noninfectious HIV-1 with gag-pol deletion, *ICV* intracerebroventricular, *GFAP* glial fibrillary acidic protein

of *ε4* status and CSF levels of ApoE protein with cognitive impairment in women with HIV infection.

Methods

Cohort description

This study is nested in the approved Hispanic-Latino Longitudinal Cohort of HIV-Seropositive Women as part of the NeuroAIDS Specialized Neuroscience Research Program (SNRP) at the University of Puerto Rico, Medical Science Campus (HLLC) with appropriate internal review (IRB) approval. This is a unique cohort of Hispanic HIV-seropositive women characterized with respect to their viral immune profiles, neurological function, and neuropsychological performance (Luo et al. 2003; Wojna et al. 2006). All seropositive women were screened at their primary HIV clinics at the Puerto Rico Medical Center (Clinica de Enfermedades Transmitidas Sexualmente and Longitudinal Materno-Infant Clinic) and community-based organizations. This study used a convenient sample of 20 HIV-seropositive and 16 HIV-seronegative women from the HLLC and seronegative controls consecutively recruited as they were evaluated in the cohort (described previously in Wojna et al. 2006). HIV-seronegative (control) CSF samples were provided by Dr. Carlos Pardo at Johns Hopkins University, Baltimore, MD (JHU) and obtained from patients examined by a neurologist for non-HIV related conditions [3/6 normal participants (no neurological disorders), 2/6 multiple sclerosis, and 1/6 with common variable immune deficiency] after obtaining appropriate consent from JHU IRB as banked samples for biological testing.

Neurocognitive assessments

The battery of neurocognitive assessments has been previous described (Luo et al. 2003; Velázquez et al. 2009; Wojna et al. 2006; Wojna et al. 2007). The assessments included five different cognitive domains (tests): frontal executive function (Stroop Color Word Test and Trail Making B), psychomotor speed (Symbol Digit Modality Test, Visual Reaction Time Nondominant Hand, and Auditory Reaction Time Nondominant Hand), verbal memory (Rey Auditory Learning Test; Trial 5, Memory Recall, Delayed Memory), and motor speed [Trial Making A, Grooved Pegboard (nondominant and dominant hand)]. All assessments where conducted in Spanish. Individual z-scores were calculated using a reference group of 35 HIV-seronegative women. No differences were seen between the reference group and HIV-seronegative or HIV-seropositive in regard to age, education, vocabulary, reading, and annual income ($p>0.05$).

HIV cognitive impairment determination

Cognitive status was determined using the American Academy of Neurology HIV dementia criteria (American Academy of Neurology AIDS Task Force 1991; American Academy of Neurology AIDS Task Force 1996), modified to include criteria for asymptomatic cognitive impairment. Asymptomatic cognitively impaired patients are defined as those who have abnormal neuropsychological test results (1 SD in two or more tests or 2 SD in one or more tests, below the normal control group), but who do not exhibit current neurological deficits during medical and neuropsychological examinations and who report no functional/emotional disturbances in quality of life questionnaires (Wojna et al. 2006). For the purposes of this study, we combined participants with normal cognitive function (n=8) and those with asymptomatic impairment (ANI, n=3) as the HIV-seropositive normal cognition group and minor cognitive motor disturbance (MCMD, n=4) and HIV-associated dementia (HAD, n=5) in the impaired group.

Memory Island test

All women were asked to enter a virtual environment designed to test spatial learning and memory (Rizk-Jackson et al. 2006), which consisted of an island with trees, house, hills, grass, sidewalks, and buildings. Women navigated through the environment using a joystick, while listening to sounds such as birds singing and water flowing through headphones that simulated movement through the environment. During training sessions (duration=10 min), the women were free to explore the environment to find (one at a time) four different targets marked by large, brightly colored flags (visible trials). The women were then asked in four consecutive trials (each restarting in the middle of the island) to navigate the island using spatial cues to locate the each of the four targets, but without flag locaters (hidden trials). During each trial, after 2 min, the subjects were given an arrow at the top of the screen to help guide them to the target. In the hidden trials, after a 15-min delay, the women were asked to find the target location with 30 s remaining to perform the task. The environment is located on a quadrant-based coordinate system, so that distance and latency to target can be measured. Cumulative distance to target was calculated by determining the distance between the participant and the target every 2 seconds. Percentage of successful trials was determined as the percent in which the participant was able to find the target without the aid of the arrow. For the probe determination, we measured time spent in each of the four quadrants. The MI test was conducted with a culturally appropriate Spanish script and visual word prompts. Copyright permission was obtained from Jacob Raber, Ph.D., Professor in the Department of Neurology at Oregon Health and Science University for the use of this program.

ApoE genotyping

Blood was collected from seropositive participants and purified as previously described (Toro-Nieves et al. 2009; Velázquez et al. 2009). Saliva was collected from the seronegative participants using Oragene DNA kits (DNA Genotek, Ottawa, Canada) and shipped or taken to Dr. Summer Acevedo's laboratory at the Ponce School of Medicine for purification. Polymerase chain reactions were performed using 5′-TCCAAGGAGCTGCAGGCGGCGCA-3′ (upstream) and 5′-ACAGAATTCGCCCCGGCCTGGTA CACTGCCA-3′ (downstream) primers, then digested overnight at 37 °C with CfoI. Products were run on 4 % agarose gels with the bands categorized by size used to identify genotypes (Wenham et al. 1991).

ELISA for total ApoE levels

ApoE levels were measured in previously frozen CSF samples of HIV-seronegatives from JHU and HIV-seropositive women samples from the HLLC using an ELISA for total human ApoE (MABTECH, 3814 West Street, Mariemount, OH, USA), based on a quantitative sandwich method in a 96-well plate. ApoE assays were performed and analyzed using manufacturer's protocol. Protein concentrations in diluted CSF samples were within the linear range of the standard curve generated for each assay, and triplicate measurements were obtained for each sample (3 μL). A standard solution was used to produce a standard curve. A Bradford protein analysis was completed for each sample to calculate total CSF protein levels, and adjustments were made to values calculated from the standard curve to equalize total protein levels produced final total ApoE protein levels (μg/mL) used for comparisons between groups.

Data analysis

All data was collected using standard data forms and entered into a Microsoft Excel spreadsheet for analysis using SPSS software, version 17. The assumption of normal distribution was tested for continuous variables (e.g., spatial learning score) using Shapiro–Wilk test. Student's t tests were conducted between genotypes (non-*ε4*, *ε4*) or HIV status (HIV-seronegative or HIV-seropositive) for neurocognitive performance and biological measures. Analysis of variance (ANOVA) were used in the comparison of HIV-seronegative subjects to HIV-seropositive subjects stratified into two cognitive performance groups (normal and impaired), with subsequent Tukey's post hoc to distinguish difference in subgroups. Values are reported as F value (degrees of freedom) and p value. For the MI test,

repeated measure ANOVAs were conducted on measures of velocity and performance (latency to target) across trials during visible and hidden trials. For the probe determination, the Wilcoxon signed rank test was used to test the median differences between the percent time spent in the target quadrant and the percent spent all other quadrants. For all analyses, $p<0.05$ was considered statistically significant. All graphs were preformed using Prism.

Results

Subject demographics

HIV-seropositive women and seronegative controls were similar in age and education (Puerto Rican and Spanish speaking) (Tables 2 and 3). When HIV-seropositive women were stratified by cognitive performance into groups with normal cognition and impaired cognition (see "Methods"), Student's *t* test indicated no differences between the groups with regard to plasma ribonucleic acid (RNA) HIV viral load, CSF HIV RNA viral load, type of antiviral treatment or protease inhibitor treatment, or central nervous system penetration effect of the cART (CPE) (Letendre et al. 2010) (Table 2). For the neuropsychological assessments, HIV-seronegative controls and HIV-seropositive women stratified by cognitive impairment showed no significant differences in reading and vocabulary status, and Beck's Depression Index (Table 2).

Neuropsychological assessments

Significant differences in performance on NP tests were seen between controls and HIV-seropositive women stratified by cognitive performance (normal vs. impaired) using ANOVA (Table 3). Overall neuropsychological testing z-scores (NPZ) and performance in the frontal executive, psychomotor speed, and motor speed cognitive domains were significantly different among groups (Table 3, $p<0.05$). Tukey's post hoc analysis indicated differences between the HIV-seronegative and HIV-seropositive impaired group for the NPZ ($p<0.04$), psychomotor speed ($p<0.02$) and motor speed cognitive domains ($p<0.03$), and individual scores on the Symbol Digit Modalities Test ($p<0.03$). These findings are consistent with those of other studies using different samples from this same cohort (Wojna et al. 2006, 2007a,b).

HIV and Memory Island test

In order to identify possible group differences in the spatial learning (visible target) and immediate memory (hidden target) trials of the MI, we first analyzed differences

Table 2 HIV-seropositive characteristic

	HIV seropositive		*p* value
	Normal	Impaired	
N	8	12	
Age	39.1±3.0	42.9±3.5	0.41
CSF HIV RNA viral load (log) (*N*)	2.1±0.2 (6)	1.7±0.1 (12)	0.06
Plasma HIV RNA viral load (log) (*N*)	2.8±0.8 (8)	2.0±0.5 (12)	0.10
CD4 nadir (cells/mm^3)	308.9±55.5	482.1±93.8	0.59
CD4 (cells/mm^3)	477.9±43.9	740.6±118.9	0.28
Antiretroviral treatment (%)			
Naive	2/8 (25 %)	2/12 (16.6 %)	
ART		1/12 (8.3 %)	
cART	6/8 (75 %)	9/12 (75 %)	
CPE (*N*)	8.7±0.7 (5)	8.8±0.9 (11)	0.71
Protease Inhibitor (%)	5/6 (83.3 %)	6/9 (66.6 %)	0.28
ApoE CSF levels (μg/mL)	4.9±1.3	5.1±1.2	0.92
HCV (% positive)	1/8 (12.5 %)	3/12 (25 %)	0.50
Toxicology (% positive) [agent (#patients)]	25 % [cocaine (1), marijuana (1)]	16.66 % [marijuana (1), cocaine/marijuana (1)]	0.66

Mean±SEM

CPE central nervous system penetration effect (Letendre et al. 2010), *HCV* hepatitis C virus, *ART* single drug antiretroviral treatment, *cART* combined antiretroviral treatment

[a] Student's *t* test

Table 3 Neurocognitive testing in HIV-seronegative and HIV-seropositive subjects

	HIV status		p value[b]	HIV-seropositive		F[c]	p value[c]
	Seronegative	Seropositive		Normal	Impaired		
N	16	20		8	12		
Age[a]	41.4±2.3	41.4±2.4	0.99	39.1±2.9	42.9±3.5	0.33	0.72
Education (years)	13.4±0.6	12.9±0.5	0.49	13.1±1.1	12.7±0.5	0.29	0.74
BDI-II[a]	7.7±2.2	9.8±2.8	0.52	4.5±1.6	13.1±3.3	2.52	0.12
Reading[a]	28.3±0.5	27.5±0.7	0.41	28.9±0.4	26.6±1.2	2.19	0.13
Vocabulary[a]	50.8±3.2	48.1±3.1	0.54	48.3±4.5	47.9±4.4	0.18	0.83
I. NPZ	0.3±0.2	0.2±0.1	0.41	0.6±0.1	−0.1±0.1[d,e]	*6.43*	*0.004*
II. Neuropsychological domains/subtests							
1. Frontal executive	0.1±0.3	0.1±0.2	0.92	0.8±0.1	−0.3±0.2[e]	*3.61*	*0.04*
a. Stroop Color/Word	0.1±0.4	0.2±0.2	0.70	0.5±0.1	0.1±0.3	0.41	0.67
b. Trail Making B	0.1±0.3	0.0±0.4	0.85	1.0±0.2	−0.6±0.5[d]	*4.32*	*0.02*
2. Psychomotor speed	0.2±0.1	−0.1±0.2	0.06	0.2±0.1	−0.4±0.2[d]	*4.40*	*0.02*
a. Symbol Digit Modality Test	0.4±0.3	−0.2±0.2	0.08	0.3±0.2	−0.5±0.2[d]	*3.40*	*0.05*
b. Visual RT nondominant hand	0.1±0.2	−0.1±0.3	0.56	0.4±0.3	−0.3±0.2	1.24	0.30
c. Auditory RT nondominant hand	0.2±0.2	−0.2±0.2	0.21	−0.0±0.2	−0.3±0.4	1.04	0.37
3. Verbal memory (RAVLT)	0.2±0.2	0.4±0.1	0.27	0.5±0.3	0.4±0.2	0.73	0.49
a. Trial 5	0.2±0.1	0.2±0.2	0.77	0.3±0.3	0.2±0.2	0.07	0.94
b. Memory Recall	0.3±0.2	0.9±0.2	0.10	0.8±0.4	0.9±0.3	1.39	0.26
c. Delayed Recognition	0.1±0.2	0.2±0.3	0.57	0.5±0.3	0.1±0.2	0.80	0.46
4. Motor speed	0.6±0.1	0.2±0.3	0.23	0.8±0.1	−0.3±0.4[d,e]	*4.63*	*0.02*
a. Trail Making A	0.1±0.3	−0.2±0.4	0.54	0.9±0.2	−0.1±0.6[e]	*3.88*	*0.03*
b. Grooved Pegboard dominant hand	0.7±0.1	0.4±0.3	0.45	0.9±0.2	0.0±0.5	1.79	0.18
c. Grooved Pegboard nondominant hand	0.1±0.1	0.4±0.3	0.12	0.7±0.3	0.2±0.5	1.91	0.16

z-scores, mean±SEM

BDI-II Spanish Version Beck Depression Inventory II, *NPZ* average of all test z-scores, *RAVLT* Rey Auditory Verbal Learning Test, *RT* reaction time

[a] Raw score±SEM

[b] Student's t test between seronegative and seropositive, significant difference $p \leq 0.05$ in italic

[c] ANOVA between seronegative, seropositive normal and seropositive impaired $p \leq 0.05$ in italic

[d] Tukey post hoc test indicate significant differences $p \leq 0.05$ between seronegative and seropositive impaired

[e] Tukey post hoc test indicate significant differences $p \leq 0.05$ between seropositive normal and seropositive impaired

between HIV-seronegative and HIV-seropositive women. Differences were seen between these groups with respect to *velocity* (virtual units/s) in the visible trials and hidden trials [$F(34)=5.70$, $p<0.03$and $F(34)=6.01$, $p<0.02$, Fig. 1a], where the seropositive group navigated more slowly than the seronegative group. However, when the HIV-seropositive group was stratified by cognitive performance using repeated measures ANOVAs, the HIV-seropositive impaired group showed a trend towards reduced velocities in both the visible trials [$F(33)=2.80$, $p=0.08$] and the hidden trials [$F(33)=2.98$, $p=0.07$, Fig. 1a]. Overall, the HIV-seropositive women navigated more slowly, with the seropositive impaired group showing a trend toward being the slowest group.

Regarding time required to find the target, *latency*, a significant difference was observed across HIV serostatus, with the seropositive group having a higher *latency* [$F(34)=5.70$, $p<0.03$, Fig. 1b]. When stratified by cognitive performance, a significant difference in *average latency* in visible trials was observed between groups [$F(33)=5.42$, $p<0.01$, Fig. 1b], where a Tukey's post hoc test ($p<0.01$) indicated impaired performance in the seropositive women compared to seronegative controls. The *average latency* in hidden trial showed a similar trend, although it was not statistically significant.

With regard to success in finding the target in the first 2 min, *cumulative distance to target*, a repeated measures ANOVA indicated differences between women of the seropositive group and those from the seronegative group during visible

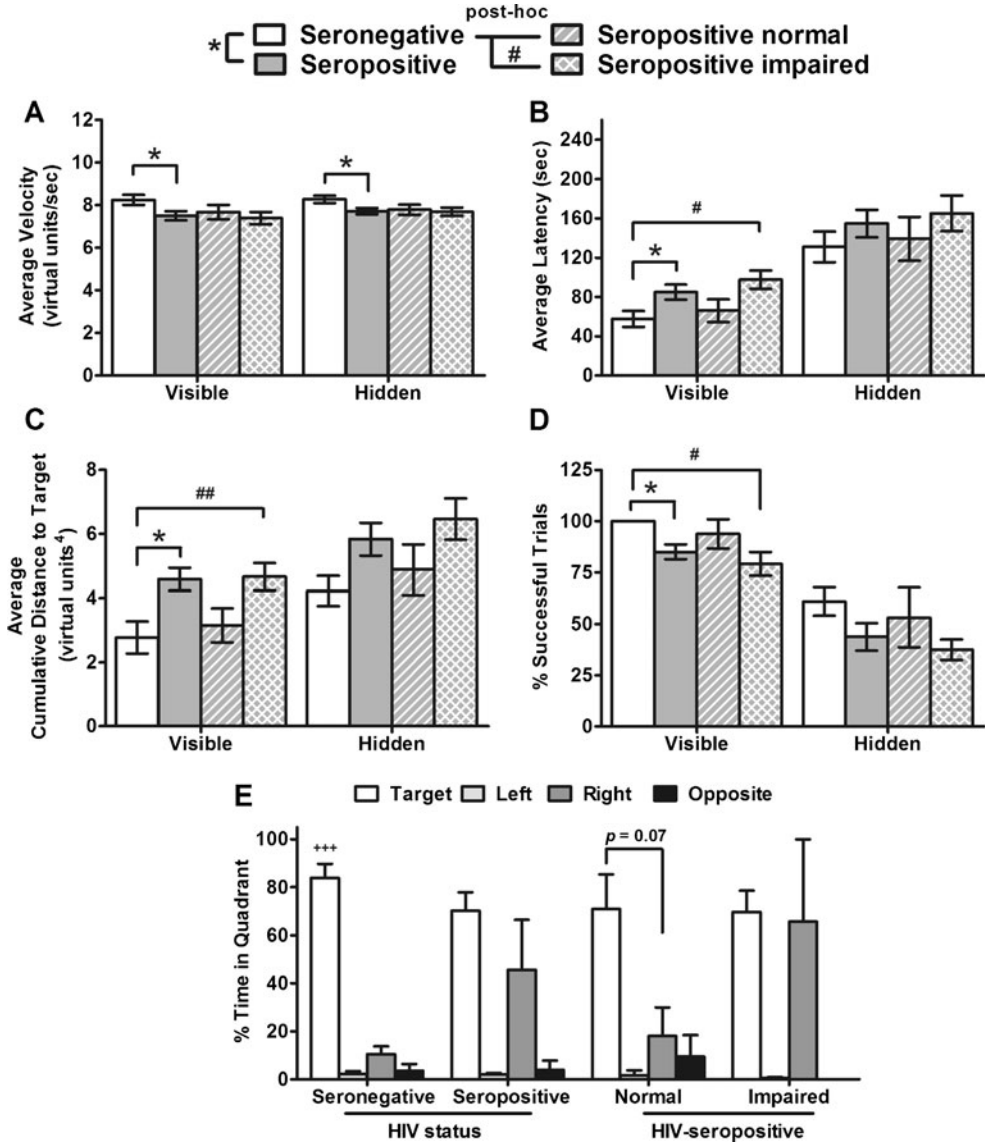

Fig. 1 Differences in MI test performance between HIV-seropositive and seronegative women. **a** There was a difference between the HIV-seropositive and HIV-seronegative groups in velocity during the visible and hidden trials (*$p<0.05$). **b** There was a difference in latency to target for between HIV status groups during the visible and hidden trials (*$p<0.05$). Tukey's post hoc suggests differences between the seronegative and seropositive, impaired group in latency in visible trials ($^{\#}p<0.05$), but not in hidden trials. **c** Differences were also found between the seropositive and seronegative groups in cumulative distance to target for both the visible trials (*$p<0.05$). In stratified ANOVAs, post hoc analysis suggested that differences are due to reduced performance in seropositive impaired group ($^{\#}p<0.05$). **d** The seronegative subjects had a greater percentage of successful trials (i.e., trials in which they found the target within 2 min) than the seropositive subjects overall (*$p<0.05$) and the seropositive, impaired ($^{\#}p<0.05$) group. **e** HIV seronegative subjects spent more time in the target quadrant during the probe trial ($^{+++}p<0.001$, Wilcoxon signed rank test of target quadrant vs. all other quadrants). Overall, seropositive subjects did not display less preference for the target quadrant. When stratified, the seropositive, normal subjects displayed higher preference for the target quadrant compared to other quadrants (75 %) with only 63 % of the impaired group spending >51 % of time in the target indicating impaired delayed memory performance. Graph was performed using prism program

trials [$F(34)=5.80$; $p<0.02$, Fig. 1c]. Stratification by cognitive performance of the HIV-seropositive group compared to seronegatives indicate difference in both visible [$F(33)=5.66$; $p<0.01$, Fig. 1c] and hidden [$F(33)=5.66$; $p<0.01$, Fig. 1c] trials with Tukey's post hoc, suggesting the HIV-seropositive cognitively impaired group had worse performance.

For all groups (both before and after stratification), there was an improvement in *velocity*, *latency*, and *cumulative distance to target* overall performance within trials, both visible and hidden ($p<0.01$, data not shown).

An ANOVA between the seropositive and the seronegative groups in the visible *percentage of successful trials*,

suggested a difference between groups during the visible trial [$F(35)=3.76$ $p<0.04$, Fig. 1d]. In addition, there were differences between the seronegative group and the seropositive women stratified by cognitive status groups in the percentage of successful visible trials [$F(35)=4.77$, $p<0.04$, Fig. 1d], with Tukey's post hoc test indicating the seropositive impaired group had the lowest success compared to seronegative subjects.

Overall, our results suggest that the HIV-seropositive impaired group had difficulties in learning in the MI test compared to the HIV-seropositive group with normal cognition and HIV-seronegative controls.

After 15 min, the subjects were given 30 s to again find the same hidden target (probe trial). By the Wilcoxon signed rank test, the HIV-seronegative participants had a higher *percent time in quadrant*, in the target quadrant during the delayed probe trial ($p<0.01$, target vs. all other quadrants, Fig. 1e), with 93.7 % showing preference (>51 % time) for the target quadrant. However, the HIV-seropositive subjects did not spend more time in the target quadrant than they did in the adjacent quadrant to the right of the intended target (Fig. 1e). When the seropositive participants were stratified by cognitive performance, the seropositive normal group spent more time in the target quadrant than they did in the left or opposite quadrant, with a small difference ($p=0.07$) in the time spent in the right quadrant (Fig. 1e). The seropositive impaired women showed equal preference for the target quadrant and the right quadrant where 63 % showed preference for the target (>51 % time spent in target quadrant, Fig. 1e).

In summary, HIV-seropositive women with a cognitive impairment displayed spatial memory impairments in the virtual reality MI task.

Correlations between Memory Island test and other neuropsychological tests

Spearman's correlation analysis including both seropositive and seronegative women demonstrated a strong correlation between performance in the MI task and other cognitive measures (Table 4). There was a significant correlation between performance on the visible latency trial (i.e., the learning portion of MI) and hidden latency trial (i.e., the immediate memory portion of MI) with overall NPZ scores (p values of 0.02 and 0.01 respectively), as indicated in Table 4. Performance on the learning and immediate memory portions of the MI task in particular correlated ($p<0.05$) to psychomotor speed cognitive domain, with strong correlations with the Trail Making B test and the Symbol Digit Modalities Test. The immediate memory portion of the task also correlated with frontal executive cognitive domain ($p<0.04$). These correlations mirror differences between neuropsychological test performance in HIV-seronegative and HIV-seropositive subjects, stratified by cognitive performance. In the MI paradigm, the probe trial assesses delayed memory; this kind of memory correlated with the psychomotor speed subtest, mostly due to the measures the auditory reaction time (RT) of the nondominant hand performance.

ApoE study population characteristics

HIV-seronegative and HIV-seropositive women were matched by age and other factors (Puerto Rican, Spanish-speaking, and economic status) and did not differ with respect to frequency of non-*ε4* (*ε3/ε3*) and the heterozygous *ε3/ε4* genotypes. In the seropositive group, Student's *t* test indicated no differences between *ε4* carrier groups with regard to RNA viral load, CSF RNA viral load, CD4 T cell counts, CD4 nadir, use of cART or protease inhibitor medications, CPE, BDI scores, or cognitive performance (Table 5). Due to the limited number of participants, stratification by cognitive performance (normal or impaired) was not possible.

ApoE genotype and neurocognition

A Student's *t* test analysis of neurocognitive assessments showed that in the HIV-seronegative group, *ε4* carriers scored lower in NPZ, in frontal executive cognitive domain (driven by the performance of the Stroop Color-Word Test), and in verbal memory cognitive domain [subtest RAVLT memory recall (trial 5)] (Table 5). Among the HIV-seropositive women, no differences were seen between the different allele carrier groups for any of the standardized neurocognitive test measures (Table 5). This finding suggests that while *ε4* status impacts cognitive performance in healthy individuals, it may not have this effect in our sample of HIV-seropositive women (at least the cognitive performance measures used and our sample size).

ApoE genotype and MI

Due to sample number, with respect to genotype, all MI analyses were conducted using repeated measures ANOVA with genotype (non-*ε4* vs. *ε4*) as between subjects and trial as within variable for each HIV status groups unless otherwise indicated. Among the genotypes, there were no differences in average *velocity* (virtual units/s) in either the visible or the hidden trials for either HIV status groups (Fig. 2a).

There was a trend towards a difference between non-*ε4* and *ε4* carriers in the seronegative group across the visible latency trials with regard to the time it took them to arrive at the target, *latency* [$F(14)=3.48$, $p=0.07$, Fig. 2b]. In the seropositive group, there was a significant difference between *ε4* carrier groups in visible latency trial [$F(18)=4.97$, $p<0.04$, Fig. 2b]. This finding suggests a potential interacting effect between being an *ε4* carrier and being HIV-seropositive in relation to spatial learning assessed by

Table 4 Spearman's correlations between Memory Island and other neuropsychological measures for all subjects

	Visible velocity (U/s)	Visible latency (s)	Hidden latency (s)
I. NPZ	0.23 (0.17)	*−0.49 (0.02)**	*−0.42 (0.01)**
II. Neuropsychological domains/subtests			
1. Frontal executive	0.29 (0.08)	−0.29 (0.13)	*−0.35 (0.04)*
a. Stroop Color/Word	0.21 (0.22)	−0.18 (0.10)	−0.19 (0.37)
b. Trail Making B	0.27 (0.11)	*−0.34 (0.05)**	*−0.46 (0.004)***
2. Psychomotor speed	*0.43 (0.008)***	*−0.48 (0.003)***	*−0.43 (0.009)***
a. Symbol Digit Modality Test	*0.35 (0.04)**	*−0.46 (0.006)***	*−0.42 (0.01)***
b. Visual RT nondominant hand	0.24 (0.16)	−0.24 (0.16)	−0.30 (0.08)
c. Auditory RT nondominant hand	0.14 (0.41)	−0.11 (0.53)	−0.13 (0.46)
3. Verbal memory (RAVLT)	−0.12 (0.47)	−0.09 (0.57)	−0.281 (0.22)
a. Trial 5	−0.10 (0.54)	−0.07 (0.69)	−0.13 (0.41)
b. Memory Recall	−0.28 (0.10)	−0.13 (0.45)	−0.11 (0.50)
c. Delayed Recognition	0.04 (0.83)	−0.21 (0.21)	−0.27 (0.10)
4. Motor speed	0.26 (0.13)	−0.36 (0.03)	−0.15 (0.37)
a. Trail Making A	0.25 (0.14)	−0.32 (0.06)	−0.23 (0.16)
b. Grooved Pegboard dominant hand	0.17 (0.32)	−0.25 (0.14)	−0.04 (0.79)
c. Grooved Pegboard nondominant hand	−0.24 (0.15)	−0.28 (0.09)	−0.10 (0.54)

Correlation coefficient (*p* value)

NPZ average of all test z-scores, *RAVLT* Rey Auditory Verbal Learning Test, *RT* reaction time

*$p<0.05$; **$p<0.01$ statistically significant in italic

MI. Although a similar trend was observed in the *latency* during the hidden trials, no significant differences were found based on genotype (Fig. 2b).

No significant differences between carrier groups for either the seropositive or seronegative women were found in either visible or hidden trails during the first two minutes measured by *cumulative distance to target* (Fig. 2c). The performance of all groups improved across trials during visible and hidden trials for *velocity*, *latency*, and *cumulative distance to target* ($p<0.01$, data not shown).

Student's *t* test indicated an overall difference in *percentage of successful hidden trials* between non-*ε4* and *ε4* carriers in the seropositive group only ($p<0.05$, Fig. 2d). Fewer HIV-seropositive *ε4* carrier women were able to find the target within the first 2 min.

Overall, there is evidence that the *ε4* carrier seropositive groups displayed the worst performance in MI for both visible and hidden trials, pointing to a potential effect of genotype on visuospatial memory that must be considered in future studies.

Lastly, after a 15-min wait, subjects were asked to again find the same hidden target during the probe trial. Seronegative participants of both genotypes spent more time in the target quadrant, *percent time in quadrant*, during the delayed probe trial ($p<0.01$ target vs. all other quadrants, Fig. 2e), with 80 and 100 % showing a preference (>51 % time) for the target quadrant. These results are similar to those reported in a previous study of healthy adults (Rizk-Jackson et al. 2008). HIV-seropositive non-*ε4* carriers also spent more time in the target quadrant compared to all other quadrants, with a 78.6 % preference ($p<0.05$, Fig. 2e). However, seropositive *ε4* carriers spent equal amounts of time in the target quadrant and the opposite quadrant, having only a 40 % target quadrant preference (Fig. 2e). Thus, for seropositive women, being an *ε4* carrier may be associated with an increased risk of impairment in delayed visual spatial memory.

CSF ApoE protein levels and neurocognition

No differences in CSF ApoE protein levels were observed between HIV-seropositive women based on HAND status (Table 2) or *ε4* carrier status (Table 5). However, according to Student's *t* test ApoE protein levels were lower in seropositive women (5.0 μg/mL±0.8; $n=20$), compared to seronegative samples (9.3 μg/mL±2.4; $n=6$) provided by the Johns Hopkins University CSF repository ($p<0.05$). No correlations were observed between protein levels and neurocognitive test measures, MI performance, or other biological measures.

Discussion

HIV has detrimental effects on cognition even in otherwise asymptomatic individuals and may affect spatial navigation

Table 5 Effects *ε4* on Neurocognitive testing in HIV-seronegative and HIV-seropositive subjects

	HIV-seronegative		*p* value	HIV-seropositive		*p* value
	Non-*ε4*	*ε4*		Non-*ε4*	*ε4*	
N	10	6		15	5	
Age	40.0±2.8	43.7+4.3	0.47	39.2±2.7	48.0±3.8	0.10
Education (years)						
Normal cognition				40 %	40 %	
Impaired cognition				60 %	60 %	
HIV RNA viral load $\log_{10}$ copies/mL						
CSF (*N*)				1.9±0.1 (13)	1.7±0.5 (5)	0.32
Plasma (*N*)				2.4±0.2 (15)	2.0±0.3 (5)	0.40
CD4 nadir (cells/mm^3)				718±100	597±134	0.53
CD4 (cells/mm^3)				457±88	462±149	0.97
Treatment						
% Native (*N*)				4/14 (28.6 %)	0/5	
% ART (*N*)				1/14 (7.1 %)	0/5	
% cART (*N*)					9/14 (64.3 %)	5/5 (100 %)
CPE (*N*)				7.2±1.0 (11)	10.8±1.4 (5)	0.06
% Protease inhibitor (*N*)				63.6 % (11)	50 % (4)	0.70
ApoE CSF levels (μg/mL)				5.1±1.0	4.8±1.9	0.89
Beck Depression Inventory				9.3±2.7	11.2±4.6	0.72
I. NPZ	0.5±0.2	−0.0±0.2	*0.05*	0.2±0.1	0.1±0.2	0.63
II. Neuropsychological domains/subtests						
1. Frontal executive	0.5±0.3	−0.6±0.4	*0.03*	0.0±0.2	0.4±0.4	0.40
Stroop Color Word	0.7±0.3	−0.1±0.4	*0.02*	0.2±0.3	0.4±0.5	0.74
Trail Making B	0.4±0.1	−0.3±0.6	0.27	−0.1±0.4	0.4±0.6	0.34
2. Psychomotor speed	0.2±0.2	0.2±0.3	0.98	−0.0±0.2	−0.5±0.3	0.33
a. Symbol Digit Modality Test	0.7±0.3	−0.1±0.4	0.19	−0.2±0.2	−0.0±0.4	0.47
b. Visual Reaction Time Nondominant Hand	0.0±0.3	0.3±0.4	0.50	0.2±0.3	−0.7±0.5	0.34
c. Auditory Reaction Time Nondominant Hand	−0.0±0.3	0.5±0.3	0.13	−0.0±0.2	−0.6±0.4	0.22
3. Verbal memory (RAVLT)	0.4±0.2	−0.0±0.2	0.08	0.4±0.2	0.5±0.3	0.97
a. Trial 5	0.3±0.2	−0.1±0.3	*0.03*	0.1±0.2	0.6±0.3	0.67
b. Memory Recall	0.4±0.2	−0.5±0.3	0.34	0.2±0.2	0.4±0.3	0.35
c. Delayed Recognition	0.5±0.3	0.0±0.4	0.36	1.0±0.2	0.4±0.4	0.25
4. Motor speed	0.8±0.3	0.3±0.4	0.12	0.3±0.3	−0.1±0.4	0.58
a. Trail Making A	0.5±0.5	−0.5±0.6	0.11	0.2±0.4	−1.5±0.7	0.08
b. Grooved Pegboard dominant hand	0.8±0.4	0.4±0.5	0.17	0.3±0.3	0.6±0.5	0.67
c. Grooved Pegboard nondominant hand	0.9±0.3	0.1±0.5	0.73	0.3±0.3	0.7±0.5	0.48

Mean±SEM, Student's *t* test non-ε4 vs. ε4 carrier; significant difference $p<0.05$ in italic

ART single drug antiretroviral treatment, *cART* combined antiretroviral treatment

functions (Olesen et al. 2007; Heaton et al. 2011). We assessed spatial learning and memory performance in HIV-seropositive women using the virtual reality-based MI task. The MI test is modeled after the MWM, which is widely used to evaluate spatial learning and memory in rodent models, and is unique in that it is performed in a naturalistic human environment. Our studies with the MI task revealed reduced average velocity for the HIV-seropositive group compared to the seronegative group during the visual but not the hidden trials. When the HIV-seropositive patients were stratified by cognitive function, the seropositive impaired group showed worse performance than the control, seronegative group that suggested difficulty in learning how to navigate to the targets. These results are consistent with those from Olesen et al. (2007), who observed that HIV-seropositive participants showed impaired performance on

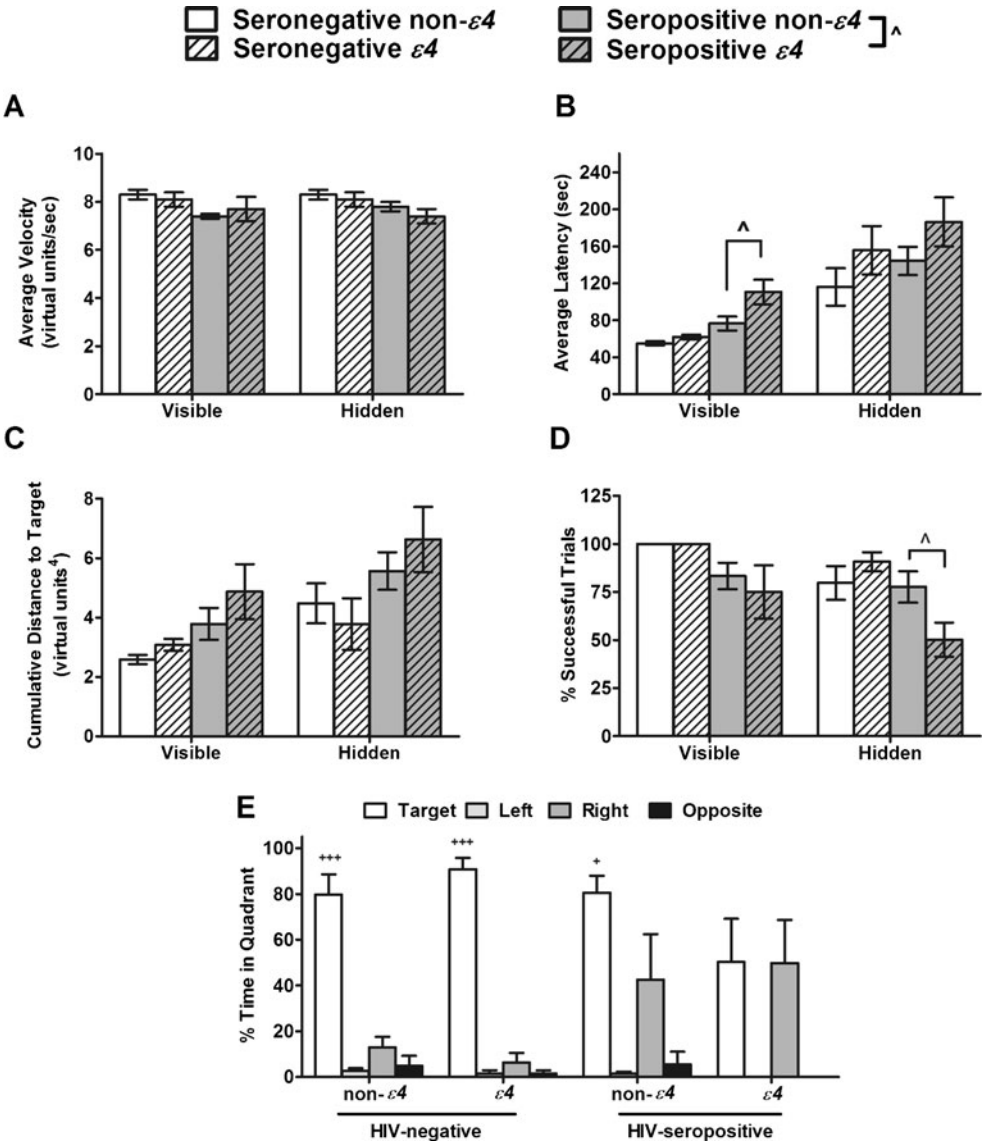

Fig. 2 Differences in MI test performance between HIV-seropositive *ε4* carriers and non-*ε4* carriers. **a** No genotype dependent differences where seen for genotype for other HIV status group. **b** Only in the seropositive group did *ε4* carriers perform worse than non-*ε4* carriers in the visible trials, with difference between the two groups in the hidden trials. **c** In cumulative distance to target during the visible trials there were no significant effects of genotype within HIV status groups. **d** All groups were highly successful in finding the four visible targets within 2 min (% successful trials). However, during the four hidden trails, the seropositive *ε4* carriers were significantly less successful in finding the target within 2 min compared to non-*ε4* carriers (^$p<0.05$). **e** The seropositive non-*ε4* carriers also displayed a preference for the target quadrant ($^{+}p<0.05$, $^{+++}p<0.001$, Wilcoxon signed rank test of target vs. all other quadrants). Only the seropositive *ε4* carriers failed to display target quadrant preference, indicating impaired delayed memory. Graph was performed using PRISM program

standard clinical NP tests of spatial learning function. The fact that no differences were found when comparing HIV-seropositive normal and impaired women in MI performance could reflect the relatively small sample sizes, or the fact that three out of the 11 women included in the HIV-seropositive "normal" group had asymptomatic cognitive impairment. Alternatively, it is possible that spatial learning is affected early in the disease and that the HIV-positive women classified as "normal" in the NP tests were actually beginning to have spatial learning deficits to which the MI test was sensitive. To further explore these issues, we currently have an ongoing study to extend our analyses with larger sample sizes and validate them with neuroimaging studies.

In our sample, we observed that the HIV-seropositive, cognitively impaired women displayed impairments in both immediate and delayed memory on the MI task. These findings are consistent with studies in HIV-1 mouse models (Table 1). Investigators have suggested that the cognitive deficits observed in these studies are associated with significant increases in astrogliosis and microgliosis, a possible explanation for our findings (Griffin et al. 2004). Additionally, there is evidence in Tat-mice (Fitting et al. 2008; personal communication) or with gp120 treatment (Sanchez-Alavez et al. 2000) that there are impairments in the induction and maintenance of long-term potentiation of the hippocampus interfering with spatial learning and memory (Table 1).

We observed strong associations between MI task performance measures and cognitive measures, as well as between the *visible trial* (learning portion) and *hidden trial* (immediate memory) portions of the MI task, with the overall NPZ score. The immediate memory portion of the MI task in particular correlated with overall psychomotor speed, with strong correlations with scores on the Trail Making B Test and Symbol Digit Modality Test. These correlations support

the idea that immediate memory performance on the MI task may be dependent on psychomotor speed, although a component of attention cannot be fully ruled out. Immediate memory portion of the task also correlated with subtests for frontal executive function and verbal memory cognitive domains. These data are consistent with earlier studies in Caucasians and other populations showing that MI performance is dependent on psychomotor speed, executive function, and memory function (Ayala-Feliciano et al. 2011; Piper et al. 2010).

We also examined the potential impact of *ε4* allele status on cognition in both HIV-seropositive and HIV-seronegative women. We found that the *ε4* allele was associated with impaired performance on the NP test battery in the control, seronegative women. In the MI task, we observed a trend in the seronegative group towards a difference between non-*ε4* and *ε4* carriers across v*isible trials in latency* to the target. Lack of *ApoE*, genotype effects on cognitive performance in seronegative women is consistent with other studies with in both Caucasian and Hispanic women of similar age (Acevedo's unpublished data).

In contrast, in the HIV-seropositive group, *ε4* carriers and noncarriers showed significant differences in their performance in certain MI test measures. In the seropositive group, ε4 carriers showed increased latency to target in the visible trials and also performed worse in the immediate memory and delayed memory portions of the task. These data suggest that for HIV-seropositive women, being an *ε4* carrier may be associated with increased risk of impairment in *delayed visual spatial memory*, supporting the idea that HIV infection and the *ε4* genotype both have an effect on neurocognitive performance in HIV-seropositive patients. These findings are also consistent with prior studies reporting that the *ε4* allele is associated with accelerated progression of HIV disease (Burt et al. 2008) and an increased risk of developing HAND (Corder et al. 1998; Valcour et al. 2004b). In contrast, other studies did not find an association between the *ε4* carriers and increased risk of cognitive deficits in HIV subjects (Joska et al. 2010; Pomara et al. 2008). There are many variable, including differences in age, gender, race/ethnicity, HIV viral strain (Joska et al. 2010), and cART, which may contribute to the differences observed between studies.

Higher levels of CSF ApoE protein have been correlated with worse cognitive performance in Alzheimer's disease patients (Fukuyama et al. 2000) and in HIV-seropositive men carrying the *ε4* allele (Andres et al. 2011). However, we observed no correlation between CSF ApoE levels and cognitive performance in our study. One possible explanation for our different findings may be that our HIV-seropositive sample consists entirely of women, whereas the sample in the study of Andres *et al.* was predominately male (Andres et al. 2011). However, our results agreed with the Andres et al. (2011) study in that both studies observed that CSF ApoE protein levels are lower in HIV patients when compared to controls. Among our HIV-seropositive women, *ε4* and non-*ε4* carriers showed no differences in CSF ApoE protein levels, consistent with a previous study done in male subjects (Andres et al. 2011). There are two possible explanations for our results: (1) that neurocognitive performance in HIV-seropositive women is not associated with changes in CSF ApoE protein levels, or (2) that our sample size is a limiting factor in detecting correlations with neurocognitive performance; both alternatives will be examined in the future.

There are a few limitations to the current study. Our sample size was small, and the HIV-seropositive subgroups were smaller after stratification by level of cognitive impairment. However, our results are consistent with those of certain previous studies in showing (1) impairment of spatial learning and memory in seropositive women using a standardized pen and pencil assessment and (2) an association of the *ε4* allele with worse cognitive performance in seropositive individuals. In addition, this is the first study to examine the use of a translational paradigm, the MI test, to assess cognitive impairment in HIV-seropositive individuals. Our results support the use of the MI test to study spatial learning and memory deficits in HAND, and to assess potential therapeutics for these deficits. Finally, our study participants were relatively young (mean age <42 years) and were all women. Given prior data implicating interactions between *ε4* genotype with age (Alexander et al. 2007; Mondadori et al. 2007; Valcour et al. 2004a) and gender (Payami et al. 1996), it will be of interest to include older individuals and men in future studies.

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