Low $CD4^+$ cell count nadir exacerbates the impacts of APOE ε4 on functional connectivity and memory in adults with HIV

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Objective: Nearly half of individuals living with HIV in the USA are now 50 or older. This rapidly ageing populace may be at an increasingly greater risk of Alzheimer's disease. However, the potential interaction between HIV-disease and Alzheimer's disease pathogenesis (i.e. Alzheimer's disease genetic risk factors) on brain function remains an open question. The present study aimed to investigate the impact of APOE ε4 on brain function in middle-aged to older people with HIV (PWH), as well as the putative interaction between ε4 and HIV disease severity.

Methods: Ninety-nine PWH participated in a cross-sectional study (56.3 ± 6.5 years, range 41–70 years, 27 women, 26 ε4 carriers and 73 noncarriers). Structural MRI and resting-state functional MRI were collected to assess alterations in brain structure and functional connectivity, respectively.

Results: APOE ε4 was associated with worse memory performance and reduced functional connectivity in the memory network. The functional connectivity reduction was centred at the caudate nucleus rather than hippocampus and correlated with worse memory performance. In ε4 carriers, low $CD4^+$ cell count nadir was associated with reduced functional connectivity in the memory network, but this association was absent in noncarriers. Furthermore, there was an indirect detrimental impact of ε4 on memory performance through memory network functional connectivity. However, this indirect effect was contingent on $CD4^+$ cell count nadir, that is the indirect effect of ε4 on memory was only significant when $CD4^+$ cell count nadir was low.

Interpretation: APOE ε4 is associated with reduced memory and reduced functional connectivity within the memory network, and low $CD4^+$ cell count nadir – indicating a history of severe immunosuppression – may exacerbate the effects of ε4.

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Introduction

APOE ε4 is a known genetic risk factor for late-onset sporadic Alzheimer's disease, atherosclerosis and worse clinical outcomes after a traumatic brain injury [1]. In people with HIV (PWH), ε4 is associated with increased amyloid pathology [2–4], but the association between ε4 and neurocognitive impairment is unclear: although some studies found that ε4 was associated with a higher risk of neurocognitive impairment or dementia [5–8], others found no association [9–14], supporting a need for additional research, especially as ε4 may predispose to damage caused by agents like HIV.

Resting-state functional MRI (fMRI) is a useful technique to study brain function [15]. In APOE ε4, studies have shown that functional connectivity is altered in ε4 carriers compared with noncarriers [16], even prior to the onset of detectable amyloid deposition [17]. A recent study found reduced functional connectivities between hippocampus and caudate, and between hippocampus and other key regions of the Papez circuit in cognitively normal middle-aged ε4 carriers compared with noncarriers (despite a lack of significant difference in memory performance), and across individuals, functional connectivity correlated with memory performance [18]. This finding is of particular interest for several reasons: first, the Papez circuit is a vital pathway in episodic memory formation and consolidation [19], and is involved in Alzheimer's disease [20]; second, the caudate nucleus [21,22] and several regions in the Papez circuit are preferentially affected in PWH, including thalamus, hippocampus and cingulate cortex [22,23], especially the caudate, which has been shown to play a critical a role in HIV-associated neurocognitive disorders (HAND) [21,22]; third, although the caudate and the hippocampus belong to separate and competing memory systems, the caudate-hippocampus functional connectivity correlates with memory performance (e.g. [18,24]). Therefore, investigating the impact of ε4 on functional connectivities between these regions (the Papez circuit and the caudate) in PWH may provide important insight into the potential interactions between HIV-disease and APOE ε4 on brain function.

The present study was conducted to understand whether HIV-disease and APOE ε4 may concomitantly and/or interactively affect brain function (with a focus on the memory network). We first examined whether ε4 was associated with worse memory performance in PWH; then using resting-state functional connectivity technique, we investigated the impact of ε4 on memory-related brain regions (focusing on the Papez circuit, and the caudate), and the potential interaction between ε4 status and $CD4^+$ cell count nadir.

Materials and methods

See supplemental materials for additional details on methods, http://links.lww.com/QAD/C21.

Participants

One hundred and four PWH were recruited from the greater Washington, DC, metropolitan area between 11 January 2015 and 28 June 2019. Blood specimens were collected to measure viral load and current $CD4^+$ cell counts. Saliva samples were collected for genotyping. Self-reported $CD4^+$ cell count nadirs and estimated duration of HIV infection were documented. In addition, we applied bootstrapping techniques to data analyses to assess the robustness of the results. Five individuals were excluded due to the lack of genotype information ($n = 3$) or MRI anomalies ($n = 2$). All procedures were performed in accordance with the guidelines and regulations from the Institutional Review Board. Written informed consent from every participant was obtained prior to enrolment.

Neuropsychological testing

A comprehensive neuropsychological battery was administered (including Hopkins Verbal Learning Test-Revised (HVLT-R), see Table S1, http://links.lww.com/QAD/C21) to assess performance of cognitive domains that are affected in PWH [25]. Neuropsychological test scores were used to calculate global deficit score (GDS) [26] and to diagnose HAND [together with the Lawton and Brody Activities of Daily Living (ADL) index] following the standard Frascati guideline [27].

MRI acquisition and preprocessing

High-resolution ($1 \times 1 \times 1\,mm^3$) T1-weighted images and one run of resting-state fMRI images ($n = 264$; resolution $3.2 \times 3.2 \times 4\,mm^2$) were acquired from each participant at the local institute.

The software package SPM12 (https://www.fil.ion.ucl.ac.uk/spm/), the Computational Anatomy Toolbox (CAT, version 12.5) (www.neuro.uni-jena.de/cat/) and the CONN functional connectivity toolbox (https://www.nitrc.org/projects/conn/) [28] were used for preprocessing and analysing MRI data, following default processing pipeline settings with default parameters.

Statistical analyses

The statistical analyses were conducted in SPSS 25.0 (Chicago, Illinois, USA), and MATLAB 2018b (Math Works, Natick, Massachusetts, USA). We divided the PWH into two groups based on their genotypes: *carriers*, PWH with at least one copy of ε4 allele; and *noncarriers*, PWH with zero copy of ε4 allele. All statistical analyses (including MRI) were two-tailed, and controlled for age, education years, sex and race. Additional corresponding MRI covariates were included in MRI analyses.

Table 1. Demographics and HIV disease information of APOE ε4 carriers and noncarriers.

	Carriers[a] ($n=26$)	Noncarriers[b] ($n=73$)	P
Age (years)	55.1 (5.9) [c]	56.8 (6.7)	n.s.[d]
Education (years)	13.62 (3.1)	14.5 (2.9)	n.s.
Sex (Female%)	26.9%	21.9%	n.s.
Race (AA%) [e]	76.9%	57.5%	n.s.
Current CD4$^+$ cell count (cells/μl)	684.5 (561.0)	612.0 (450.3)	n.s.
CD4$^+$ cell count nadir (cells/μl)	152 (330)	200 (285)[f]	n.s.
Disease duration (years)	26.0 (9.8)	26.0 (9.3)	n.s.
GDS [g]	0.34 (0.29)	0.34 (0.44)	n.s.
HAND diagnosis [h]	26.9%	26.0%	n.s.
On stable cART [i]	100%	97.3%	n.s.
Undetectable VL [j]	84.6%	82.2%	n.s.
History of illicit drug use [k]	53.8%	45.2%	n.s.
Taking medications for			
Hypertension	42.3%	45.2%	n.s.
Diabetes	19.2%	11.0%	n.s.
Cholesterol level [l]	46.2%	41.1%	n.s.

[a]ε2/ε4 ($n=2$), ε3/ε4 ($n=21$), ε4/ε4 ($n=3$).
[b]ε2/ε2 ($n=4$), ε2/ε3 ($n=13$), ε3/ε3 ($n=56$).
[c]Age, education, disease duration, and GDS were presented as mean (standard deviation), versus current CD4$^+$ cell count and CD4$^+$ cell count nadir were presented as median (IQR).
[d]n.s., not significant.
[e]AA, African–Americans, similar results were observed in the AA subgroup ($n=62$) (see Table S3, http://links.lww.com/QAD/C21 and Fig. S7 to S10, http://links.lww.com/QAD/C21).
[f]One noncarrier did not provide CD4$^+$ cell count nadir (treated as a missing value).
[g]GDS, global deficits score, which was calculated from the seven neurocognitive domains [26].
[h]HAND, HIV-associated neurocognitive disorders, seven carriers [six with asymptomatic neurocognitive impairment (ANI), and one with mild neurocognitive disorder (MND)], and 19 noncarriers (18 with ANI, and one with MND) met the HAND criteria [27].
[i]cART, combination antiretroviral therapy.
[j]Individuals with undetectable plasma viral load (VL) (<20 copies/ml), including 22 carriers and 60 noncarriers [similar results were observed in this subgroup ($n=82$), see Table S2, http://links.lww.com/QAD/C21 and Fig. S3 to S6, http://links.lww.com/QAD/C21], and only six PWH (two carriers, four noncarriers) had a VL higher than 200 copies/ml in their blood specimens.
[k]Individuals who have at least one drug abuse/dependent diagnoses based on Composite International Diagnostic Interview. Note that individuals with current illicit use is not qualified to participate the current study. In additional analyses, we included the history of illicit drug use and diabetes as covariates and obtained equivalent results.
[l]Eleven APOE ε4 carriers and 26 noncarriers are taking medications for dyslipidaemia, and one carrier and four noncarriers are taking medications for the purpose of general heart health.

Contingency χ^2 tests, and two-sample *t*-tests were used to examine group differences in demographics, HIV disease (current CD4$^+$ cell counts, CD4$^+$ cell count nadir and disease duration), HAND diagnoses between carriers and noncarriers, and history of illicit drug use (see Table 1). As our sample of PWH was predominantly African–American, race was defined as a dichotomous variable: African–American (1), non-African–American (0).

The CAT software package was used to test the effect of ε4 status on cortical thickness and GMv, using a nonparametric permutation-based approach [29] at a threshold of P value less than 0.001 (uncorrected, at least 50 contiguous voxels).

Three different types of functional connectivity analyses were conducted using the CONN software package: region of interest (ROI) based (ROI-to-ROI), seed-to-voxel and multivariate seed-to-voxel. The Papez circuit and bilateral caudate ROIs were identified, including thalamus (THA), caudate (CAU), mammillary body, anterior and posterior hippocampus (aHIP, pHIP), entorhinal cortex, parahippocampal cortex (PHC), anterior and posterior cingulate cortex (ACC, PCC) ROIs. On the baiss of the results of ROI-to-ROI analyses, the right caudate (CAUr) and the right anterior hippocampus (aHIPr) were chosen as the seed ROIs for the seed-to-voxel and the multivariate seed-to-voxel analyses. The multivariate seed-to-voxel analyses were conducted to compare the roles of hippocampus and caudate in the functional disruptions in ε4 carriers: when the CAUr was chosen as the seed region, the time series in the aHIPr were controlled; when the aHIPr was chosen as the seed region, the time series in the CAUr were controlled. A threshold of P value less than 0.05 [false discovery rate (FDR) corrected] was applied in ROI-to-ROI functional connectivity analysis. Seed-to-voxel functional connectivity analyses and multivariate seed-to-voxel functional connectivity analyses used a threshold of voxel-wise P value less than 0.001 (uncorrected), cluster-wise P value less than 0.05 (FDR corrected).

A moderated mediation analysis was conducted in SPSS toolbox PROCESS V3.4 to investigate the indirect (via caudate-hippocampal functional connectivity, $FC_{CAUr-aHIPr}$) effect of ε4 status on memory performance, with the indirect effect contingent on CD4$^+$ cell count nadir.

Results

There was no significant difference in demographics, HIV disease, HAND diagnosis and GDS between carriers and noncarriers (Table 1). Among the study sample, 22 carriers and 60 noncarriers had undetectable viral load and were on cART. The results in the virologically suppressed subgroup did not differ from those in the entire study sample (see Table S2, http://links.lww.com/QAD/C21 and Fig. S3-S6, http://links.lww.com/QAD/C21). Similar results were found in the African–America subgroup (see Table S3, http://links.lww.com/QAD/C21 and Fig. S7-S10, http://links.lww.com/QAD/C21).

ANCOVA analysis revealed significant differences between carriers and noncarriers in two HVLT-R scores that are related to memory. After controlling for age, education, sex and race, we found that ε4 carriers had worse HVLT-R retention rate ($F(1,93) = 6.42$, $P = 0.012$, Fig. 1a) and delayed recall ($F(1,93) = 4.92$, $P = 0.029$, Fig. 1b). As expected, no significant group differences were found in any other neurocognitive domains (Table S1, http://links.lww.com/QAD/C21), supporting that memory was the primary neurocognitive domain affected in these ε4 carriers. Additional analyses revealed no significant interaction between age and ε4 status on any memory scores.

For both cortical thickness and GMv, there was no significant difference between the carriers and noncarriers at a threshold of P value less than 0.001 uncorrected. In addition, we extracted the GMv of the medial temporal lobe (MTL) subregions, and there were no significant differences between carriers and noncarriers (Table S1, http://links.lww.com/QAD/C21).

ROI-to-ROI functional connectivity analysis revealed that, compared with noncarriers, carriers had significantly lower functional connectivities between the CAU and several key regions (aHIP, pHIP, PHC and THA) in the Papez circuit (Fig. 2). After correction for multiple comparisons, the effect was still significant in the right (Fig. 2b) but not in the left hemisphere (Fig. 2a). The strongest group difference in functional connectivity was between the CAUr and the aHIPr ($FC_{CAUR-aHIPr}$, $F(1,93) = 12.42$, $P = 0.0007$). Additional seed-to-voxel analyses and multivariate seed-to-voxel analyses confirmed the central role of CAUr. In seed-to-voxel analyses, CAUr as the seed region revealed reduced FCs between CAUr and a large cluster in the right limbic system, including hippocampus, thalamus, parahippocampus, putamen and occipital cortex, in carriers compared with noncarriers (Fig. 2c), whereas aHIPr as the seed region revealed a group difference largely limited to bilateral caudate nuclei (Fig. 2d). Similar results were found in multivariate seed-to-voxel analyses. After controlling for BOLD timeseries in the aHIPr, CAUr as the seed region revealed reduced FCs between CAUr and putamen, thalamus, posterior hippocampus, posterior cingulate cortex and occipital cortex, in carriers compared with noncarriers (Fig. 2e). In contrast, when aHIPr as the seed region and controlling for BOLD timeseries in the CAUr, there was no significant group difference (Fig. 2f). There were no increased functional connectivities in carriers compared with noncarriers with either seed region.

In addition, the ROI-to-ROI functional connectivity between CAUr and aHIPr ($FC_{CAUr-aHIPr}$) was significantly correlated with HVLT-R retention rate ($r = 0.220$, $P = 0.029$, $p_{permutation} = 0.027$ with 10 000 permutations,

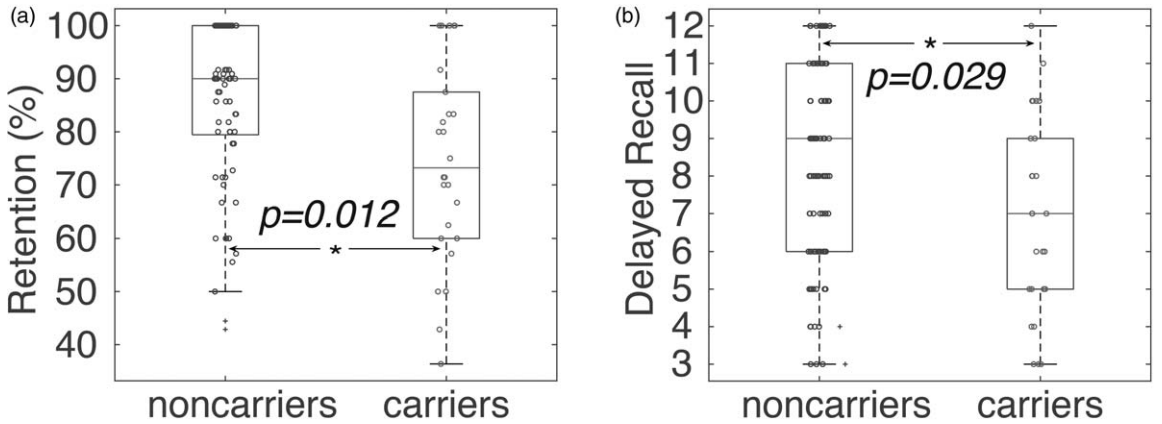

Fig. 1. Group differences in HVLT-R retention and delayed recall. APOE ε4 carriers had significantly lower HVLT-R retention rate (a) and delayed recall scores (b) than noncarriers. On each box, the central mark indicates the median, the bottom and top edges of the box are the 25th and 75th percentiles of the samples, respectively, and the whiskers extend to the most extreme data points not considered outliers. The two outlier individuals (depicted as +) in Figure 1a were identified using the *isoutlier* function in MATLAB. Similar results were obtained when the two outlier subjects were excluded (retention rate, $F(1,91) = 9.77$, $P = 0.002$; delayed recall, $F(1,91) = 7.08$, $P = 0.008$). HVLT-R, the Hopkins Verbal Learning Test–Revised. $^*P < 0.05$.

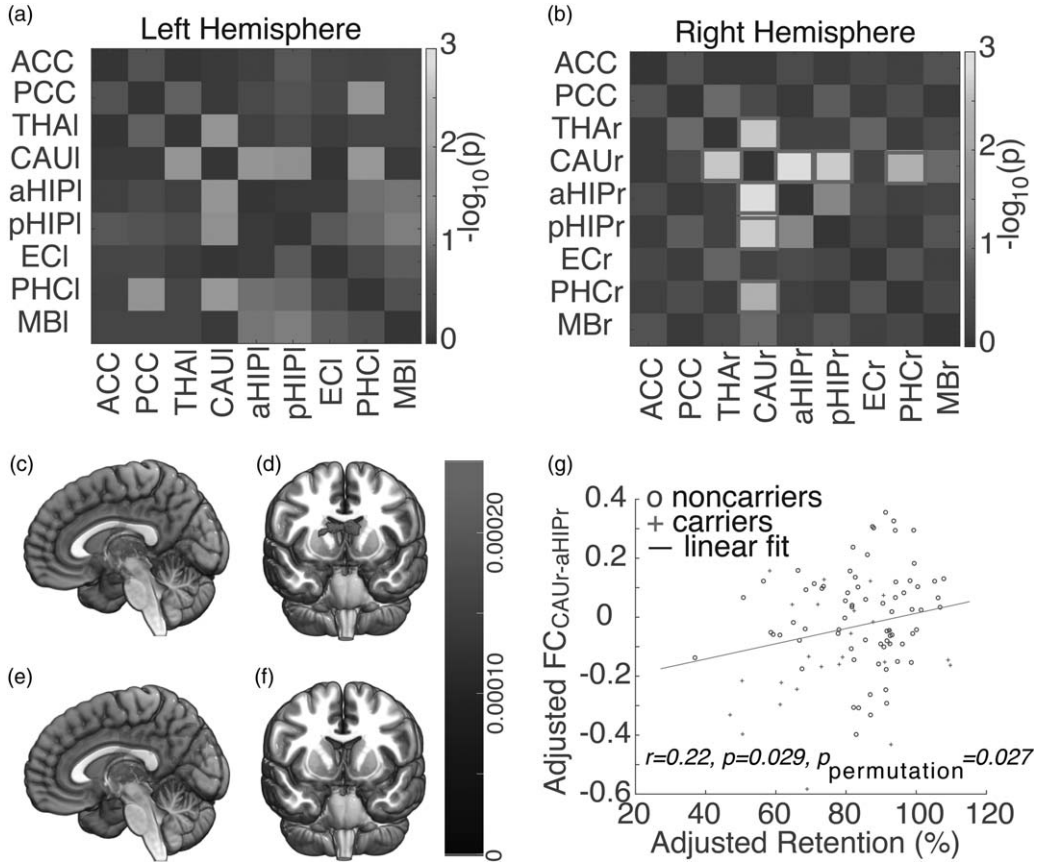

Fig. 2. Reduced functional connectivity in carriers compared with noncarriers, and the correlation between functional connectivity and memory performance. The group comparisons (carriers versus noncarriers) of the ROI-to-ROI functional connectivity (FC) in the (a) left and (b) right hemisphere, respectively (each with nine ROIs). The pairwise ROI-to-ROI FC comparisons that reached significant difference (with FDR correction) were highlighted with a square box □. The colormap represented negative log *p*-values of group comparisons. Seed-to-voxel analysis with (c) the right caudate (CAUr) and (d) the right anterior hippocampus (aHIPr) as the seed regions, respectively. (e) Multivariate seed-to-voxel analysis with the right caudate (CAUr) as the seed region, after controlling for the BOLD timeseries in the right anterior hippocampus (aHIPr). (f) Multivariate seed-to-voxel analysis with the right anterior hippocampus (aHIPr) as the seed region, after controlling for the BOLD timeseries in the right caudate (CAUr). (g) Pearson correlation revealed a significant correlation between the adjusted $FC_{CAUr\text{-}aHIPr}$ and adjusted HVLT-R retention. Crosses, carriers; circles, noncarriers. ACC/PCC, anterior/posterior cingulate cortex; aHIP/pHIP, anterior/posterior hippocampus; CAU, caudate; FC, functional connectivity; FDR, false discovery rate; MB, mammillary body; OC, occipital cortex; ROI, region-of-interest; PUT, putamen. THA, thalamus; -l/-r: left/right (e.g. CAUl/CAUr, left and right caudate, respectively).

Fig. 2g). There was no significant correlation between $FC_{CAUr\text{-}aHIPr}$ and HVLT-R delayed recall ($r = 0.105$, $P = 0.301$).

A general linear model (GLM) analysis revealed a significant interaction between CD4$^+$ cell count nadir and ε4 status on $FC_{CAUr\text{-}aHIPr}$ ($F(1,90) = 7.68$, $P = 0.006$, Fig. 3a). Posthoc correlation analyses revealed a significant correlation between CD4$^+$ cell count nadir and $FC_{CAUr\text{-}aHIPr}$ in carriers ($r = 0.441$, $P = 0.024$, $p_{permutation} = 0.022$ with 10 000 permutations), but not in noncarriers ($P = 0.505$), suggesting low CD4$^+$ cell count nadir has a negative impact on the $FC_{CAUr\text{-}aHIPr}$, but only in carriers. We further divided the PWH into four groups based on ε4 status (carriers versus noncarriers) and CD4$^+$ cell count nadirs (<200 versus ≥200 cells/μl) (Fig. 3b). ANCOVA analysis on ε4 status (carriers versus noncarriers) and CD4$^+$ cell count nadir counts (<200 versus ≥200 cells/μl) revealed a main effect of ε4 status ($P = 0.003$) and a significant interaction between ε4 and CD4$^+$ cell count nadirs ($P = 0.048$), further supporting that low CD4$^+$ cell count nadir (i.e. 200 cells/μl or lower) might exacerbate the detrimental effects of ε4, which was also supported by the moderated mediation analysis below. By contrast, there were no interactions between $FC_{CAUr\text{-}aHIPr}$ and disease duration nor current CD4 (at least $P > 0.1$).

As shown in Fig. S11, http://links.lww.com/QAD/C21, the moderated mediation analysis (Fig. 4A) was motivated

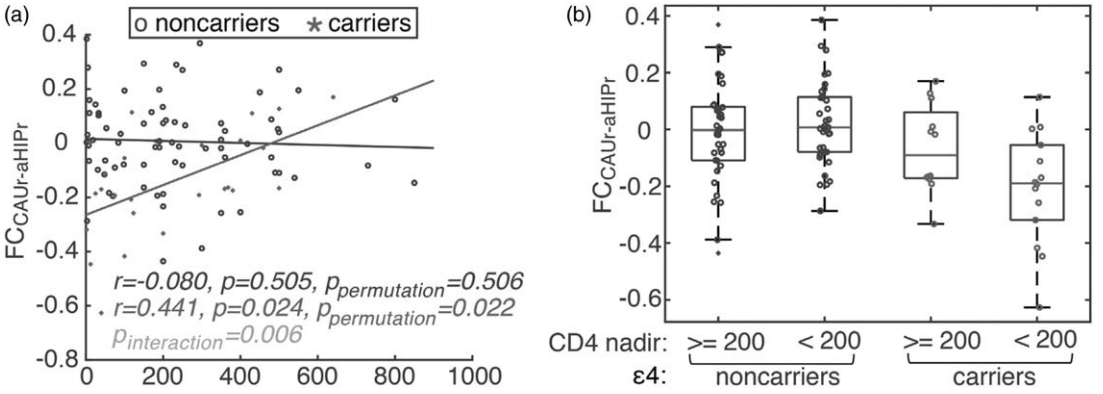

Fig. 3. The interaction of ε4 status and CD4$^+$ cell count nadir on functional connectivity between CAUr and aHIPr ($FC_{CAUr\text{-}aHIPr}$). (a) A general linear model (GLM) analysis revealed significant interaction of ε4 and CD4$^+$ cell count nadir on FC between CAUr and aHIPr ($FC_{CAUr\text{-}aHIPr}$). For carriers: crosses, data of each individual participant; gray line, fitted regression line; text, correlation coefficient between $FC_{CAUr\text{-}aHIPr}$ and CD4$^+$ cell count nadir in carriers. Noncarriers were shown in black colour [markers (circles), line and text]. (b) The individuals were further divided into four groups, ε4 status (carriers versus noncarriers) x CD4$^+$ cell count nadir (<200 versus ≥ 200 cells/μl).

by findings from two previous studies [18,24] and the results in Figs. 2 and 3. This analysis revealed a significant moderated mediation effect (index = 0.009) with 95% confidence interval (95% CI) ranging from 0.0002 to 0.02213, which did not encompass zero, suggesting a significant model (Fig. 4b). In short, the moderated mediation analysis revealed two key findings: ε4 was associated with reduced $FC_{CAUr\text{-}aHIPr}$, but the association depended on nadir CD4$^+$ cell count (in line with Fig. 3); and ε4 had an indirect detrimental effect on memory performance (HVLT-R retention rate) through $FC_{CAUr\text{-}aHIPr}$, but the indirect effect was significant only when CD4$^+$ cell count nadir was low (i.e. 199.5 cells/μl or lower) and not when CD4$^+$ cell count nadir was high (i.e. 462.4 cells/μl or higher). The detailed results can be found in the supplementary materials (http://links.lww.com/QAD/C21).

Discussion

In this sample of PWH, ε4 was associated with reduced verbal memory performance and reduced functional connectivity between the caudate and regions in the Papez circuit, especially the hippocampus. The caudate (but not the hippocampus) assumed the predominant role in this functional disruption. There was a significant correlation between the functional connectivity between right caudate and right anterior hippocampus ($FC_{CAUr\text{-}aHIPr}$) and memory performance. Low CD4$^+$ cell count nadir was associated with reduced $FC_{CAUr\text{-}aHIPr}$ in ε4 carriers, but not in noncarriers; this interaction was further supported by the moderated mediation analysis. In addition, the moderated mediation analysis revealed an indirect detrimental effect of ε4 status on memory performance through $FC_{CAUr\text{-}aHIPr}$, but the indirect effect was contingent on CD4$^+$ cell count nadir.

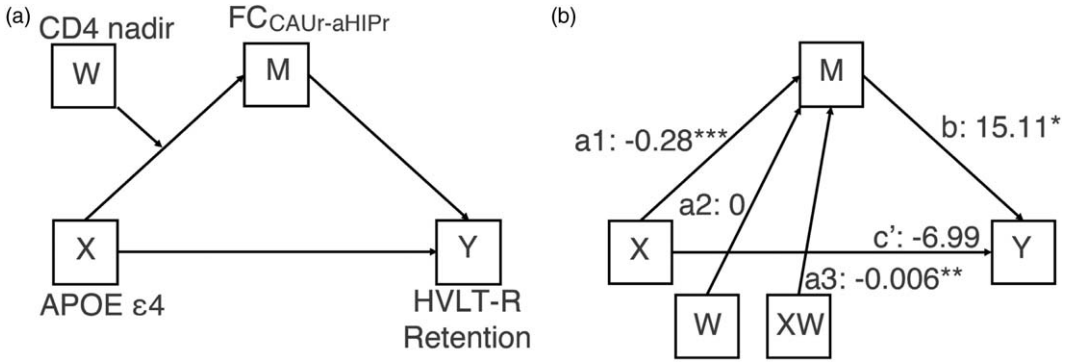

Fig. 4. The moderated mediation analysis. (a) The conceptual diagram of the moderated mediation model. **X**, APOE ε4 status; **Y**, HVLT-R retention rate; **M**, $FC_{CAUr\text{-}aHIPr}$; **W**, CD4$^+$ cell count nadir. (a) The statistical diagram of the moderated mediation model. a_1, the effect of ε4 (X) on $FC_{CAUr\text{-}aHIPr}$ (M); a_2, the effect of CD4$^+$ cell count nadir (W) on $FC_{CAUr\text{-}aHIPr}$ (M); a_3, the interaction effect between ε4 (X) and CD4$^+$ cell count nadir (W) on $FC_{CAUr\text{-}aHIPr}$ (M); b, the effect of mediator $FC_{CAUr\text{-}aHIPr}$ (M) on HVLT-R retention rate (Y); c′, the direct effect of ε4 (X) on HVLT-R retention rate (Y). * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Impaired episodic memory is the cognitive hallmark of Alzheimer's disease, and ε4 is associated with reduced episodic memory in HIV-uninfected [30] and HIV-infected [31–33] 'cognitively normal' adults, suggesting the presence of early neural injury to the memory network in some of the 'cognitively normal' ε4 carriers. Reduced executive function is also highly prevalent [8,30,32,34]. In the present study, we did not find a significant impact of ε4 on executive function, nor global cognition, but rather the effect of ε4 was limited to episodic memory. Thus, memory may be the most affected cognitive domain in these HIV-positive ε4 carriers, similar to HIV-uninfected ε4 carriers [30]. The lack of interaction between age and ε4 on memory performance might be due to a relatively young sample of PWH (with an average age of 56 years), along with a relatively narrow age range (41–70 years). The narrow age range was intentional by study design to investigate a critical transitional period (from middle-age to old age) and to produce a relatively homogeneous group of PWH (to improve sensitivity).

The lack of a significant effect of ε4 on HAND diagnosis in the present study is in line with many previous studies [9–14,31–34], but in contrast to several other studies [5–8]. The inconsistency may be partially due to differences in study samples: the PWH in these studies [5–8] either have poor immune restoration [8] or low education (5.5 years) (which in turn implicates low cognitive reserve) [7], or are at more advanced stages of HIV brain disease (i.e. 25–26% of the study sample [5] or the older subgroup [6] are demented); in contrast, the cohort of PWH in the present study are relatively healthy (Table 1). Taken together, this and previous studies suggest that ε4 may be associated with increased risk of neurocognitive decline, especially memory, implicating an early and mild neural injury that may be largely confined to brain regions/networks involving memory (or plus executive function). The mild neural injury may make HIV-infected ε4 carriers more susceptible to neurocognitive impairment or even dementia, especially when combining with additional comorbidities [5–8].

Using resting-state functional connectivity technique, we investigated the neural mechanisms underlying the impact of ε4 on memory. The functional connectivity analyses revealed that ε4 in PWH was associated with reduced functional connectivities between the caudate and several key regions in the Papez circuit (especially the hippocampus), with a stronger effect in the right than the left hemisphere (Fig. 2a and b). Future studies are needed to investigate potential hemispheric difference. In line with a previous study with HIV-uninfected middle-aged adults [18], across ε4 carriers and noncarriers, there was a significant correlation between $FC_{CAUr\text{-}aHIPr}$ and memory performance, suggesting altered functional connectivity between caudate and hippocampus might contribute to reduced memory in both HIV-infected and uninfected ε4 carriers. However, a key and important difference between the previous study [18] and the present study is that the ε4-associated network disruption is centred at the hippocampus in the previous study with HIV-uninfected adults [18], versus at the caudate in the present study with PWH (Fig. 2c–f, especially Fig. 2e, f). This difference is interesting: although the hippocampus (along with other MTL subregions) is at the centre of Alzheimer's disease pathology, the caudate (along with other subcortical regions) has been proposed to be at the centre of HAND pathology [21,22]. The inconsistency suggests that, in addition to common ε4 disease shared with HIV-uninfected carriers, unique ε4 pathology may exist in HIV-infected carriers, that is injury to the caudate and other subcortical regions, probably due to interactions between ε4 and HIV-disease severity. Amyloid PET scans may help to examine whether amyloid deposition is more prominent in caudate (or other subcortical regions) than MTL in HIV-infected ε4 carriers, similar to individuals with Down syndrome or autosomal dominant Alzheimer's disease [35,36].

Low CD4$^+$ cell count nadir, which indicates a history of severe immunosuppression, is a strong predicator of neurocognitive impairment in PWH [25,37–39]. This suggests that the depth of immunosuppression (represented by a low CD4$^+$ cell nadir count) may have caused irreversible neural injury persisting years later, or it may have triggered certain neuropathology 'cascades' in some patients (e.g. due to interaction with host genes) that evolve over time. Both mechanisms may contribute to the high prevalence of HAND in the cART era. However, it remains largely unknown whether and how CD4$^+$ cell count nadir and host genes interactively impacts brain health/function. In the present study, we observed a significant interaction between ε4 and CD4$^+$ cell count nadir on the $FC_{CAUr\text{-}aHIPr}$, suggesting that the memory network is more vulnerable to low CD4$^+$ cell count nadir in ε4 carriers. Interestingly, two previous studies have found an interaction of ε4 and current immunosuppression on HAND status [5,8]. The PWH in the present study had successful immune restoration (Table 1), thus we could not assess the potential interaction of ε4 and severity of current immunosuppression. Nevertheless, the results suggest that in PWH, the co-existence of ε4 allele and low CD4$^+$ cell count nadir may result in an increased risk of neurocognitive impairment, especially in the memory domain (along with disruption to the memory network). The underlying neural mechanisms might be due to an interaction of Alzheimer's disease pathology (through ε4) and HIV-disease pathology (i.e. immunosuppression).

Multiple factors may have contributed to the impact of CD4$^+$ cell count nadir on the $FC_{CAUr\text{-}aHIPr}$ in HIV-positive ε4 carriers. The association of ε4 with alterations in brain structure and function in PWH is consistent with a model wherein ε4 predisposes to damage caused by

other agents, such as acute injuries or ageing. This predisposition could be related to inflammation or lipid homeostasis [40], conditions that could be present in the brains of PWH and might correlate with HIV disease severity. For instance, both ε4 [1] and HIV-disease (including low $CD4^+$ cell count nadir) [41] are risk factors for atherosclerosis. Therefore, the findings of the interactive impact of low $CD4^+$ cell count nadir and ε4 on the memory network in the present study may be due to a double-hit – low $CD4^+$ cell count nadir and APOE ε4 – perhaps mediated by atherosclerosis. Another potential contributing factor is dopamine deficit. In older adults, the availability of D2 dopamine receptors (D2DR) in caudate correlates with functional connectivity between the caudate and the hippocampus, as well as episodic memory performance (the latter two also correlated with each other) [24]. In this earlier study [24], a mediation analysis further revealed an indirect of D2DR in the caudate on episodic memory through the caudate-hippocampus functional connectivity, suggesting that dopamine deficits in PWH might contribute to reduced caudate-hippocampus functional connectivity and worse memory performance in ε4 carriers with low $CD4^+$ cell count nadir. However, it is not clear whether there is an interaction of ε4 and immune suppression (current or history) on dopamine deficits in the caudate of PWH. Future studies are necessary to understand the biological mechanisms underlying the interaction between APOE ε4 and immunosuppression.

There are several limitations of this study. First, the participants in the present study were relatively young, with only six of them older than 65 and none of them older than 70, limiting our capability to detect the potential age X ε4 interaction. The young age might also contribute to the relatively weak group difference in memory, similar to other studies [18]. Second, the ε4 allele has a higher prevalence and probably a reduced strength in people with African ancestry than people of other races [42–44], but the impact of race (i.e. with African ancestry) on ε4 in PWH is unknown. In the present study, nearly two-thirds of participants were African–American, and the ε4 allele was more prevalent in AA participants (32.3%) than non- African–American participants (16.2%) (Table 1). We did find similar results in the African–American-subgroup (see Table S4, http://links.lww.com/QAD/C21 and Fig. S7-S10, http://links.lww.com/QAD/C21), but due to limited sample size, we could not directly compare African–American versus non-African–American subgroups. Third, female sex is a risk factor for Alzheimer's disease in APOE ε4 carriers [43] (but also see [45]). In the present study, sex was always included as a covariate in data analyses, and additional posthoc data analyses revealed no significant effect of sex ($P > 0.5$). However, the lack of significance may be due to a small number of female participants, and thus lack of statistical power. Fourth, due to a lack of medical records more than 10 years old, $CD4^+$ cell count nadirs were based on self-report. Although self-reported $CD4^+$ cell count nadir is largely accurate [38,46], future large cohort studies with evidence from medical records is needed to further investigate the impact of $CD4^+$ cell count nadir, current $CD4^+$ cell count and disease duration. Fifth, previous studies suggest a stronger effect of ε4 in PWH at more advanced stages of HAND [5,6], but it is unclear whether and how more advanced stages of HAND would interfere with the interaction between ε4 and $CD4^+$ cell count nadir, as only two PWH met the MND criteria in the current study. Sixth, a combination of multimodality imaging and other techniques (such as CSF specimens) is necessary for a better understanding of how ε4 impacts brain health/function in PWH, by acting alone as well as interactively with HIV disease severity. For example, amyloid PET scans can help to assess and compare amyloid deposition at different regions (i.e. caudate versus hippocampus), as well as the relationship between functional connectivities and amyloid deposition at different regions.

In summary, we provide evidence that ε4 is associated with reduced memory and reduced functional connectivity within the memory network. In this functional disruption, the caudate (but not the hippocampus) assumed the predominant role. In addition, low $CD4^+$ cell count nadir has a negative impact on memory network functional connectivity, but only in ε4 carriers and not in noncarriers, suggesting that HIV disease severity may exacerbate the effects of ε4 on brain in middle-aged and older PWH.

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F.N.Y. analysed the data. M.B. and M.D. helped with neurobehavioral data analysis. M.B., M.D. played major roles in data acquisition. P.K. recruited patients from her clinic. S.A.F. did APOE genotyping. F.N.Y. and X.J. wrote the initial manuscript. G.W.R., R.S.T., S.A.F., D.J.M. and R.J.E. revised the manuscript. X.J. is the PI and conceived of the study. All authors read and approved the final manuscript.

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Conflicts of interest

There are no conflicts of interest.

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