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Rapid Eye Movement Sleep, Neurodegeneration, and Amyloid Deposition in Aging

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Objective: Rapid eye movement (REM) sleep is markedly altered in Alzheimer's disease (AD), and its reduction in older populations is associated with AD risk. However, little is known about the underlying brain mechanisms. Our objective was to investigate the relationships between REM sleep integrity and amyloid deposition, gray matter volume, and perfusion in aging.

Methods: We included 121 cognitively unimpaired older adults (76 women, mean age 68.96 ± 3.82 years), who underwent a polysomnography, T1-weighted magnetic resonance imaging, early and late Florbetapir positron emission tomography scans to evaluate gray matter volume, perfusion, and amyloid deposition. We computed indices reflecting REM sleep macro- and microstructural integrity (ie, normalized electroencephalographic spectral power values). Voxel-wise multiple regression analyses were conducted between REM sleep indices and neuroimaging data, controlling for age, sex, education, the apnea-hypopnea index, and the apolipoprotein E $\epsilon 4$ status.

Results: Lower perfusion in frontal, anterior and posterior cingulate, and precuneus areas was associated with decreased delta power and electroencephalographic slowing (slow/fast frequencies ratio), and increased alpha and beta power. To a lower extent, similar results were obtained between gray matter volume and delta, alpha, and beta power. In addition, lower REM sleep theta power was more marginally associated with greater diffuse amyloid deposition and lower gray matter volume in fronto-temporal and parieto-occipital areas.

Interpretation: These results suggest that alterations of REM sleep microstructure are associated with greater neurodegeneration and neocortical amyloid deposition in older adults. Further studies are warranted to replicate these findings, and determine whether older adults exhibiting REM sleep alterations are more at risk of cognitive decline and belonging to the Alzheimer's continuum.

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Introduction

Rapid eye movement (REM) sleep is a sleep stage that classically occupies approximately 20 to 25% of total sleep time. It

is characterized by an intense cortical activity, mainly in the theta (4–8 Hz) and alpha (8–12 Hz) frequency ranges, resulting from the activation of the cholinergic system.^{1, 2}

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Patients with Alzheimer's disease (AD) dementia show REM sleep alterations both at the macrostructural and microstructural levels. Indeed, REM sleep is significantly reduced and fragmented in AD patients. Furthermore, they show a global slowing of REM sleep electroencephalographic (EEG) rhythms, corresponding to an increase of spectral power in low frequencies (delta and theta) over fast frequencies (alpha and beta), especially in temporo-parietal regions.^{3–5}

Importantly, REM sleep changes seem to be detectable before the onset of dementia and predictive of worse cognitive outcome. Indeed, several studies have shown that REM sleep is reduced in patients with amnesic mild cognitive impairment (aMCI),^{6–8} who are more at risk of developing dementia, especially in those who will later convert to dementia⁹ and in apolipoprotein ε4 (APOE4) carriers.¹⁰ The slowing of EEG rhythms seems to be also already measurable in aMCI patients.¹¹ In addition, reduced REM sleep is predictive of cognitive decline and dementia risk in cognitively unimpaired older adults.^{12, 13} To sum up, REM sleep is altered in AD and changes are noticeable early, notably in aMCI patients.

However, the relationships between REM sleep alterations and brain integrity remain largely unclear. It is well established that the brainstem, basal forebrain, and medial temporal lobe are among the first regions to degenerate in AD.^{14, 15} The brainstem and basal forebrain are also involved in REM sleep generation and maintenance, and the neocortex and the hippocampal region are widely activated during REM sleep.^{16, 17} This may explain, at least in part, why patients with AD and MCI show REM sleep alterations. Furthermore, greater cortical thickness in frontal, superior, and medial temporal areas, as well as in insular areas, has been related to alterations in REM sleep microstructure (ie, higher delta power and lower alpha power during REM sleep).¹⁸

To deepen our understanding of the relationships between early REM sleep alterations and AD pathophysiology, we investigated the neural substrates of REM sleep macro- and microstructural integrity in a group of cognitively unimpaired older adults, including amyloid deposition, known to start several years before the onset of cognitive deficits. Our hypothesis was that reduced REM sleep proportion and/or alterations in REM sleep microstructure in cognitively unimpaired older adults may be associated with brain alterations suggestive of early AD pathology, including neurodegeneration and greater neocortical amyloid deposition.

Methods

Participants

The present study was performed using baseline data from the Age-Well randomized controlled trial of the Mediterranean European project, sponsored by the French

National Institute of Health and Medical Research ("Institut National de la Santé et de la Recherche Médicale" [INSERM]). The Age-Well randomized controlled trial was approved by the local ethics committee (CPP Nord-Ouest III, Caen; trial registration number: EudraCT: 2016–002441-36; IDRCB: 2016-A01767-44; [ClinicalTrials.gov](https://clinicaltrials.gov) Identifier: NCT02977819), and written informed consent was collected for all participants before the examinations. The protocol has been previously described.¹⁹ Briefly, participants were all recruited from the general population, aged over 65 years, native French speakers, retired for at least 1 year, had at least 7 years of education, and performed within the normal range for age and educational levels on standardized cognitive tests of a neuropsychological diagnostic battery. The main exclusion criteria were safety concerns in relation to magnetic resonance imaging (MRI) or positron emission tomography (PET) scanning, evidence of a major neurological or psychiatric disorder (including alcohol or drug abuse), history of cerebral disease (eg, vascular disease, such as transient ischemic disease or stroke, neurodegenerative diseases, physical malformation, tumor, or head trauma with loss of consciousness for >1 hour), presence of a chronic disease or acute unstable illness, and current or recent medication that may interfere with cognitive functioning. At baseline, in a maximum period of 3 months, participants underwent a comprehensive neuropsychological assessment, an ambulatory polysomnography (PSG), APOE genotyping, and structural MRI and ¹⁸F-Flortapir PET scans.

Polysomnography Recording

Data Acquisition. Participants underwent a full-night PSG at home using an ambulatory device (Siesta[®]; Compumedics, Abbotsford, Vic., Australia). Of the 121 participants, 68% (82 participants) underwent 2 PSG recordings and the first one (habituation night) was not analyzed, whereas the remaining 32% (39 participants) only had 1 night of PSG, which was included in the analyses.

The PSG montage was composed of an electroencephalogram (EEG), electrooculogram, electrocardiogram, and chin electromyogram. Respiratory movements were recorded using thoracic and abdominal belts, respiratory airflow using nasal and oral thermistors, and oxygen saturation using a finger pulse oximeter. For the EEG recording, 20 electrodes were placed over the scalp according to the international 10–20 system (Fp1, Fp2, F3, F4, F7, F8, Fz, C3, C4, Cz, T3, T4, P3, P4, Pz, O1, O2, vertex ground, and a bi-mastoid reference), with impedances kept <5 kΩ. A subset of 67 participants underwent a 3-minute quiet resting-state wakefulness recording in the late afternoon, immediately after the set-up, during which

they were asked to close their eyes, relax without falling asleep, and avoid moving. The EEG signal was digitized at a sampling rate of 256 Hz, high-pass and low-pass filters were applied, respectively, at 0.3 Hz and 35 Hz. The recordings were then visually scored by experts in 30-second epochs according to the international scoring rules of the American Academy of Sleep Medicine.²⁰ Standard sleep parameters, including total sleep time, sleep

latency, and the proportion of each sleep stage, as well as respiratory parameters, such as the apnea-hypopnea index (AHI), were computed and are detailed in Table 1.

Spectral Analyses. Quantitative EEG analyses were based on automatic scoring by the ASEECA algorithm, after automatic artefact rejection (version 4.1.71; PHYSIP, Paris, France). The validation of this algorithm has been

TABLE 1. Participants' Characteristics

Characteristics	Mean	Standard deviation	Range
Demographics			
Age (yr)	68.96	3.82	65–83
Women, n (%)	76 (62.80)	N/A	N/A
Education (yr)	12.93	3.04	7–22
MMSE	29.03	1.06	26–30
MADRS	1.08	1.31	0–6
STAI-B	34.55	7.10	20–54
Body mass index (kg/m ²)	26.32	4.36	18.10–44.18
Current sleep medication us, n (%) ^a	10 (8.26)	N/A	N/A
Florbetapir SUVr ^b	0.98	0.21	0.72–1.76
Amyloid positive participants, n (%)	26 (21.67)	N/A	N/A
APOE4 carriers, n (%)	32 (26.45)	N/A	N/A
Sleep			
Total sleep time (min)	358.26	64.57	192–550.50
Sleep efficiency: %	76.79	10.16	49.60–96.60
NREM-1: min (% TST)	48.53 (13.67)	25.39 (7.08)	9 (3.50)–142.50 (43.90)
NREM-2: min (% TST)	173.65 (48.53)	44.45 (8.74)	88 (24.40)–313.50 (69.70)
NREM-3: min (% TST)	69.49 (19.48)	33.34 (8.97)	0 (0)–167.50 (48.10)
REM sleep: min (% TST)	66.59 (18.32)	24.76 (5.69)	0 (0)–120 (33.30)
Apnea-Hypopnea Index (No. events/h)	25.31	14.75	0.7–75.50
Normalized REM sleep delta power (%)	63.31	7.98	42.62–84.44
Normalized REM sleep theta power (%)	17.19	4.04	7.84–27.18
Normalized REM sleep alpha power (%)	8.77	3.19	3.14–21.12
Normalized REM sleep beta power (%)	8.84	3.32	2.75–23.41
REM sleep slowing ratio	13.33	13.21	2.71–113.77

Abbreviations: APOE4, apolipoprotein E allele ε4; MADRS, Montgomery–Asberg Depression Rating Scale; min, minutes; MMSE, Mini-Mental State Examination; N/A, not applicable; NREM, non-rapid eye movement sleep; REM, rapid eye movement sleep; STAI-B, State–Trait Inventory form B; SUVr, standard uptake value ratio; TST, total sleep time.

^aUse of sleep medication on a regular basis (>1/week), excluding phytotherapy and homeopathy.

^bn = 120 participants with a valid Florbetapir positron emission tomography scan.

described elsewhere,²¹ and it has been previously used in older adults.^{22, 23} A comparative visual check was performed between hypnograms obtained from visual and automatic scoring for each participant, and participants with (1) poor overall scoring agreement kappa scores ($n = 3$), and (2) warnings regarding REM sleep scoring provided by the ASEEGA algorithm ($n = 1$) were excluded from the analysis sample. In our sample, the mean accordance rate with visual scoring was 75.5%, with a Cohen's kappa coefficient of 0.67 for all sleep stages, and a sensitivity of 81.5% and specificity of 95.5% for REM sleep detection specifically. First, a general approach was used by computing absolute EEG spectral power on artefact-free 30-second epochs on the bipolar Cz-Pz derivation, using a fast Fourier transform with Hanning window, in the following frequency bands: delta (0.1–4 Hz), theta (4–8 Hz), alpha (8–12 Hz), and beta (13–30 Hz). Absolute EEG spectral power values were normalized to the global spectral power for each epoch. Log-transformed normalized REM sleep EEG power values were used in the analyses, to take into account interindividual differences in total spectral power. Based on absolute spectral power data, we also computed a REM sleep slowing ratio, as follows: $(\text{delta} + \text{theta})/(\text{alpha} + \text{beta})$, due to its sensitivity to MCI.¹¹

For complementary analyses and following the same methodology, we also averaged relative spectral power from monopolar electrodes on topographically-specific sites, as follows: frontal (F3, Fz, F4), central (C3, Cz, C4), temporal (T3, T4), parietal (P3, Pz, P4), and occipital (O1, O2) areas, all referenced to the mastoids. In addition, relative spectral power in the theta (4–8 Hz) and alpha (8–12 Hz) frequency bands was also extracted from artefact-free epochs of eyes closed resting-state recordings in a subset of 67 participants.

Neuroimaging Examinations

Participants underwent three neuroimaging examinations in separate sessions, at the Cyceron Center (Caen, France), on the same MRI and PET cameras (Philips Achieva 3.0 T (Eindhoven, The Netherlands) and a GE Healthcare Discovery RX VCT 64 PET-CT (Milwaukee, Wis) scanners, respectively).

Structural MRI. A high-resolution T1-weighted anatomical image was acquired using a 3D fast-field echo sequence (3D-T1-FFE sagittal, repetition time 7.1 ms, echo time 3.3 ms, flip angle 6°, 180 slices with no gap, slice thickness 1 mm, field of view $256 \times 256 \text{ mm}^2$, in-plane resolution $1 \times 1 \times 1 \text{ mm}^3$). During the MRI session, participants were equipped with earplugs, and their head was stabilized with foam pads to minimize head motion.

T1-weighted images were segmented using fluid-attenuated inversion recovery images (3D-IR sagittal, TR/TE/TI 4,800/272/1,650 ms; flip angle 40°; 180 slices with no gap; slice thickness 1 mm; field of view $250 \times 250 \text{ mm}^2$; in-plane resolution $0.98 \times 0.98 \text{ mm}^2$), spatially normalized to the Montreal Neurological Institute template, modulated using the SPM12 segmentation procedure (<http://www.fil.ion.ucl.ac.uk>), and smoothed with an 8-mm full-width at half-maximum Gaussian filter. Images were then masked to exclude non-GM voxels from the analyses.

PET Imaging. A dual-phase Florbetapir PET scan was acquired with a resolution of $3.76 \times 3.76 \times 4.9 \text{ mm}^3$ (field of view 157 mm). Forty-seven planes were obtained with a voxel size of $1.95 \times 1.95 \times 3.27 \text{ mm}^3$. A transmission scan was performed for attenuation correction before the PET acquisition. Each participant underwent a 10-minute PET scan beginning at the intravenous injection of $\sim 4 \text{ MBq/Kg}$ of Florbetapir (ie, early acquisition), composed of 10 1-minute dynamic frames. Early Florbetapir PET images were reconstructed from 1 to 6 minutes, and reflect cerebral blood flow, which has been shown to be highly coupled to brain glucose metabolism.²⁴ In addition, a 10-minute PET scan beginning 50 minutes after the intravenous injection (ie, late acquisition), reflecting brain amyloid burden, was acquired.

PET images were coregistered on their corresponding anatomical MRI, voxel-wise corrected for partial volume effects using the three-compartmental voxel-wise Müller-Gärtner method, and were then normalized to the Montreal Neurological Institute template using deformation parameters derived from the anatomical MRI. The resulting images were scaled using cerebellar GM as a reference. A smoothing kernel of 10 mm Gaussian filter was applied, and images were masked to exclude non-GM voxels from the analyses. Partial volume effect-corrected normalized and scaled images were also used to extract the individual global cortical amyloid standard uptake value ratio using a predetermined neocortical mask including the entire GM, except the cerebellum, occipital and sensory motor cortices, hippocampi, amygdala, and basal nuclei.²⁵ The threshold for amyloid positivity was defined as >0.99 , and corresponded to the 99.9th percentile of the neocortical standard uptake value ratio distribution among 45 healthy young individuals, aged <40 years.²⁵

Statistical Analyses

Voxel-wise multiple regression analyses were performed using SPM12, between neuroimaging data (ie, GM volume, perfusion and amyloid burden), and REM sleep

macro- (ie, REM sleep proportion) and microstructural indices (ie, normalized EEG power values in each frequency band and the EEG slowing ratio). Age, sex, education, the AHI, and the APOE4 status were included as covariates. Results were considered significant at a $p < 0.005$ (uncorrected) threshold combined with a cluster-level threshold of $p < 0.05$ corrected for family-wise errors (FWE). For transparency, we also provide the results at a lower statistical threshold (ie, $p < 0.005$ unc., $k = 100$) in the Supplementary Material. For the sake of completeness, we compared REM sleep macro- and microstructural indices between amyloid-positive ($n = 94$) and negative ($n = 26$) participants by performing ANCOVAs separately for each REM sleep parameter as dependent variables, amyloid positivity as a between-participants factor, and age, sex, education, the AHI and APOE4 status as covariates. Post-hoc comparisons were performed using bootstrapping (1,000 replicates), and 95% confidence intervals and effect sizes were reported.

Then, sensitivity and specificity analyses were conducted to verify the robustness of the results. The results are available in the Supplementary Material.

First, we aimed at verifying whether we obtained similar results in a subsample of 45 participants free of potential comorbidities or confounders (ie, excluding participants with an AHI ≥ 30 , only 1 PSG night, occasional sleep medication use, and poor agreement between REM sleep automatic and visual scoring, defined as a difference between visual and automatic REM sleep duration, and proportion < 10 th and > 90 th percentile of the whole cohort). For this purpose, neuroimaging signal values were extracted from clusters significantly associated with REM sleep in voxel-wise analyses. We performed multiple regressions between REM sleep variables and brain integrity in the whole sample ($n = 121$ for MRI data, $n = 119$ for perfusion data, and $n = 117$ for amyloid data), as well as in the subsample ($n = 45$ for MRI data, $n = 44$ for perfusion data, and $n = 42$ for amyloid data), controlling for age, sex, education, and the AHI.

Second, to assess whether the main results were replicated on other derivations, we performed voxel-wise multiple regressions between neuroimaging data and relative spectral power from monopolar electrodes averaged separately on frontal, central, temporal, parietal, and occipital sites. These analyses were controlled for the same covariates as in the main analyses (ie, age, sex, education, the AHI, and APOE4 status), and the results were provided both at the $p < 0.005$ unc., combined with a FWE cluster-level correction threshold, and the more permissive $p < 0.005$ unc., $k = 100$ threshold.

Finally, we checked whether theta and alpha power during REM sleep and resting-state wakefulness were

significantly associated. In a subset of 67 participants with available resting-state EEG data, we conducted multiple regression analyses between REM sleep and wakefulness theta and alpha relative spectral power, in separate models, controlling for age, sex, education, the AHI, and APOE4 status.

Results

Sample Characteristics

From the 137 participants enrolled in the Age-Well randomized controlled trial at baseline, 121 participants completed a polysomnography, neuroimaging scans, neuropsychological evaluation, blood sampling, and were included in the final analysis sample (see the flowchart in Fig 1). Their characteristics are described in Table 1.

Gray Matter Volume

REM sleep macrostructure, as reflected by REM sleep proportion, was not significantly associated with gray matter (GM) volume in cognitively unimpaired older adults.

At the microstructural level, we found a positive relationship between spectral power in the theta band and GM volume in widespread brain regions (ie, frontal, anterior cingulate, insular, lateral temporal, parietal, and occipital bilateral cortices; p -values ranging from $p_{FWE-corrected} < 0.001$ to 0.041, t -values ranging from 4.91 to 4.04; Table 2 and Fig 2). Thus, lower REM sleep theta power was associated with lower GM volume in these areas. In addition, beta power was negatively associated with GM volume in the anterior cingulate cortex ($p_{FWE-corrected} = 0.44$, $t = 4.08$). In contrast, delta and alpha power, and the REM sleep slowing ratio were not associated with GM volume.

Brain Perfusion

REM sleep proportion was not associated with regional brain perfusion.

Delta power was positively associated with perfusion in anterior, posterior cingulate areas and the precuneus ($t = 5.17$, $p_{FWE-corrected} < 0.001$), as well as frontal areas ($t = 5.08$, $p_{FWE-corrected} < 0.001$; Table 2 and Fig 2). Furthermore, theta power was negatively related to perfusion in fronto-parietal cortices ($p_{FWE-corrected} = 0.001-0.031$, $t = 4.73-5.04$). Alpha power was negatively associated with perfusion in middle and posterior cingulate regions, and the precuneus ($p_{FWE-corrected} = 0.016$, $t = 4.63$). Finally, beta power negatively correlated with perfusion in anterior and middle cingulate areas ($p_{FWE-corrected} < 0.001$, $t = 4.23$; Table 2 and Fig 2). No significant association was obtained with the slowing ratio.

To summarize, higher REM sleep delta power was associated with greater perfusion in fronto-cingulate areas,

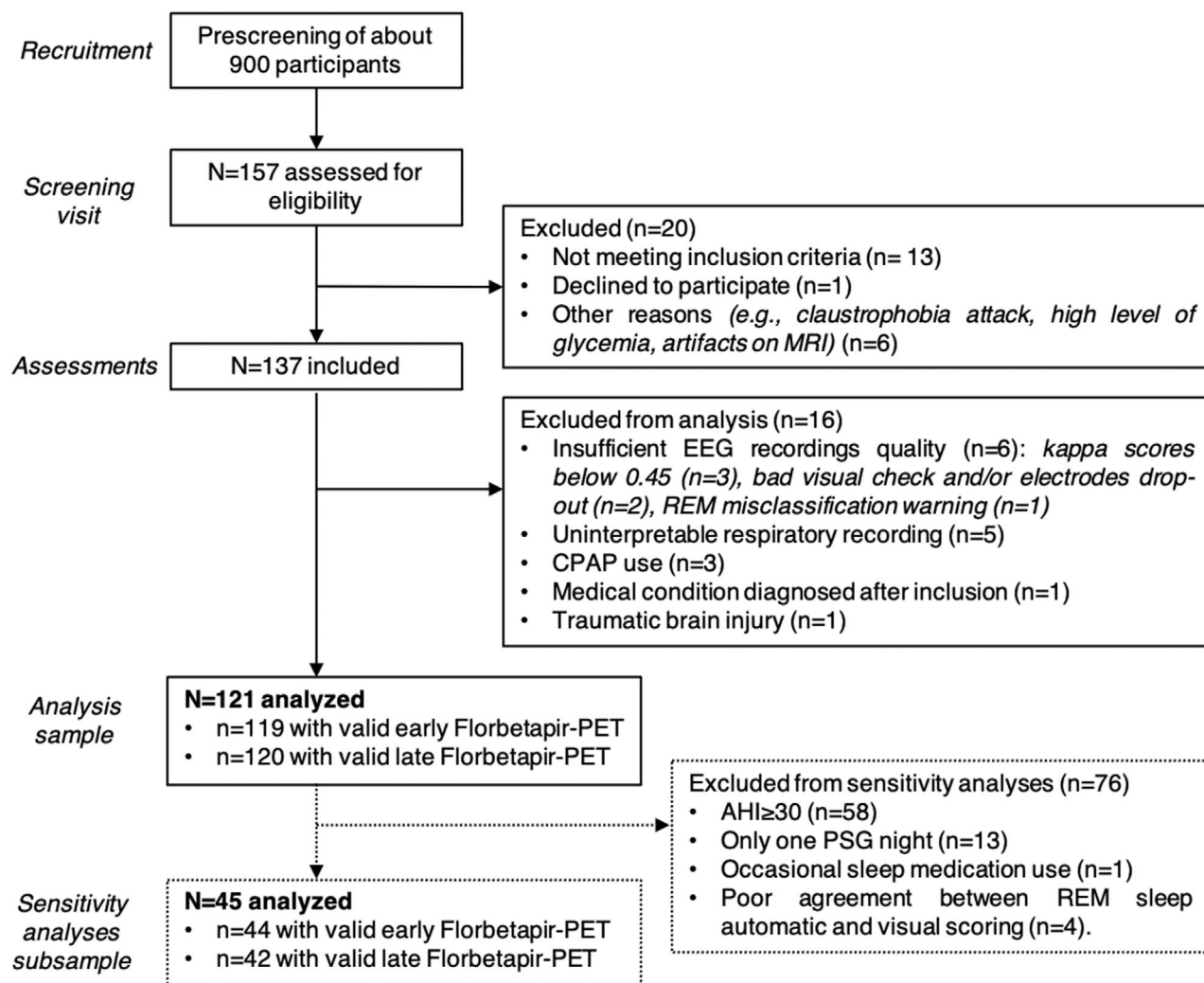


FIGURE 1: Flowchart of the inclusion process. Abbreviations: AHI, Apnea-Hypopnea Index; APOE, apolipoprotein E; CPAP, continuous positive airway pressure; MRI, magnetic resonance imaging; PET, positron emission tomography.

and higher REM sleep theta, alpha, and beta power were associated to lower perfusion in fronto-cingulate and parietal areas.

Amyloid Deposition

Voxel-Wise Analysis. REM sleep theta power was the only variable associated with amyloid deposition. Indeed, theta power was negatively associated with amyloid deposition in a large cluster encompassing frontal, anterior, middle, and posterior cingulate areas, as well as in temporal and parietal cortices ($p_{\text{FWE-corrected}} < 0.001$, $t = 4.97$; Table 2 and Fig 2).

Between-Group Comparisons. As a complementary analysis, we compared REM sleep parameters between amyloid positive and negative participants (Table 3). This analysis revealed that only REM sleep theta power was significantly different between amyloid-positive and negative individuals, with lower values in amyloid-positive

participants (mean difference 0.166, 95% CI 0.044–0.292, $p = 0.003$, Cohen's $d = 0.713$).

Sensitivity and Specificity Analyses

First, to ensure the robustness of our results, we performed multiple regressions analyses between REM sleep spectral power and neuroimaging signal values extracted from relevant significant clusters described above, in the whole sample, as well as in a subsample of participants free of potential bias, controlling for age, sex, education, and the AHI. These analyses showed that all associations remained significant in the subsample (Table S1 and Fig S1). Of note, the associations between (1) theta power and GM volume ($\beta = 0.32$, $p = 0.043$, partial $\eta^2 = 0.10$), (2) beta power and GM volume ($\beta = -0.32$, $p = 0.040$, partial $\eta^2 = 0.10$), and (3) theta power and amyloid burden ($\beta = -0.32$, $p = 0.049$, partial $\eta^2 = 0.10$) were still significant in the subsample, but effect sizes were lower than in the whole sample.

TABLE 2. Results of Voxel-Wise Multiple Regressions Analyses between Rapid Eye Movement Sleep Normalized Electroencephalographic Spectral Power Values and Neuroimaging Data

Frequency band	Correlation	Neuroimaging modality and brain region	Nb of voxels	MNI coordinates			t-value	<i>p</i> _{FWE-corrected}
				<i>x</i>	<i>y</i>	<i>z</i>		
GM volume								
Theta	+	L temporal inf, temporal pole mid	1,152	-45	12	-42	4.91	0.007
		R occipital mid/sup/inf, parietal Inf, Angular, temporal mid, lingual	3,277	50	-72	28	4.80	<0.001
		L temporal mid/inf, occipital Mid/inf, angular	3,697	-66	-27	2	4.63	<0.001
		L temporal sup, Heschl, supramarginal	820	-58	-10	10	4.54	0.041
		L frontal inf tri/inf orb/mid orb/sup orb/med orb/mid/sup med, cingulate ant	3,661	-52	34	8	4.45	<0.001
		L insula	1,385	-38	-3	-8	4.30	0.002
		R temporal pole mid/sup, insula	1,577	50	15	-34	4.18	0.001
		R frontal inf tri/mid/inf orb	1,044	52	38	10	4.04	0.012
Beta	-	B cingulate ant	812	-3	27	-9	4.08	0.044
Perfusion								
Delta	+	B cingulate mid/ant/post, rectus, precuneus	10,592	2	-10	38	5.17	<.001
		L frontal sup/inf tri/mid/sup orb/mid orb, precentral	4,322	-18	34	38	5.08	<0.001
Theta	-	L frontal mid/sup	3,727	-32	20	45	5.04	0.001
		R parietal sup/inf						
		R frontal sup	1970	14	28	52	4.73	0.031
Alpha	-	L cingulate mid/post, precuneus	2,300	-6	-34	36	4.63	0.016
Beta	-	B cingulate ant/mid	5,341	6	40	3	4.27	<0.001
Amyloid burden								
Theta	-	B frontal sup/sup med/mid, rectus, cingulate ant/mid/post, supp motor area, postcentral, precuneus, calcarine	54,762	21	-6	57	4.97	<0.001
		R frontal sup orb/inf oper/inf tri, putamen, insula, temporal sup/mid, precentral, rolandic operc, Heschl, parietal sup/inf, angular, occipital mid/inf, lingual, fusiform						

Results are presented at the $p < 0.005$ (uncorrected) level combined with a FWE cluster-level correction, controlling for age, sex, education, the Apnea-Hypopnea Index, the apolipoprotein E allele $\epsilon 4$ status, and sleep medication use (see legend of Table 2 for details about sleep medication use). The first region mentioned corresponds to the statistical peak, and the others regions listed compose the rest of each cluster.

Abbreviations: AHI, Apnea-Hypopnea Index; APOE4, apolipoprotein E allele $\epsilon 4$; B, bilateral; FWE, family-wise error; GM, gray matter; L, left; MNI, Montreal Neurological Institute; MRI, magnetic resonance imaging; PET, positron emission tomography; R, right; REM, rapid eye movement.

Second, we aimed at replicating the main voxel-wise results using relative spectral power averaged from monopolar electrodes located in frontal, central, temporal,

parietal, and occipital sites. Overall, these analyses demonstrated that fronto-cingular GM volume and perfusion were positively associated with delta power and EEG

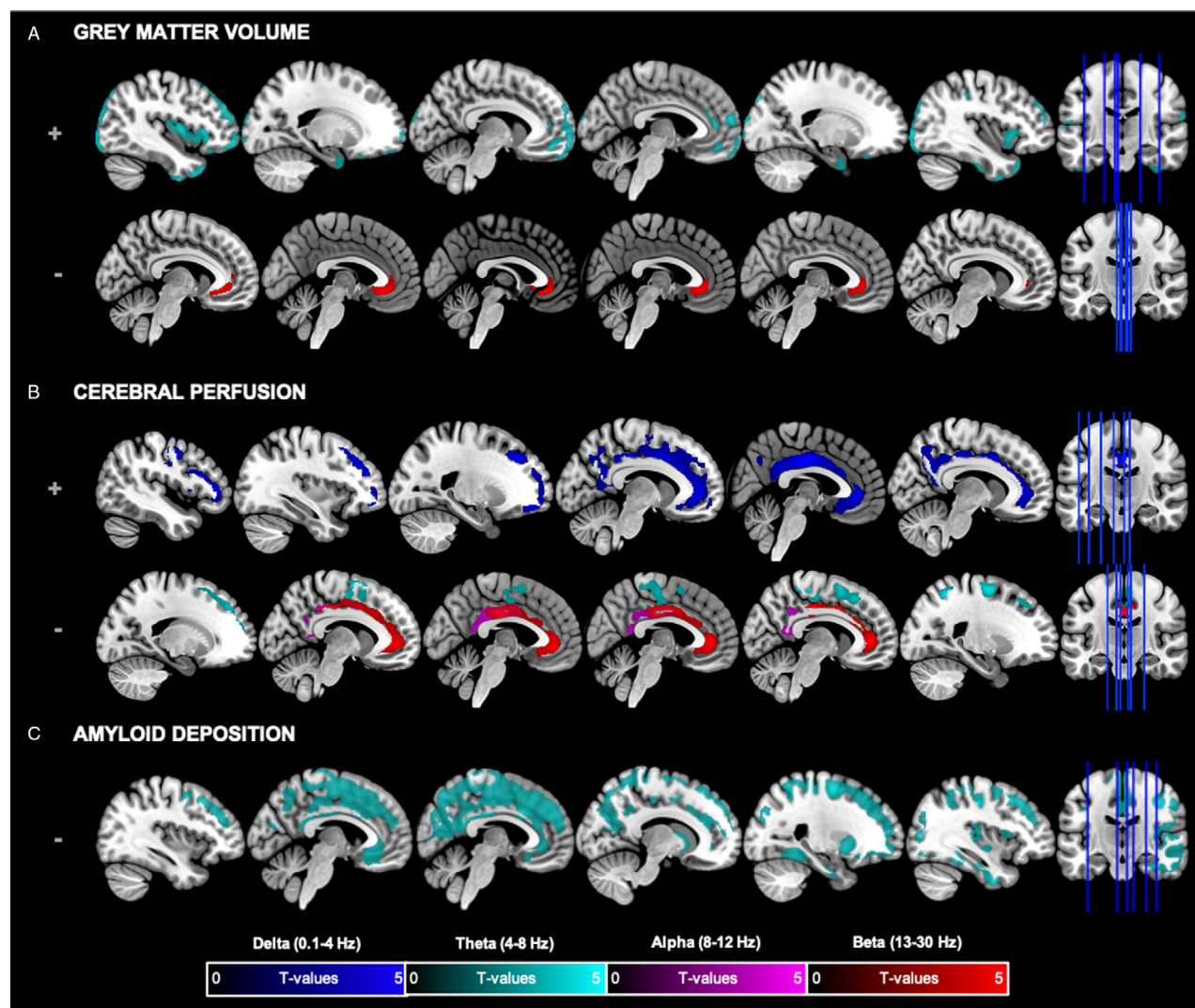


FIGURE 2: Multimodal neuroimaging correlates of rapid eye movement sleep normalized electroencephalographic spectral power in cognitively unimpaired older adults. Results of voxel-wise multiple regressions showing significant positive and/or negative associations between normalized spectral power in the delta (0.1–4 Hz), theta (4–8 Hz), alpha (8–12 Hz), and beta (13–30 Hz) frequency bands during rapid eye movement sleep, and (A) gray matter volume, (B) perfusion, and (C) amyloid burden. Results are presented at the $p < 0.005$ (uncorrected) level, combined with a family-wise error cluster-level correction. Analyses were controlled for age, sex, education, the Apnea-Hypopnea Index, and the apolipoprotein E allele $\epsilon 4$ status.

slowing ratio, and negatively associated with alpha and beta power on almost all derivations (Figs S2 and S3). In addition, theta power was negatively associated with brain perfusion in the same pattern of regions across all derivations, but at a lower statistical threshold with monopolar derivations (Fig S3). However, the positive association between theta power and GM on the one hand (Fig S2), and the negative association between theta power and amyloid deposition on the other hand (Fig S4), were not entirely replicated, even at a lower statistical threshold. For amyloid, we obtained a negative association between theta power measured on posterior electrodes (ie, parieto-occipital areas) and amyloid deposition in posterior cortical regions at the trend level. This association reached

significance when theta power was measured on the Pz derivation alone (Fig S4).

Finally, to investigate whether changes in theta and alpha power were specific to REM sleep, we checked whether theta and alpha power during REM sleep and eyes-closed quiet wakefulness were reciprocally associated. We observed that REM sleep theta power, measured on the CzPz derivation based on automatic scoring, was not significantly associated with eyes-closed resting-state theta power measured on several brain areas (Table S2). However, REM sleep alpha power was highly correlated with resting-state EEG alpha power. Taken together, this indicates that changes in theta power may be specific to REM sleep, whereas alterations of alpha power are not.

TABLE 3. Comparison of Rapid Eye Movement Sleep Theta Power between Amyloid Positive and Negative Individuals

Variables	Sum of squares	Degrees of freedom	F	p	η_p^2	Post-hoc analysis			
						Mean difference	95% CI	p _{Bonferroni}	Cohen's d
Amyloid status	0.507	1	9.092	0.003	0.074	0.166	0.051–0.31	0.003	0.713
<i>Covariates</i>									
Age	4.580×10^{-4}	1	0.008	0.928	7.271×10^{-5}	-	-	-	-
Sex	0.058	1	1.037	0.311	0.009	-	-	-	-
Education	0.003	1	0.052	0.820	4.610×10^{-4}	-	-	-	-
AHI	0.108	1	1.938	0.167	0.017	-	-	-	-
APOE4 status	0.055	1	0.986	0.323	0.009	-	-	-	-
<i>Residuals</i>	6.299	113	-	-	-	-	-	-	-

Results of the ANCOVA show significant differences in rapid eye movement sleep normalized theta power (4-8 Hz) in amyloid positive (n = 26) versus negative (n = 94) individuals, controlling for age, sex, education, the Apnea-Hypopnea Index, and apolipoprotein E allele $\epsilon 4$ status. Post-hoc results were obtained after bootstrapping (1,000 replicates). No other rapid eye movement sleep parameter was significantly different between the two groups. Abbreviations: AHI, Apnea-Hypopnea Index; APOE4, Apolipoprotein E allele $\epsilon 4$; CI, confidence interval; η_p^2 , partial eta-square.

Discussion

The goal of the present study was to establish the structural, functional, and molecular cerebral correlates of REM sleep macro- and microstructure in cognitively unimpaired older adults. Taken together, our results mainly show that lower perfusion in fronto-cingulate areas, extending to the precuneus in some cases, was robustly associated with decreased power in slow frequencies (ie, delta power and slowing ratio values), and greater power in faster frequencies (ie, alpha and beta power). To a lower extent, the same pattern of associations was obtained between GM volume and delta, alpha, and beta power. In addition, lower theta power was related to greater perfusion in superior fronto-parietal and lateral temporal areas, and marginally to lower GM volume in widespread fronto-temporal and parieto-occipital cortices (ie, the latter association was only obtained on the bipolar centro-parietal derivation). Finally, lower theta power, mainly on posterior derivations, was associated with greater diffuse amyloid load, and amyloid-positive participants showed lower theta power.

According to the latest recommendations for research of the NIA-AA,²⁶ individuals showing significant amyloid deposition, even in the absence of cognitive deficits, are considered engaged in the pathological path of Alzheimer's disease.²⁶ Investigating which factors may be

associated with amyloid pathology is thus crucial both to better understand AD pathophysiology and to motivate future therapeutical interventions. In older populations, several changes in sleep have previously been associated with increased amyloid pathology, such as reduced slow wave activity,²⁷⁻²⁹ sleep apnea,^{25, 30} or excessive daytime sleepiness.^{31, 32}

To the best of our knowledge, we show for the first time that lower REM sleep theta power may be associated with greater neocortical amyloid deposition in cognitively unimpaired older adults, although this link was restricted to posterior EEG derivations. This result is consistent with the marked alteration of REM sleep observed in patients with an Alzheimer's clinical syndrome of dementia,^{3, 4, 33} and with the fact that reduced REM sleep duration in asymptomatic older adults predicts the conversion to MCI and dementia.^{12, 13} Furthermore, it has been previously reported that amyloid-beta (A β) aggregation, reflected by lower levels of A β 42 in the cerebrospinal fluid, is associated with a reduction of REM sleep in patients with cognitive deficits ranging from subjective cognitive decline to MCI and dementia.³⁴ In these studies, the authors suggested that the degeneration of cholinergic structures in AD may deregulate the balance between the cholinergic and orexinergic systems, in favor of the overexpression of orexins, a wake-promoting neuropeptide.³⁴ Indeed, the

brainstem and basal forebrain are known to degenerate early in patients with MCI and asymptomatic amyloid-positive older adults.^{35–38} In addition, theta rhythm is known to have a hippocampal origin,³⁹ and to be modulated by cholinergic neurons of the basal forebrain and brainstem.¹ Thus, the degeneration of cholinergic regions is likely to underlie early changes in theta rhythms in individuals showing AD pathology. Importantly, we observed that theta power during REM sleep and eyes-closed quiet wakefulness were not reciprocally correlated, alterations in theta rhythm may be specific to REM sleep.

In patients with dementia, the degeneration of the cholinergic system is thought to underlie a global slowing in EEG rhythms during REM sleep, with an increased spectral power in low frequencies (delta and theta rhythms) and a decreased spectral power in fast frequencies (alpha and beta rhythms).^{11, 33} The use of anticholinesterase inhibitors, such as donepezil, rescuing cholinergic activity, has been shown to reduce REM sleep EEG slowing.⁴⁰ Thus, one would expect greater theta activity to be related with AD pathology. However, here, we rather show that lower theta power is associated with amyloid pathology and GM atrophy in cognitively unimpaired older adults, although the relationship with GM volume was more marginal. Some studies suggest that the cholinergic system may upregulate at early stages of the disease,^{41, 42} which might potentially explain why theta activity may be reduced or preserved in cognitively unimpaired older adults and in predementia stages.^{43–45}

Interestingly, our observations may also suggest that the associations between REM sleep microstructure and AD pathophysiology may be non-linear across the Alzheimer's continuum. Indeed, the present results reveal that reduced theta power is related to greater perfusion in fronto-temporo-parietal areas, where amyloid deposition is known to accumulate. Greater perfusion was also associated with EEG slowing in centro-temporo-parietal areas, which seemed to be mainly driven by increased delta activity during REM sleep, as all other frequency bands were negatively associated with brain perfusion. We hypothesize that greater perfusion in regions showing amyloid deposition and GM atrophy could potentially reflect a compensatory, but temporary, increase in neuronal activity, to help maintain cognition within the normal range. However, this phenomenon may have detrimental long-term effects, as neuronal hyperactivity is known to promote amyloid accumulation,⁴⁶ and might precede aberrant increases in theta activity observed at dementia stages.

Finally, the regions we found to be associated with theta activity are consistent with previous reports. Indeed, it has been shown that decreased metabolism and perfusion in temporo-parietal areas correlate with greater theta activity in patients with AD dementia.⁴⁷ Further studies

should confirm these hypotheses by including patients with amnesic MCI and dementia, and investigate the impact of cognitive status on these associations. Indeed, if REM sleep regulatory nuclei in the brainstem and basal forebrain are among the first regions to degenerate, patients with cognitive deficits may also present with more severe REM sleep alterations than cognitively unimpaired participants (ie, not restricted to REM sleep microstructure) due to the spreading of neurodegeneration.

The present study had some strengths, including analyses performed on a large sample of cognitively unimpaired individuals recruited in the community, who underwent both polysomnography and multimodal neuroimaging examinations, with analyses controlled for multiple confounders, such as sleep apnea or the APOE4 status. However, our study also presents limitations. Indeed, our sample was composed of highly-educated, cognitively unimpaired older adults, showing modest AD-like brain changes and REM sleep alterations, which may likely have impacted our results. Notably, the associations between theta power, amyloid deposition and GM volume were relatively marginal. Importantly, the negative association between theta power and amyloid deposition was restricted to parietal areas and not generalizable to all derivations, and no association was observed between amyloid deposition and REM sleep macrostructure. Therefore, at this stage of evidence, our data do not indicate that REM sleep theta power may represent a reliable and clinically-meaningful predictor of amyloid deposition at the individual level. Further studies will need to (i) replicate this observation in larger samples of cognitively unimpaired older adults, and (ii) extend these findings using cohorts including participants with cognitive deficits (ie, MCI or dementia), which may exhibit greater REM sleep alterations. In addition, our PSG set-up did not include leg electrodes, so periodic limb movements could not be assessed and controlled for. If none of the participants presented with a formal diagnosis or treatment for periodic limb movements, nor reported kicking or twitching limbs during sleep, the absence of periodic limb movements assessment is a limitation of our study, given that this disease is frequently underdiagnosed in older populations. Further studies combining both amyloid and tau-PET are required to determine their specific contribution to REM sleep alterations, and the links with cognitive performance will need to be investigated.

To conclude, we showed that alterations in REM sleep microstructure are associated with functional and structural changes in regions known to be sensitive to aging and AD, including fronto-cingulate areas and the precuneus. Our results also indicate that REM sleep theta rhythm may be sensitive to amyloid pathology, although this observation warrants replications in larger cohorts and clinical populations.

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Author Contributions

C.A., G.R., G.C., V.d.L.S., and D.V. contributed to the conception and design of the study. C.A., P.C., S.R., E.K., E.T., V.O., G.L.D., S.S., B.L., and F.M. contributed to the acquisition and analysis of data. C.A., G.R., and G.C. contributed to manuscript drafting or figures preparation. Members of the Medit-Ageing Research Group and their respective contributions are detailed in Table S3.

Potential Conflicts of Interest

Nothing to report.

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