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RESEARCH ARTICLE

SV2A PET shows hippocampal synaptic loss in cognitively unimpaired *APOE* ε4/ε4 homozygotes

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Abstract

INTRODUCTION: We investigated hippocampal synaptic density using synaptic vesicle 2A positron emission tomography (PET), and its association with amyloid beta (Aβ) and cognitive performance in healthy apolipoprotein E (*APOE*) ε4 carriers.

METHODS: Synaptic density was assessed in 46 individuals (*APOE* ε4/ε4 $n = 14$; *APOE* ε3/ε4 $n = 16$; *APOE* ε3/ε3 $n = 16$) with [^{11}C]UCB-J-PET standardized uptake value ratios (SUVRs), by using the centrum semiovale as a reference region. Differences in hippocampal [^{11}C]UCB-J SUVRs were analyzed with analysis of variance (ANOVA) and linear models. Associations among [^{11}C]UCB-J SUVR, Aβ, hippocampal volume, and cognitive variables were analyzed with Spearman correlation.

RESULTS: Hippocampal synaptic density was different among the *APOE* groups ($P_{ANOVA} = 0.016$): *APOE* ε4/ε4 carriers had lower [^{11}C]UCB-J SUVRs compared to *APOE* ε3/ε3 ($p = 0.013$). Hippocampal synaptic density did not correlate with Consortium to Establish a Registry for Alzheimer's Disease (CERAD) total score (rho = −0.052, $p = 0.74$), Alzheimer's Prevention Initiative Preclinical Cognitive Composite (APCC) score (rho = 0.17, $p = 0.28$), or [^{11}C]PiB uptake (rho = −0.10, $p = 0.50$).

DISCUSSION: Hippocampal synaptic loss emerges early in the AD continuum and is measurable in vivo in cognitively unimpaired high-risk individuals.

KEYWORDS
[^{11}C]UCB-J, Alzheimer's disease, amyloid beta, apolipoprotein E, biomarker, cognition, hippocampus, positron emission tomography, preclinical, synaptic density, synaptic vesicle 2A

Highlights

- Synaptic density was studied in vivo in healthy older adults using [^{11}C]UCB-J positron emission tomography.
- Apolipoprotein E (*APOE*) ε4/ε4 carriers had lower hippocampal synaptic density compared to *APOE* ε3/ε3.
- Synaptic density was not associated with cognitive performance in this population.

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- Hippocampal synaptic alterations occur before clinical symptoms in *APOE* ε4/ε4 carriers.

1 | INTRODUCTION

Loss of synapses, the structures by which neurons communicate, is one of the key characteristics of Alzheimer's disease (AD). Synapses seem to degenerate early during the disease process based on neuropathological studies that have measured synaptic density markers such as synaptophysin or used unbiased stereological counting.[1–3] Synaptic loss also correlates better with cognitive deficits than histopathological measures of the hallmark AD pathologies, that is, amyloid beta (Aβ) deposits and neurofibrillary tangles.[4] Apolipoprotein E (*APOE*) genotype, the most important genetic risk factor for sporadic AD,[5] is also known to influence synaptic proteins.[6–8] However, there is still a paucity of research on synaptic loss in *APOE* ε4 carriers, a risk population for AD.

Biomarkers for the hallmark AD pathologies are already widely available both for clinical evaluation and research settings. However, reliable biomarkers for early synaptic changes would be valuable both for diagnostics purposes and for monitoring the effect of novel drugs targeted at restoring synaptic functions.[9] During recent years, synaptic alterations in neurodegenerative diseases have become measurable in vivo with positron emission tomography (PET) using synaptic vesicle 2A (SV2A) targeted radioligands such as [^{11}C]UCB-J.[10,11] SV2A is a membrane protein expressed in synaptic vesicles at the presynaptic terminals. Due to the ubiquitous expression of SV2A in the brain,[12] and binding of [^{11}C]UCB-J to all gray matter structures of the human brain,[13] SV2A PET has been suggested to serve as an alternative in vivo synaptic density marker for synaptophysin.[10]

In AD, previous histopathological studies have shown that the entorhinal cortical neurons are the first to show early degeneration.[14] Because the entorhinal cortex is connected to the hippocampus via the perforant pathway, the earliest deficits in synaptic density have been hypothesized to be present in the hippocampus.[15] In line with this, the first study evaluating synaptic density in AD in vivo found a 41% decrease in hippocampal [^{11}C]UCB-J binding to SV2A in AD patients compared to healthy controls.[16] Subsequent SV2A PET imaging studies have since shown consistent reductions in [^{11}C]UCB-J binding in this region both in early AD dementia patients[17] and amnestic mild cognitive impairment (MCI).[18] Similar findings have also been reported in amyloid-positive patients (MCI, mild and moderate probable AD dementia) using another, ^{18}F-labeled SV2A PET ligand, [^{18}F]UCB-H.[19] Hippocampal [^{11}C]UCB-J binding has also been reported to be associated with global Aβ deposition in amnestic MCI patients ($N = 14$), but not in mild AD dementia ($N = 24$).[20] In addition to the hippocampus, AD dementia patients showed reductions in synaptic density in other cortical and subcortical regions,[17,19] and global synaptic density estimated by [^{11}C]UCB-J correlated with cognitive performance in a study including 45 participants with early AD dementia.[15] However, because previous SV2A PET studies have included only individuals from MCI to advanced AD dementia, it is still unclear when the earliest synaptic dysfunction emerges, and whether it is possible to detect such changes in preclinical AD or in individuals at increased genetic risk of clinical AD in vivo with SV2A PET.

Here, we aimed to fill the previously mentioned gaps in the literature by evaluating differences in hippocampal synaptic density in vivo using [^{11}C]UCB-J PET in cognitively unimpaired individuals with one, two, or no *APOE* ε4 alleles and thus at varying genetic risk for sporadic AD. In addition, we studied the associations among hippocampal and cortical synaptic density, brain Aβ load, and cognitive performance in the whole cognitively unimpaired study sample. According to our working hypothesis, lower synaptic density would be present in the hippocampus of cognitively unimpaired *APOE* ε4/ε4 homozygotes compared to *APOE* ε3/ε3 controls. We also hypothesized that lower synaptic density would be associated with lower cognitive performance, which was evaluated using a preclinical cognitive composite score designed to detect subtle cognitive alterations related to preclinical AD.

2 | METHODS

2.1 | Study sample

This cross-sectional study includes 47 cognitively unimpaired individuals from the ASIC-E4 study[21] with either *APOE* ε4/ε4 ($n = 14$), *APOE* ε3/ε4 ($n = 16$), or *APOE* ε3/ε3 ($n = 16$) genotype. The study was conducted at Turku PET Centre, Finland. All included subjects participated in [^{11}C]UCB-J PET, magnetic resonance imaging (MRI), and neuropsychological testing from 2020 to 2022 and in [^{11}C]PiB PET at the time of recruitment (2018–2020, median 21 [19–22] months prior to [^{11}C]UCB-J scans). A detailed description of the recruitment process, inclusion and exclusion criteria, and study protocol for ASIC-E4 has been previously published.[21] Briefly, all included individuals had either *APOE* ε4/ε4, *APOE* ε3/ε4, or *APOE* ε3/ε3 genotype; Mini-Mental State Examination (MMSE) > 25 and Consortium to Establish a Registry for Alzheimer's Disease (CERAD) total score > 62 points at baseline; and no dementia or cognitive impairment, including subjective memory complaints. A flowchart describing the sample selection for this study is presented in Figure 1.

2.2 | Ethics

The study has been approved by the ethics committee of the Hospital District of Southwest Finland and the Hospital District of Southwest

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RESEARCH IN CONTEXT

1. **Systematic review**: The authors performed a literature search for publications describing in vivo evaluation of synaptic alterations using synaptic vesicle 2A (SV2A) positron emission tomography (PET) in the Alzheimer's disease (AD) continuum. Regional synaptic changes had not been previously studied in vivo in a cognitively normal population at risk for AD. However, the authors found several studies in which synaptic changes have been investigated using SV2A PET in early AD and amnestic mild cognitive impairment (MCI), and these findings are properly cited throughout this work.
2. **Interpretation**: Our findings in a clinically unimpaired at-risk population suggest that hippocampal synaptic loss is an early event measurable in vivo already in individuals at increased risk of AD. Early synaptic loss in this population is associated with apolipoprotein E ε4/ε4 genotype, rather than brain amyloid beta load.
3. **Future directions**: Future follow-up on the study population will further clarify whether early synaptic loss detected using [^{11}C]UCB-J PET predicts cognitive decline or conversion to amnestic MCI or AD.

Finland. All study participants signed a written informed consent. Declaration of Helsinki, Good Clinical Practice, and general data protection regulations were followed.

2.3 | Imaging

All included participants underwent [^{11}C]UCB-J PET scans at Turku PET Centre and structural brain MRI at Turku University Hospital during the years 2020 through 2022. MRI was conducted using 3T Philips Ingenia 3.0 T systems (Philips Healthcare), and a 20-channel dS head coil. T1-weighted sequences were used for co-registration, the definition of volumes of interest (VOIs), and for calculating hippocampal volumes.

[^{11}C]UCB-J was synthesized as previously reported.[22] This synthesis method was modified from the methods presented previously.[23–25] Briefly, [^{11}C]CH_3I was produced with a Synthra MeIplus device and transferred to a reaction vessel in an in-house–built methylation device. Previously activated 3-pyridyl trifluoroborate precursor was added to the reaction vessel and the solution was heated to 100°C for 5 minutes. The product was purified with high-performance liquid chromatography (HPLC) and extracted from the HPLC mobile phase using solid-phase extraction. The product was then formulated in a 10% ethanol/0.9% saline solution and sterile filtered prior to use. All [^{11}C]UCB-J PET scans were performed using an ECAT High Resolution Research Tomograph (HRRT, Siemens Medical Solutions). [^{11}C]UCB-J (mean dose 493 [37.5] MBq) was injected intravenously, and dynamic emission data was collected for 90 minutes, followed by a transmission scan of 5 minutes. List mode data was histogrammed into 29 timeframes (6 × 30 seconds, 7 × 60 seconds, 16 × 300 seconds) and reconstructed using OP-OSEM3D algorithm with 16 subsets and 10 iterations and with point spread function (PSF) modeling to reduce the partial volume effect (PVE)[26,27]. Reconstructed images were consequently post-processed with a 2.5 mm FWHM (full width at half maximum) Gaussian filter.

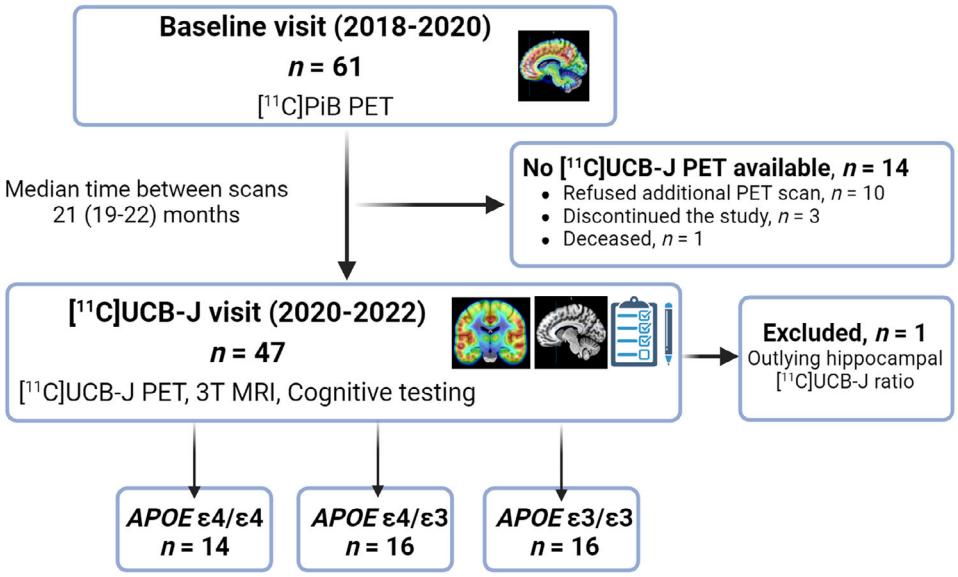

FIGURE 1 Flowchart and study design. Sixty-one individuals were recruited to the ASIC-E4 study during 2018 to 2020, and from those, 47 participated in [^{11}C]UCB-J imaging and additional MRI and neuropsychological testing during 2020 through 2022. APCC, Alzheimer's Prevention Initiative Preclinical Cognitive Composite; *APOE*, apolipoprotein E; CERAD, Consortium to Establish a Registry for Alzheimer's Disease; MRI, magnetic resonance imaging; PET, positron emission tomography

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Amyloid [^{11}C]PiB-PET was performed for all participants 21 months (median, interquartile range [IQR] 19–22) prior to [^{11}C]UCB-J scans as previously described[28] and by using the same ECAT High Resolution Research Tomograph. Emission data were collected for a 50 minute time period from 40 to 90 minutes post-injection.

2.4 | Image analysis

Preprocessing of the data was done using an in-house automated image analysis pipeline (Magia[29]) running on MATLAB (MathWorks, Inc.). MRI images were processed using FreeSurfer to define VOIs and to obtain hippocampal volumes and total intracranial volumes. For each subject, mean hippocampal volume was calculated, normalized to estimated total intracranial volume (eTIV), and finally expressed as % eTIV.

For the PET data, all scans were quantified as mean standardized uptake value ratios (SUVRs) for the 60- to 90-minute period[30] using the centrum semiovale (CS) as a reference region.[31,32] This simplified quantification method was chosen based on its previous validation in AD patients compared to the simplified reference tissue model 2 (SRTM2) method.[30] A small CS VOI similar to that previously reported in Rossano et al.[32] was first manually drawn to Montreal Neurological Institute space and then registered to all [^{11}C]UCB-J images in individual space, and restricted to locate inside FreeSurfer white matter segment. All reference VOIs were further visually inspected to avoid leaking into the ventricular and cortical regions. The primary region of interest in this study was the hippocampus, which is expected to show the earliest synaptic loss due to neurodegeneration of the entorhinal cortical neurons. In addition, $SUVR_{CS}$ were calculated for exploratory cortical regions including (1) entorhinal cortex, (2) medial temporal cortex (including hippocampus, amygdala, and entorhinal cortex), (3) prefrontal cortex, (4) parietal cortex, (5) lateral temporal cortex, (6) precuneus, (7) anterior cingulum, (8) posterior cingulum, and (9) a volume-weighted composite of the aforementioned regions.

To ensure that the simplified $SUVR_{CS}$ method did not differ from the SRTM2 method, we used the dynamic [^{11}C]UCB-J data to estimate binding potential (BP_{ND}) using SRTM2 with CS as a reference region, and where fixed CS K_{2ref} value (0.0568) was calculated as the median of all considered regional SRTM k_2/R_1 estimates. As also the whole cerebellum has been proposed to serve as a reference region in AD research and to show reductions in [^{11}C]UCB-J uptake in wider cortical regions in AD subjects compared to cognitively normal controls,[17] we also used the indirect way of calculating the distribution volume ratio ($DVR = BP_{ND} + 1$) with respect to whole cerebellum (CER) by dividing the SRTM2 DVR_{CS} with whole cerebellum DVR, as described previously.[17]

[^{11}C]PiB PET was quantified as mean SUVRs of 60 to 90 minutes post-injection using the cerebellar cortex as the reference region as previously described in detail,[21] and the global amyloid load was estimated using a volume-weighted composite VOI similar to previously described for [^{11}C]UCB-J using the same VOI set as for [^{11}C]UCB-J.

2.5 | Cognitive testing

All participants performed the Consortium to Establish a Registry for Alzheimer's Disease (CERAD) neuropsychological test battery (including the Mini-Mental State Examination [MMSE]), the Repeatable Battery for the Assessment of Neuropsychological Status (RBANS[33]), and a subset of Raven's matrices. Association between hippocampal [^{11}C]UCB-J $SUVR_{CS}$ and cognitive performance was then tested using two cognitive variables of interest: the CERAD total score,[34] and the Alzheimer's Prevention Initiative Preclinical Cognitive Composite (APCC[35]), which comprises tests from RBANS, MMSE, and Raven's matrices.

2.6 | Statistics

All statistical analyses were performed using JMP Pro 17.0.0 (SAS Institute Inc.). The threshold for statistical significance was set to $p < 0.05$ (two-tailed). Characteristics of the study sample are described as mean (standard deviation) for normally distributed variables and median (IQR) for variables with a skewed distribution. The normality of data was evaluated visually from the histograms and Q–Q plots using Shapiro–Wilk tests. For demographic data, differences among the three *APOE* groups were tested using one-way analysis of variance (ANOVA) for continuous normally distributed variables Kruskal–Wallis test for skewed variables, and Fisher exact test for categorical variables. For testing differences in regional [^{11}C]UCB-J $SUVR_{CS}$, DVR_{CS}, and DVR_{CER} among the *APOE* groups, unadjusted one-way ANOVA was first used (Model 1). If a significant difference was present, Tukey–Kramer honest significance difference was used post hoc to evaluate pair-wise differences between groups. Then, multivariate linear models further adjusted for age, sex, education (Model 2: Model 1 + age, sex, and education), and hippocampal volume (Model 3: Model 2 + hippocampal volume as % of eTIV) were performed. Effect sizes were calculated as Cohen d. One *APOE* ε4/ε4 carrier was excluded due to outlying hippocampal [^{11}C]UCB-J $SUVR_{CS}$. However, we repeated the analyses, including this outlier, to verify that the results were not driven by this individual.

Associations between hippocampal density estimated by [^{11}C]UCB-J $SUVR_{CS}$ and both global amyloid PET (composite [^{11}C]PiB $SUVR_{CER}$) and cognitive composites (CERAD total score, APCC score) were first tested using Pearson or Spearman correlation. Then, associations were further tested using linear models adjusted for age, sex, and education, and also for age, sex, education, and hippocampal volume. The associations between hippocampal volumes and cognitive composite scores were analyzed similarly.

An exploratory voxel-wise comparison of [^{11}C]UCB-J $SUVR_{CS}$ differences between the *APOE* ε4/ε4 and *APOE* ε3/ε3 groups was conducted a non-parametric two-sample *t* test with the FSL randomise function, using 5000 permutations. Multiple comparisons were corrected using threshold-free cluster enhancement (TFCE) with a family-wise error (FWE) rate at a significance level of $p < 0.05$.

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TABLE 1 Demographics of the study participants.

Variable	Whole sample	*APOE* ε4/ε4	*APOE* ε3/ε4	*APOE* ε3/ε3	P_{ANOVA}
n	**46**	**14**	**16**	**16**	
Age (y), median (IQR)	71 (65–73)	71 (65–73)	71 (64–75)	72 (67–73)	0.96
Sex (M/F), *n* (%)	16/30 (35/65)	6/8 (43/57)	4/12 (25/75)	6/10 (37	0.64
Education, *n* (%)					
Primary school	12 (26)	4 (29)	2 (12.5)	6 (37.5)	
Middle or comprehensive school	7 (15)	3 (21)	2 (12.5)	2 (12.5)	
High school	18 (39)	6 (43)	6 (37.5)	6 (37.5)	
College or university	9 (20)	1 (7)	6 (37.5)	2 (12.5)	
BMI (kg/m^2), mean (SD)	26.9 (4.30)	26.2 (4.32)	26.3 (3.99)	27.9 (4.64)	0.49
CERAD total score, median (IQR)	89 (82–94)	83 (74–93)	92 (85–95)	89 (86–90)	0.22
MMSE, median (IQR)	29 (27–30)	27.5 (27–29)	29 (28–30)	28.5 (27–30)	0.16
APCC score, median (IQR)	72 (67–79)	66 (57–77)[a]	75 (72–81)	73 (70–80)	**0.03**
Hippocampal volume (%eTIV)	0.0049 (0.0046–0.0059)	0.0048 (0.0044–0.0057)	0.0051 (0.0046–0.0065)	0.0050 (0.0046–0.0059)	0.60
[^{11}C]PiB composite $SUVR_{CER}$, median (IQR)	1.54 (1.42–2.01)	2.03 (1.56–2.79)[b]	1.49 (1.40–1.75)	1.47 (1.39–1.77)	**0.034**
Hippocampal [^{11}C]UCB-J					
$SUVR_{CS}$, mean (SD)	3.12 (0.41)	2.90 (0.30)[a]	3.09 (0.47)	3.33 (0.35)	**0.016**
DVR_{CS}, mean (SD)	3.22 (0.49)	2.99 (0.33)[a]	3.21 (0.60)	3.44 (0.39)	**0.036**
DVR_{CB}, mean (SD)	1.04 (0.069)	1.03 (0.057)	1.02 (0.071)	1.07 (0.071)	0.14

Notes: The data are presented as mean (SD) for variables with a normal distribution and as median (IQR) for skewed variables.
Abbreviations: %eTIV, % estimated total intracranial volume; APCC, Alzheimer's Prevention Initiative Preclinical Cognitive Composite; *APOE*, apolipoprotein E; BMI, body mass index; CERAD, Consortium to Establish a Registry for Alzheimer's Disease; CS, centrum semiovale; DVR, distribution volume ratio; IQR, interquartile range, MMSE, Mini-Mental State Examination; SD, standard deviation; SUVR, standardized uptake value ratio.
Statistically significant *p*-values appear in bold font.
[a]$p < 0.05$ for pairwise comparison *APOE* ε4/ε4 < *APOE* ε3/ε3.
[b]$p < 0.05$ for pairwise comparison *APOE* ε4/ε4 > *APOE* ε3/ε4.

Regional correlations among $SUVR_{CS}$, DVR_{CS}, and DVR_{CER} were evaluated using Pearson correlation.

3 | RESULTS

3.1 | Participant characteristics

Demographic characteristics of the whole study sample and three *APOE* groups are presented in Table 1. From the recruited 47 participants, one *APOE* ε4/ε4 carrier was excluded from the final analysis due to outlying hippocampal [^{11}C]UCB-J $SUVR_{CS}$ (*z* score −3.22). Thus, the final sample included 46 individuals of whom 30 were females (65.2%). The median age was 71 years (IQR 65–73 years). There were no significant differences in age ($p = 0.96$), sex ($p = 0.64$), education ($p = 0.41$), or time between recruitment and [^{11}C]UCB-J scan ($p = 0.24$) among the three *APOE* groups. In addition, no significant differences between groups were found for the SUVs calculated for CS, used as the reference for calculating regional $SUVR_{CS}$ ($p = 0.54$, one-way ANOVA, Figure S1 in supporting information). There was also no significant difference in hippocampal volume among the groups ($p = 0.60$, Kruskal–Wallis).

3.2 | $SUVR_{CS}$ method versus DVR_{CS} and DVR_{CER} methods for [^{11}C]UCB-J analyses

The correlations among hippocampal $SUVR_{CS}$, DVR_{CS}, and DVR_{CER} are presented in Figure S2 in supporting information. Hippocampal DVR_{CS} showed a very high correlation between hippocampal $SUVR_{CS}$ ($r = 0.96$, $P < 0.0001$), whereas the correlation between DVR_{CER} and $SUVR_{CER}$ was only modest, albeit statistically significant ($r = 0.42$, $p = 0.0038$). All the following main results in the article are presented as $SUVR_{CS}$. For transparency and for the comparison between the CS and the CER reference regions the regional DVR_{CS} and DVR_{CER} values are presented in Table S1 (DVR_{CS}) and Table S2 (DVR_{CER}) in supporting information, and analyses adjusted for age, sex, education, and hippocampal volume are presented in Table S3 (DVR_{CS}) and Table S4 (DVR_{CER}) in supporting information.

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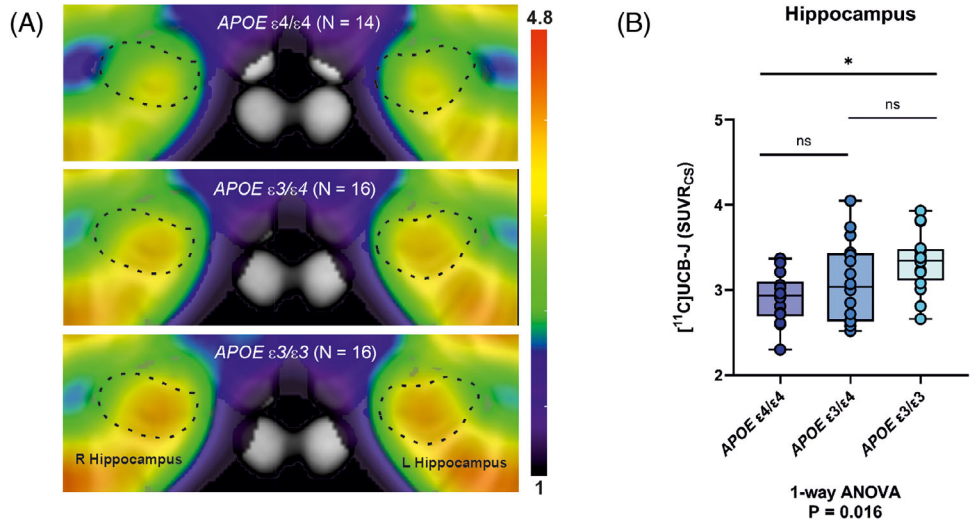

FIGURE 2 Mean coronal [^{11}C]UCB-J binding $SUVR_{CS}$ maps for *APOE* ε4/ε4, *APOE* ε3/ε4, and *APOE* ε3/ε3 groups for visual comparison of the hippocampal retention (delineated with a dashed line). Gene dose–related reduction of [^{11}C]UCB-J retention was present both visually (A) and when quantified using the hippocampal region of interest ($p = 0.016$, one-way analysis of variance, *pairwise comparison *APOE* ε4/ε4 < *APOE* ε3/ε3 $p = 0.013$) (B). *APOE*, apolipoprotein E; CS, centrum semiovale; SUVR, standardized uptake value ratio

3.3 | Differences in hippocampal [^{11}C]UCB-J retention by *APOE* genotype

Mean parametric $SUVR_{CS}$ maps at the hippocampal plane for each *APOE* genotype are presented in Figure 2. Visually, *APOE* ε4/ε4 homozygotes showed lower [^{11}C]UCB-J binding in the hippocampus compared to *APOE* ε3/ε4 heterozygotes and *APOE* ε3/ε3 controls (Figure 2A). Regional quantification showed that the difference between the hippocampal $SUVR_{CS}$ was also statistically significant ($F = 4.53$, $p = 0.016$, one-way ANOVA, Figure 2B). Post hoc pair-wise comparisons among all groups further revealed that hippocampal [^{11}C]UCB-J $SUVR_{CS}$ was significantly lower in the *APOE* ε4/ε4 homozygotes (mean 2.90, standard deviation [SD] 0.30) compared to *APOE* ε3/ε3 controls (mean 3.33, SD 0.35; $p = 0.013$, Tukey–Kramer honestly significant difference [HSD]; effect size = −1.32). No significant difference was present between *APOE* ε3/ε4 (mean 3.09, SD 0.47) and *APOE* ε4/ε4 ($p = 0.39$, Tukey–Kramer HSD), or *APOE* ε3/ε4 and *APOE* ε3/ε3 ($p = 0.21$, Tukey–Kramer HSD). Similar findings were obtained with DVR_{CS} obtained from SRTM2 ($F = 3.59$, $p = 0.036$, one-way ANOVA; *APOE* ε3/ε3 [mean 3.44, SD 0.12] vs. *APOE* ε4/ε4 [mean 2.99, SD 0.12], $p = 0.028$; *APOE* ε3/ε4 [mean 3.21, SD 0.12] vs. *APOE* ε4/ε4, $p = 0.33$; *APOE* ε3/ε3 vs. *APOE* ε3/ε4, $p = 0.33$, Tukey–Kramer HSD; Table S3). There was no difference among the *APOE* groups when the cerebellum was used as a reference region (DVR_{CER}: $p = 0.37$, one-way ANOVA; Table S4).

The observed *APOE* ε4 effect became even stronger when its sensitivity was tested by adding the excluded outlier *APOE* ε4/ε4 individual into the analysis ($F = 5.53$, $p = 0.0072$, one-way ANOVA, post hoc *APOE* ε4/ε4 vs. *APOE* ε3/ε3 $p = 0.0050$). In addition, the *APOE* ε4 effect not only remained statistically significant, but became stronger, when subsequently tested using a linear model further adjusted for age, sex, education, and hippocampal volume ($F = 5.56$, $p = 0.0077$; post hoc *APOE* ε4/ε4 vs. *APOE* ε3/ε3 $p = 0.0087$, Tukey–Kramer HSD; Table 2, Table S3). There were no differences among the *APOE* genotype groups in hippocampal [^{11}C]UCB-J DVR_{CER} in the adjusted linear models when using the cerebellum as the reference region (Table S4).

In the whole study sample, hippocampal [^{11}C]UCB-J $SUVR_{CS}$ was not correlated with hippocampal volume (rho = −0.082, $p = 0.59$, Figure S3A in supporting information). Contrary to the findings with [^{11}C]UCB-J PET, no significant differences in hippocampal volumes were found among the *APOE* groups ($p = 0.60$, Kruskal–Wallis test, Figure S3B).

3.3.1 | Differences in cortical [^{11}C]UCB-J retention by *APOE* genotype

Full parametric [^{11}C]UCB-J $SUVR_{CS}$ maps for the three *APOE* groups are presented in Figure 3A. Visually, *APOE* ε4/ε4 homozygotes showed reduced retention in most cortical regions in a gene dose (i.e., the number of ε4 alleles)–dependent manner compared to *APOE* ε3/ε4 and *APOE* ε3/ε3. However, due to large individual variation, our additional exploratory analysis for cortical regions showed no significant differences in any of the prespecified regions related to AD pathology ($P > 0.068$ for all, one-way ANOVA, Figure 3B; Table 3). When the analyses were adjusted for age, sex, and education, differences in the medial temporal cortex ($p = 0.056$), parietal cortex ($p = 0.069$), and precuneus ($p = 0.085$) had a tendency, albeit not statistically significant, for gene dose–related lower [^{11}C]UCB-J binding in *APOE* ε4 carriers. There was no difference among the *APOE* genotype groups in any of the cortical regions examined in the DVR_{CS} (Table S1) or the DVR_{CER} (Table S2) analyses.

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TABLE 2 Associations between hippocampal [^{11}C]UCB-J $SUVR_{CS}$ and *APOE* ε4 gene dose.

	Model 1				Model 2				Model 3			
	$R2_{Adj}$	13.60%			$R2_{Adj}$	15.70%			$R2_{Adj}$	15.50%		
	β	95% CI		P	β	95% CI		P	β	95% CI		P
Number of *APOE* ε4 alleles [1]	−0.20	−0.37	−0.037	**0.018**	−0.17	−0.34	−0.002	**0.048**	−0.2	−0.37	−0.018	**0.032**
Age (y)					−0.003	−0.02	0.023	0.8	−0.009	−0.03	0.02	0.54
Sex [M]					0.037	−0.094	0.17	0.57	0.0085	−0.14	0.15	0.91
Education [1]					−0.19	−0.39	0.021	0.077	−0.2	−0.41	0.011	0.063
Hippocampal volume (%eTIV)									−65.5	−205	74.4	0.35

Note: Model 1 unadjusted; Model 2 adjusted for age, sex, and education; Model 3 additionally adjusted for hippocampal volume.
Abbreviations: %eTIV, % estimated total intracranial volume; *APOE*, apolipoprotein E; CI, confidence interval; CS, centrum semiovale; SUVR, standardized uptake value ratio.
Statistically significant *p*-values appear in bold font.

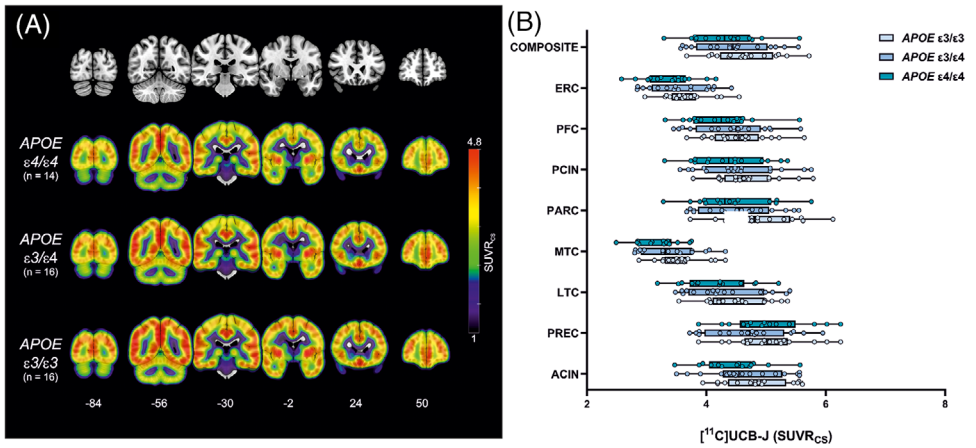

FIGURE 3 Mean coronal [^{11}C]UCB-J $SUVR_{CS}$ maps for *APOE* ε4/ε4, *APOE* ε3/ε4, and *APOE* ε3/ε3 groups for visual comparison. Gene dose–related reduction of [^{11}C]UCB-J retention was visible throughout cortical regions, with *APOE* ε3/ε3 showing the highest retention and *APOE* ε4/ε4 the lowest. ACIN, anterior cingulum; *APOE*, apolipoprotein E; COMPOSITE, a volume-weighted composite of the aforementioned regions; ERC, entorhinal cortex; LTC lateral temporal cortex; MTC, medial temporal cortex; PAR, parietal cortex; PCIN, posterior cingulum; PFC, prefrontal cortex; PREC, precuneus; SUVR, standardized uptake value ratio

TABLE 3 Regional [^{11}C]UCB-J binding stratified by *APOE* genotype.

[^{11}C]UCB-J binding ($SUVR_{CS}$)								
	APOE ε4/ε4		*APOE ε3/ε4*		*APOE ε3/ε3*			
Region	Mean	SD	Mean	SD	Mean	SD	P_a	P_b
Entorhinal cortex	3.38	0.44	3.55	0.50	3.67	0.39	0.22	0.20
Prefrontal cortex	4.23	0.58	4.41	0.61	4.54	0.52	0.35	0.27
Parietal cortex	4.42	0.67	4.53	0.63	4.88	0.62	0.13	0.069
Anterior cingulum	4.45	0.53	4.65	0.65	4.83	0.52	0.21	0.12
Posterior cingulum	4.37	0.62	4.54	0.67	4.70	0.53	0.33	0.26
Precuneus	4.62	0.71	4.69	0.68	5.05	0.64	0.19	0.085
Lateral temporal cortex	4.15	0.57	4.32	0.65	4.52	0.51	0.23	0.15
Medial temporal cortex	3.20	0.36	3.38	0.49	3.56	0.38	0.068	0.056
Cortical composite	4.29	0.59	4.44	0.62	4.65	0.54	0.24	0.16

Notes: Data presented as mean (SD). P_a, one-way ANOVA. P_b, adjusted for age, sex, and education.
Abbreviations: ANOVA, analysis of variance; *APOE*, apolipoprotein E; CS, centrum semiovale; SD, standard deviation; SUVR, standardized uptake value ratio.

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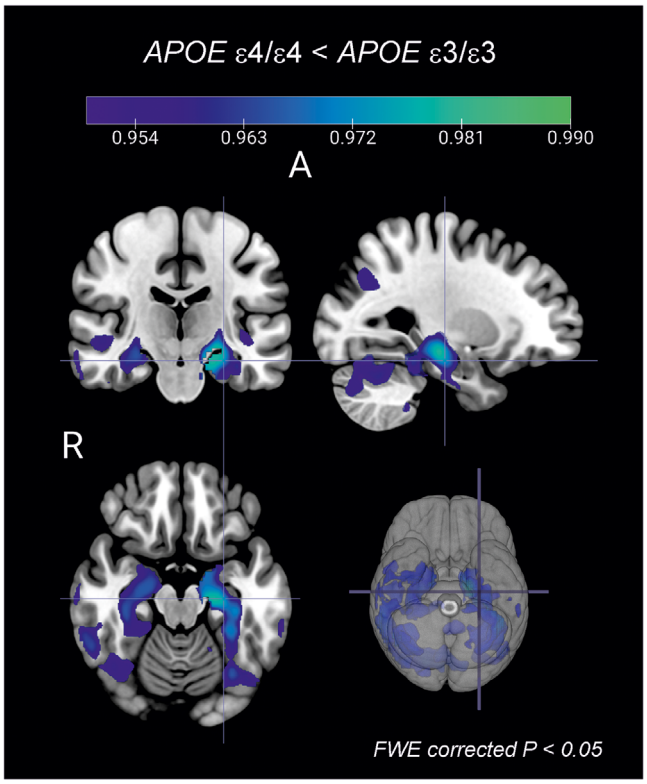

FIGURE 4 Voxel-level comparison showed reduced [^{11}C]UCB-J binding in the hippocampus of *APOE* ε4/ε4 ($n = 14$) compared to *APOE* ε3/ε3 ($n = 16$). The figure shows statistical parametric maps obtained with a non-parametric two-sample *t* test with FSL randomization, and correction of multiple comparisons with a threshold-free cluster enhancement (TFCE) and FWE-corrected $p < 0.05$, overlaid on the Montreal Neurological Institute template MRI. Scale = (1-p). *APOE*, apolipoprotein E; FWE, family-wise error; MRI, magnetic resonance imaging

3.4 | Voxel-level differences in [^{11}C]UCB-J retention by *APOE* genotype

Exploratory voxel-level comparisons between *APOE* ε4/ε4 and *APOE* ε3/ε3 verified the region of interest–level findings: lower [^{11}C]UCB-J binding was present in the hippocampus of the *APOE* ε4/ε4 group compared to *APOE* ε3/ε3 controls (Figure 4; FWE-corrected $p < 0.05$).

3.5 | Association among hippocampal [^{11}C]UCB-J binding, hippocampal volume, and cognitive performance

There were no correlations between hippocampal [^{11}C]UCB-J binding and CERAD total score (rho = −0.052, $p = 0.73$) or APCC score (rho = 0.17, $p = 0.28$; Figure 5A). The results did not change after adjusting for age, sex, and education (CERAD total score: $\beta = -0.61$, $p = 0.85$; APCC score: $\beta = 2.90$, $p = 0.29$), nor after adjusting also for hippocampal volume (CERAD total score: $\beta = -0.10$, $p = 0.97$; APCC score: $\beta = 3.41$, $p = 0.15$). Hippocampal volume showed a moderate positive correlation with both CERAD total score (rho = 0.63, $P < 0.0001$) and APCC score (rho = 0.46, $p = 0.0014$) in the study sample (Figure 5B). The observed associations remained significant when further tested using a linear model adjusted for age, sex, and education (CERAD total score adjusted $R^2 = 0.40$, hippocampal volume effect $p = 0.015$; APCC adjusted $R^2 = 0.50$, hippocampal volume effect $p = 0.0025$).

3.6 | Correlation between hippocampal [^{11}C]UCB-J and global Aβ load

We did not find a statistically significant correlation between hippocampal [^{11}C]UCB-J binding and global Aβ load estimated by composite [^{11}C]PiB $SUVR_{CER}$ (rho = −0.10, $p = 0.50$, Figure S4 in supporting information). The results did not change after adjusting for age, sex, and education ($\beta = -0.16$, $p = 0.42$) or for age, sex, education, and hippocampal volume ($\beta = -0.18$, $p = 0.39$). Similarly, our exploratory analysis with all predefined VOIs revealed no significant correlation between regional [^{11}C]UCB-J and [^{11}C]PiB binding (rho from −0.14 to 0.0070 and $P > 0.34$ for all VOIs). Similarly, there was no correlation between composite [^{11}C]PiB $SUVR_{CER}$ and hippocampal [^{11}C]UCB-J DVR_{CS} or DVR_{CER} (Figure S4).

4 | DISCUSSION

Here, we investigated hippocampal synaptic density in vivo with [^{11}C]UCB-J $SUVR_{CS}$ in *APOE* ε4 homozygotes, heterozygotes, and non-carriers. We found reduced hippocampal [^{11}C]UCB-J $SUVR_{CS}$ in cognitively unimpaired *APOE* ε4/ε4 homozygotes, who are at increased genetic risk for sporadic AD, compared to the most common *APOE* ε3/ε3 genotype. This reduction in synaptic density was independent of age, sex, education, and hippocampal volume. Similar findings have previously been reported in amnestic MCI and early AD[16–19] but, to our knowledge, this study is the first to show lower synaptic density in asymptomatic individuals at risk for clinical AD. In this clinically unimpaired sample, the reduction of hippocampal synaptic density was not associated with global Aβ load or cognitive performance. We did not find differences in hippocampal synaptic density between the intermediate-risk group (*APOE* ε3/ε4) compared to the high-risk group (*APOE* ε4/ε4) or the control group (*APOE* ε3/ε3).

Of note, there was no difference among the *APOE* groups when the cerebellum was used as a reference region. This is in contrast to a previous study that showed widespread cortical differences in [^{11}C]UCB-J DVR_{CER} between AD patients and cognitively normal controls.[17] Larger studies would be needed to evaluate if the cerebellum reference could be used to study differences in synaptic density among cognitively normal individuals at risk for AD.

Previous SV2A-targeted PET studies investigating AD dementia and amnestic MCI have shown the most prominent reductions of synaptic density in vivo in the hippocampus[16–19]. These effects are most likely

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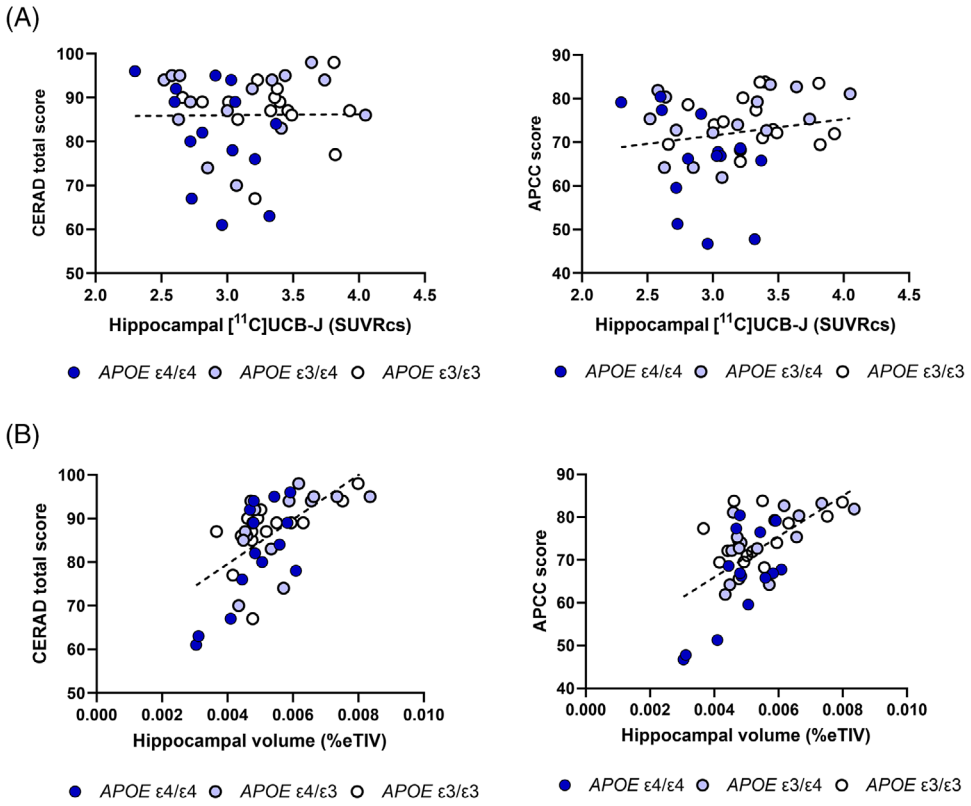

FIGURE 5 Correlations between hippocampal [^{11}C]UCB-J binding (A), hippocampal volume (B), and cognitive performance estimated with the CERAD total score and APCC score in the whole study sample. *APOE* ε4/ε4, dark blue circles; *APOE* ε3/ε4 light blue circles; *APOE* ε3/ε3 white circles. %eTIV, % estimated total intracranial volume; APCC, Alzheimer's Prevention Initiative Preclinical Cognitive Composite; *APOE*, apolipoprotein E; CERAD, Consortium to Establish a Registry for Alzheimer's Disease; CS, centrum semiovale; SUVR, standardized uptake value ratio

due to the early neurodegeneration process present in the entorhinal cortical neurons, resulting in downstream synaptic deficits in the hippocampus[36]. Interestingly, a recent study found lower hippocampal levels of synapsin-1 and synaptophysin in cognitively normal individuals with a high level of AD pathology, compared to individuals with normal cognition and low AD pathology[3]. These findings are in line with our in vivo results, and support the concept of synaptic dysfunction being an early event in individuals with preclinical AD.

To ensure that our findings on the differences in hippocampal synaptic density were not due to lower hippocampal volume in *APOE* ε4/ε4 homozygotes, we included hippocampal volume as a predictor in multivariate linear models. There was no difference in hippocampal volume among the *APOE* groups. Also, *APOE* ε4 gene dose predicted hippocampal [^{11}C]UCB-J binding after adjusting for age, sex, education, and hippocampal volume. Thus, we are confident that the differences found between *APOE* ε4 homozygotes and non-carriers in synaptic density are not due to possible hippocampal atrophy in the high-risk *APOE* ε4/ε4 individuals. In addition, because PVE can also affect the quantification accuracy, particularly in small brain structures, we reconstructed the PET data using PSF reconstruction, which improves the spatial resolution and is independent of the assumptions of post-reconstruction PVE correction methods[37]. Our $SUVR_{CS}$ findings were also replicated by DVR_{CS} estimated from the dynamic data (0–90 minutes) using SRTM2.

We did not find differences in synaptic density in cortical [^{11}C]UCB-J $SUVR_{CS}$, although differences in the medial temporal cortex, parietal cortex, and precuneus showed a trend toward statistical significance ($P < 0.085$ for all). Previous PET studies in early AD dementia patients have shown reduced synaptic density also in the parahippocampus, thalamus, parietal, prefrontal, temporal, occipital, and basal forebrain[19]. However, the strongest effect sizes have been reported for the hippocampus. Considering our relatively healthy at-risk sample, we did not expect to find significant group differences outside the hippocampus. Also, although there was a trend toward a gene dose effect in hippocampal synaptic density, there were no differences in hippocampal synaptic density between *APOE* ε3/ε4 heterozygotes and ε4/ε4 homozygotes, nor between heterozygotes and ε3/ε3 controls. This underscores the value of recruiting *APOE* ε4/ε4 homozygotes instead of ε4 carriers in general, considering that most previous studies do not stratify for hetero- and homozygotes.

Our results of decreased hippocampal synaptic density are indirectly supported by a previous *post mortem* study that examined synaptic proteins in the superior temporal cortex of cognitively normal controls (mean age 75 years) and AD brains (mean age 80 years)[6]. In the controls, *APOE* ε4 carriers had a trend toward lower levels of presynaptic proteins (syntaxin 1 and synaptophysin) compared to *APOE* ε3 and ε2 carriers. There was no association between the *APOE* genotype and these proteins in AD brains[6]. In another study investigating AD

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dementia patients, expression of synaptophysin was lower in the hippocampus and in the inferior temporal cortex in AD dementia cases with at least one *APOE* ε4 allele, compared to AD dementia cases without *APOE* ε4[38]. An association between the *APOE* ε4 allele and early synaptic damage has also been suggested by several fluid biomarker studies.[7,39,40] MCI patients with at least one *APOE* ε4 allele showed elevated cerebrospinal fluid (CSF) concentrations of a presynaptic protein, SNAP-25[39]. Similarly, CSF SNAP-25 was elevated in cognitively normal *APOE* ε4 carriers and Aβ-positive individuals. In Aβ-negative individuals, *APOE* ε4 carriers had significantly higher CSF SNAP-25 concentrations compared to those without *APOE* ε4[7]. Levels of CSF neurogranin, a postsynaptic protein, were also significantly higher in *APOE* ε4 carriers than in non-carriers with MCI, but not AD dementia patients, and CSF neurogranin levels increased in an *APOE* ε4 gene dose-dependent fashion in the whole sample ($N = 399$). A comparison between ε4 carriers and non-carriers with unimpaired cognition showed a similar trend[40]. The results of these previous studies, as well as the present study, indicate that the ε4 allele is associated with premorbid synaptic disruption that could contribute to an increased vulnerability of neurons against an ongoing disease process, and thus also cognitive impairment.

Based on histopathological findings, synaptic density is suggested to be the best predictor of cognitive performance in AD[4,41,42]. Accordingly, a recent in vivo [^{11}C]UCB-J PET study including 45 early AD dementia patients showed that global synaptic density predicted cognitive impairment better than gray matter volume[15]. In another study, MMSE scores were correlated with ^{18}F-UCB-H binding in the hippocampus, prefrontal cortex, and temporal cortex in early AD patients[19]. In the present study, no correlation was found between hippocampal or global synaptic density and cognitive performance. This finding is understandable, considering the clinically unimpaired study population who did not have significant cognitive impairment and who might be resilient to early synaptic loss in the hippocampus. Also, the detection of subtle cognitive impairment might require more sensitive tests than CERAD and APCC. Interestingly, although there was no *APOE* group difference in hippocampal volume and hippocampal volume was not associated with synaptic density in the hippocampus, lower hippocampal volume showed an association with poorer global cognition in the total study sample. It is possible that lower hippocampal volumes detected on structural MRIs might represent a different process than the early synaptic loss detected by [^{11}C]UCB-J that seems to be driven by the *APOE* ε4/ε4 genotype, independently of structural changes or Aβ pathology. Even though the lack of an association between hippocampal synaptic density and hippocampal volumes in the present study may seem controversial, it could be explained by studies that indicate that [^{11}C]UCB-J could only bind to SV2A in functional synapses where the synaptic vesicles are attached to the plasma membrane[43]. Thus, although synapses might still be present (as suggested by hippocampal volumetrics) they might not be functional.

Aβ deposition estimated by [^{11}C]PiB has previously been shown to associate with hippocampal synaptic density in patients with amnestic MCI that are expected to be in the Aβ accumulation phase, but not in patients with AD dementia in whom Aβ load is expected to already reach its peak[20]. Based on this finding, we expected to find an inverse association between hippocampal synaptic density and global Aβ load in our cognitively well-preserved sample. However, even though Aβ load in the whole ASIC-E4 cohort has previously been shown to increase according to *APOE* ε4 gene dose[28], we did not find any association in the whole sample or when stratified by *APOE* ε4 gene dose even after adjusting for time between [^{11}C]PiB and [^{11}C]UCB-J scans.

The strength of the study is a well-balanced cohort including cognitively well-preserved *APOE* ε4 heterozygotes, homozygotes, and non-carriers. This study also has limitations: Due to delay in tracer availability and the COVID-19 pandemic, [^{11}C]UCB-J-PET was performed on average 20 months after recruitment, and [^{11}C]PiB-PET and our sample size was reduced from the original ASIC-E4 study population. In addition, cognitive tests needed to be repeated at the time of [^{11}C]UCB-J scan, and thus a learning effect is likely. According to previous *post mortem* studies,[44] it is possible that [^{11}C]UCB-J binding in AD patients also reflects other underlying causes than synaptic density. In a very recent *post mortem* validation study, immunoblot analyses demonstrated a correlation between SV2A and AT8 (demonstrating larger phosphorylated tau species) immunoreactivity in frontal cortex nuclear fraction of AD dementia brains[44]. In AD, synaptic density has been suggested to be closely associated with tau pathology[45], but unfortunately, tau PET was not included in our protocol. Therefore, we could not evaluate whether entorhinal or hippocampal tau would have confounded our results. Longitudinal studies are needed to investigate whether synaptic dysfunction can predict cognitive decline and further conversion to MCI and AD dementia among *APOE* ε4 carriers.

5 | CONCLUSION

Here, we demonstrate that hippocampal synaptic density, estimated with [^{11}C]UCB-J PET, was lower in cognitively unimpaired *APOE* ε4 homozygotes compared to *APOE* ε3/ε3 controls, and that this difference was independent of age, education, and hippocampal volume. These results suggest that synaptic dysfunction and loss is an early feature of the AD pathological process that seems to be associated with the most common genetic risk factor for sporadic AD, the *APOE* ε4 genotype.

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CONFLICT OF INTEREST STATEMENT

J.O.R. serves as a consultant or a member of the advisory board for BioArctic/Eisai Nordic and Novartis and a member of a global expert panel for Novo Nordisk and a data monitoring committee for Lundbeck. Other authors report no declaration of interest. Author disclosures are available in the supporting information.

CONSENT STATEMENT

All study participants provided written informed consent and the Declaration of Helsinki, Good Clinical Practice, and general data protection regulations were followed.

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