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Original Article

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Sleep arousals are associated with the polygenic risk for developing Alzheimer's disease and with cognitive change in healthy late middle-aged individuals

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Abstract

Study objectives: Sleep disturbances are increasingly recognized as early features of Alzheimer's disease (AD) neuropathology. Specifically, spontaneous arousals during sleep have been associated with the burden of Amyloid beta in the brain of healthy late middle-aged individuals. However, it remains unclear whether heterogeneity of arousals relates to genetic risk for AD in younger adults or to cognitive change later in life. Here, we evaluated the association between arousals, polygenic risk scores (PRS) for AD, and cognitive performance and change in healthy young and late-middle-aged individuals.
Methods: We classified spontaneous arousals using in-lab EEG sleep recordings in 453 younger individuals (22 ± 2.7y; 49 women) and 87 late middle-aged individuals (59.3 ± 5.3y; 59 women) based on their association with sleep stage transitions and changes in muscle tone. We examined the associations between arousal types, AD-PRS, baseline cognitive performance and, in late middle-aged individuals, cognitive change over 2 and 7 years.
Results: The prevalence of arousals associated with sleep stage transition was higher in late middle-aged vs. younger individuals. In late middle-aged but not younger individuals, transition arousals with muscle tone increases correlated with lower AD-PRS, better attentional performance and lower memory change over follow-ups, whereas transition arousals without muscle tone increases were linked to higher AD-PRS, poorer baseline attention, and greater memory change across follow-up periods.
Conclusions: The heterogeneity in spontaneous arousals during sleep may reflect their physiological intensity or underlying neural activation, and may indicate vulnerability to AD in late middle-aged individuals. The findings may help identify early markers of neurodegenerative risk.

Key words: Alzheimer's disease; arousal; polygenic risk score; sleep

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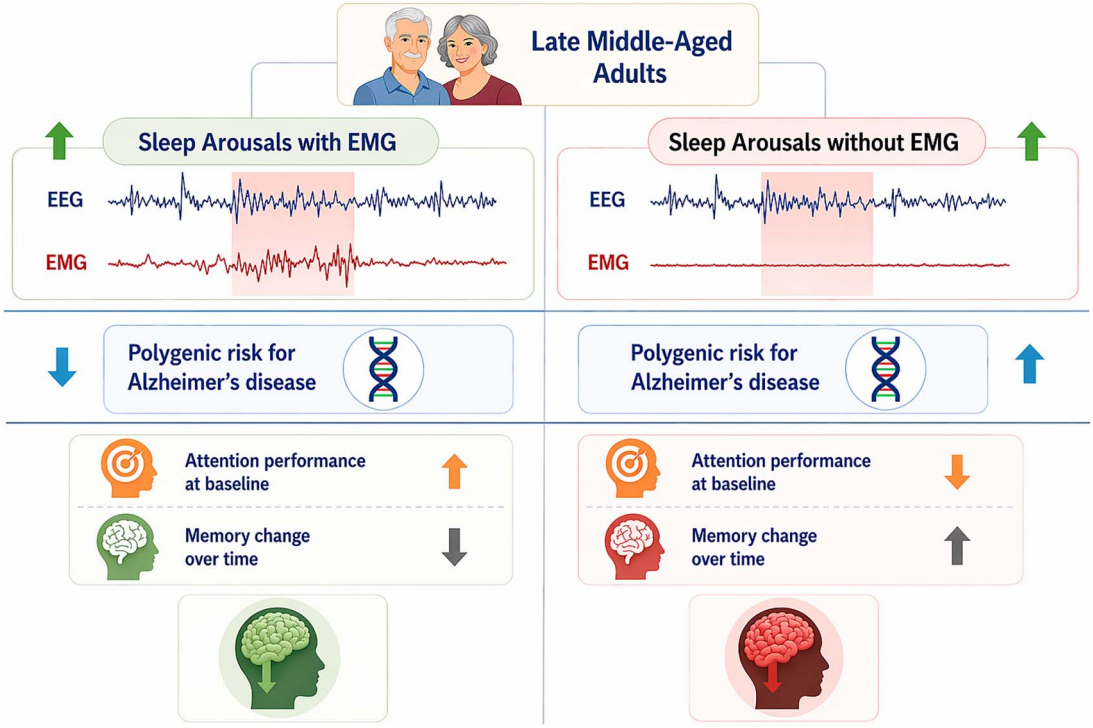
Graphical Abstract



Statement of Significance

Sleep arousals are often considered disruptive, yet their physiological diversity may provide key insights into brain health. This study shows that distinct subtypes of spontaneous sleep arousals are differentially associated with genetic vulnerability to Alzheimer's disease (AD) and future cognitive change in healthy late middle-aged adults. Arousals linked to sleep stage transitions without muscle activation were related to higher polygenic risk for AD and greater memory change, whereas those with muscle tone increases showed the opposite pattern. These results suggest that subtle variations in sleep microstructure reflect neurobiological vulnerability to AD before symptoms appear. Identifying electrophysiological markers tied to genetic risk and cognition highlights the potential of sleep-based biomarkers for early neurodegenerative detection.

Introduction

Individuals with Alzheimer's disease (AD)—who are characterized by cognitive deficits, particularly in memory—also show decreased total sleep time, sleep efficiency, N3 sleep and REM sleep, and increased number of awakenings compared with controls [1] and these changes have been correlated with the severity of their cognitive impairment [1]. Alterations in sleep are also related to the hallmarks of AD pathogenesis (i.e. Amyloid-Beta (Aβ) and tau accumulation) in healthy individuals, prior to any clinical manifestations of the disease [2]. In addition, sleep disturbances have been linked to the future risk of both cognitive decline and AD pathology [3]. Notably, individuals at higher risk for AD exhibit more frequent nocturnal arousals—transient/abrupt shift of EEG frequency not associated with full awakenings– than those at lower risk [4]. Additionally, arousals related to movement are associated with increased risk of developing AD and cognitive decline [5]. In cognitively healthy late middle-aged individuals, sleep arousals were associated with early Aβ burden depending on whether arousals were linked to sleep stage transitions (T+/T–) and to detectable increase in muscle tone (M+/M–) [6]. Specifically, T + M– arousals were associated with higher Aβ burden while T–M+ arousals were linked to lower Aβ accumulation and better cognitive performance, particularly in the attentional domain.

Recent studies in rodents indicate that the heterogeneity in arousals may be related to the activity of locus coeruleus (LC), which is among the first sites of tau protein aggregation in the brain [7] and is likely to contribute to the alteration in sleep regulation found in preclinical AD [8]. Stronger activity of the LC during slow wave sleep would be associated with increased arousal density while lower level of LC activation would lead to decreased arousal density [9]. The heterogeneity in sleep arousal appears therefore to be directly related to a key structure for sleep and for AD, and as a result, may constitute a marker of the vulnerability for AD. How early this vulnerability can be detected through arousals and whether it bears prediction for future cognitive change is unknown.

Aβ protein accumulation in the brain typically begins to increase over the sixth decade in humans [10] and is therefore not a useful biomarker for assessing AD risk in younger individuals. In contrast, genetic approaches offer unique tools to assess the variability for the risk for complex diseases, including AD. AD is recognized to be polygenic [11, 12] and genome-wide association studies (GWAS) have identified over 70 genes which are associated with AD [13]. Due to the polygenic nature of AD, polygenic risk

scores (PRS)—which aggregate the risks associated with common genetic variants—have proven to be an effective method for assessing AD risk. Studies have demonstrated that PRS can distinguish between AD cases and healthy controls, reaching a prediction accuracy up to between 75% and 84% [11, 14]. The PRS would be particularly useful for studying asymptomatic individuals of any age for studying AD risk long before classical pathological hallmarks are detectable [15]. Importantly, PRS may capture a different dimension of AD risk than Aβ burden. In cognitively healthy individuals, PRS has generally not been associated with baseline Aβ levels but predicts longitudinal increases in Aβ over time [16–18].

In this study, we examined the associations between PRS for AD, different types of sleep arousals—classified by the presence of muscle tone increase and sleep stage transitions— and cognitive performance in a relatively large sample of cognitively unimpaired individuals (N = 540). Our aim was to determine whether arousal types previously associated with Aβ accumulation were also linked to PRS for AD in late middle-aged individuals aged 50 to 70y and in much younger adults aged 18 to 31y. We anticipated that more T + M− and T−M+ arousals would be linked, respectively, to higher and lower PRS values. We further assess their link to cognitive performance and, in late-middle aged individuals, with cognitive change over 2y and 7y follow-up periods. We hypothesize that, in late middle-aged individuals, T−M+ arousals would be associated with better attentional performance in the baseline (as observed in our previous study [6]) while they would be associated with reduced memory change at follow-up, given that memory deterioration is a core feature of AD.

Materials and methods

Participants

A total of 540 healthy participants [453 younger individuals aged 18–31 years (22.04 ± 2.69y; 49 women;) and 87 late middle-aged individuals aged 50-69 years (59.28 ± 5.3y; 59 women)] part of different multi-modal studies were included in the present analyses (Table 1) [6, 19]. Data from young adults were collected across six different studies while data from late middle-aged individuals were collected as part of a single study. One of the studies in young adults contributed the majority of the sample, and including 357 Caucasian young men (to reduce variability as the study was originally designed as a genetic study). This means that vast majority of our young sample consist of men (~90%) while a minority of men constituted the late-middle aged sample (~32%). This constitutes an inherent limitation of the present study, even if sex is included as covariates in all statistical analyses.

All studies collected quantitative sleep parameters and blood samples or buccal swabs to assess PRS for AD (Figure 1). Extensive cognitive evaluation was conducted in the largest studies of the younger dataset (N = 357) and in all late middle-aged individuals, while the later part of the sample also completed follow-up cognitive assessment after 2 and 7 years.

In all studies, participants were excluded if they had a body mass index (BMI) less than 18 and greater than 27 kg/m^2 (greater than 29 kg/m^2 for late middle-aged individuals), a history of psychiatric disorders or severe brain injury, documented/diagnosed sleep pathologies such as insomnia and REM behavior disorder, substance addiction, chronic use of medication affecting the central nervous system, smoking, excessive alcohol consumption (more than 14 units per week), high caffeine intake (more than three cups per day for younger participants, or more than five cups per day for older participants), shift work within the past 6 months, trans-meridian travel within the preceding two months, moderate to severe subjective anxiety or depression, as indicated by a score greater than 16 on the Beck Anxiety Inventory [20] or greater than 19 on the Beck Depression Inventory-II [21]. Poor sleep quality (Pittsburgh Sleep Quality Index score > 7) [22], excessive daytime sleepiness (Epworth Sleepiness Scale score > 14) [23], significant sleep apnea (apnea-hypopnea index >15 events per hour), and parasomnia as determined during an in-laboratory screening night using polysomnography (PSG) and based on the 2017 American Academy of Sleep Medicine (AASM) criteria (version 2.4) [24], also led to exclusion. Old individuals were also excluded if they had clinical symptoms of cognitive impairment (dementia rating scale <130; mini mental state examination <27).

All study procedures were approved by the Ethics Committee of the Faculty of Medicine at the University of Liège (Belgium). Written informed consent was obtained from all participants prior to inclusion and participants received financial compensation for their participation. The study was conducted in accordance with the Declaration of Helsinki and the World Medical Association's International Code of Medical Ethics.

Protocol

Data from young adults were obtained from six separate studies [19, 25–30], whereas data from late middle-aged individuals were derived from a single study [6] and we detail here only those aspects that are relevant for the present study.

For the majority of young participants (N = 357 young men), for three weeks prior to the in-lab experiment, participants adhered to a regular sleep schedule based on their usual sleep times (within ±30 minutes for the first two weeks and within ±15 minutes for the final week), verified through actigraphy data (Actiwatch 4, CamNtech, Cambridge, UK). Prior to the experiment, participants completed a urine drug screen (Multipanel Drug Test, SureScreen Diagnostics Ltd) and an adaptation night in the laboratory aligned with their habitual sleep–wake schedule. During this night, full PSG recordings were performed to screen for sleep-related breathing disorders and periodic limb movements. On the second day, participants left the lab in the morning and were instructed not to nap during the day, with adherence confirmed via actigraphy. They returned to the lab in the evening, 3.5 hours before their scheduled lights-off time, and had a baseline night of sleep recorded in complete darkness. The timing of this night was centered on the average sleep midpoint from the preceding week. Older participants and the rest of young participants (N = 96 young individuals) first completed an in-laboratory adaptation night to get familiar with the lab environment and allow for detection of any sleep disturbances. For the seven days before the baseline night, participants adhered to a regular sleep–wake schedule (within ±30 minutes) according to their habitual bed and wake-up times, verified using sleep diaries and wrist actigraphy (Actiwatch©, Cambridge Neurotechnology, UK). All participants were instructed to avoid daytime naps and to refrain from unusually intense physical activity during the final three days of the fixed sleep schedule. Their habitual sleep was then recorded in complete darkness using EEG. The current study focuses exclusively on the baseline night of sleep in both younger and late middle-aged individuals.

EEG acquisitions

Polysomnographic sleep data of young participants were acquired using either V-Amp 16 amplifiers (Brain Products, Germany) [19, 25–27], a QuickAmp-72 (Brain Products, Germany) [28] or a N7000 amplifiers (EMBLA, Natus Medical Incorporated, Planegg,

Table 1. Characteristics of the study sample

Characteristic	Young Mean (SD)	Late middle-aged—Mean (SD)	*p*-value
Sample size (N)	453	87	-
Sex (Men)	89.37%	32.18%	<0.0001
Age (years)	22.04 (2.69)	59.28 (5.35)	<0.0001
Body Mass Index (BMI) ($kg*m^{-2}$)	22.15 (2.31)	24.83 (2.85)	<0.0001
Anxiety	2.56 (2.94)	2.78 (2.86)	0.5
Depression	2.96 (3.53)	5.52 (4.55)	<0.0001
Sleep Quality	3.46 (1.76)	4.63 (2.59)	0.0001
Daytime sleepiness	5.94 (3.54)	6.12 (4.00)	0.67
Chronotype	50.10 (8.26)	53.68 (7.88)	0.0003
Total sleep time (min)	451.4 (41.53)	392.3 (40.90)	<0.0001
T + M+ arousals number	12.92 (5.53)	15.16 (6.57)	0.004
T + M− arousals number	9.50 (5.21)	13.59 (7.29)	<0.0001
T−M+ arousals number	106.2 (33.12)	55.60 (25.88)	<0.0001
T−M− arousals number	127.9 (45.59)	95.71 (44.08)	<0.0001
T + M+ arousals density (number/h)	1.72 (0.72)	2.33 (1.02)	<0.0001
T + M− arousals density (number/h)	1.27 (0.72)	2.11 (1.19)	<0.0001
T−M+ arousals density (number/h)	14.16 (4.17)	8.54 (3.98)	<0.0001
T−M− arousals density (number/h)	17.11 (6.07)	14.59 (6.34)	0.0005
Aβ burden (centiloid units)	-	−5.19 (8.68)	-

The *p*-values shown in the table correspond to two-sample t-tests except for sex that were compared using a Chi-square test. Sleep quality was assessed by the Pittsburgh Sleep Quality index (PSQI) [22]. Daytime sleepiness was measured by the Epworth Sleepiness Scale [23], Chronotype was assessed by the Morningness–Eveningness Questionnaire (MEQ) [84]. Anxiety was estimated by the Beck Anxiety Inventory [20] and Depression was estimated by the 21-item Beck Depression Inventory II [21]; Total sleep time was extracted from polysomnography recordings. All late–middle-aged individuals and the largest young adult dataset (N = 357) had measures of anxiety, depression, sleep quality, daytime sleepiness, and chronotype. These measures were not available for the remaining young adult datasets (N = 96). Women were largely under-represented in the younger group, due to the selection criteria of one of the studies composing the sample (see methods), while they represent a large majority of the late-middle-aged group. This constitutes an inherent limitation of the present study, even if sex is included as covariates in all statistical analyses. SD: Standard deviation.

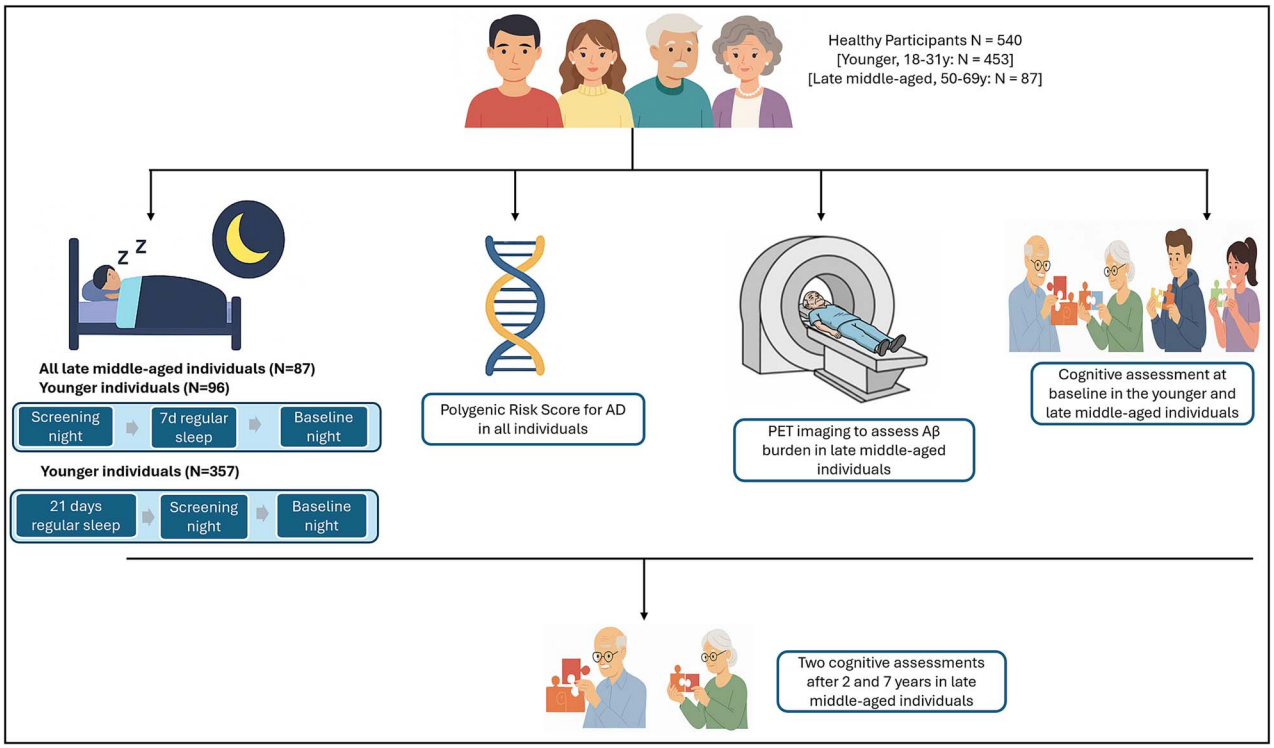


Figure 1. Overview of the study design. The habitual sleep of young and older adults was recorded in a laboratory setting to identify sleep arousals. Blood samples or buccal swabs were then collected to measure Alzheimer's disease polygenic risk scores (PRS), and PET imaging was conducted in late middle-aged individuals to assess amyloid-beta (Aβ) burden. Participants subsequently completed a battery of cognitive tests. A subset of late middle-aged adults who initially participated in the study also returned for follow-up cognitive assessments after 2 and 7 years.

Germany) [29]. The electrode montage consisted of at least nine EEG channels (F3, Fz, F4, C3, Cz, C4, Pz, O1, O2; reference to right mastoid), two bipolar EOGs, two bipolar EMGs, and two bipolar ECGs. For late-midlife group, participants sleep EEG data were acquired using N7000 amplifiers (EMBLA, Natus Medical Incorporated, Planegg, Germany) with 10 EEG channels (F3, Fz, F4, C3, Cz, C4, P3, Pz, P4, O1, O2), two bipolar EOGs, two bipolar EMGs, and two bipolar ECGs [6]. For both age groups, acquisition

on the screening night of sleep also included respiration belts, oximeter and nasal flow, two electrodes on one leg, but included only Fz, C3, Cz, Pz, Oz, and A1 channels. Baseline EEG data were re-referenced off-line to average mastoids.

Arousal detection

Sleep stage scoring and arousal detection were carried out in separate steps by two independent algorithms. Sleep stage scoring was performed in 30-second windows using a validated algorithm (ASEEGA, Physip) [31, 32]. Automatic arousal detection was then computed as it is objective and reproducible [33]. We used an individually tailored validated algorithm based on the AASM definition [24] of arousal but without using sleep stage information. Automatic scorings were visually inspected following computation.

In brief, arousal detection is performed over all electrodes on whole-night recordings split into 1-second epochs in two successive steps computed over the power in the broad-α (7–13 Hz), β (16–30 Hz), and lower-θ (3–7 Hz) frequency bands, excluding the σ band (11–16 Hz)—i.e. corresponding to frequency of sleep spindles—which cannot be considered as arousals. A fixed threshold is first applied to detect abnormal EEG activity relatively to the whole-night recording: any 1-second epoch with power in any of the 3 frequency bands higher than the whole-night median value in each frequency band is considered as a potential arousal. The second step adapts the threshold to account for the specific EEG background activity in a shorter time window. A specific threshold is computed for each 30-second window: all 1-second epochs without concomitant EMG tone increase are selected, as well as the first ten 1-second epochs without EMG increase before and after the 30-second window being evaluated; threshold of each frequency band consists in the median power over the selected 1-second epochs. Events composed of at least three consecutive 1-second epochs with changes in EEG frequencies higher than twice the local median and 1 median of the whole recording for that frequency band were considered as arousals. For detailed explanations on the method, see ref. [33].

As previously [6], we classified arousals according to two criteria, which we considered relevant to both research settings and clinical practice. The first criterion addressed whether arousals were associated with a sleep stage transition (T+), defined as occurring within 15 seconds on either side of a sleep stage transition—that is, during the final 15 seconds of the 30-second epoch preceding a stage change or the first 15 seconds of the subsequent epoch scored as a different stage. Sleep staging is performed in 30-second epochs according to AASM criteria, and stage transitions are defined at epoch boundaries [24]. Because a transition may begin within the latter part of the preceding epoch and stabilize during the early part of the subsequent epoch, a $\pm$15-second window (i.e. half an epoch on either side of the boundary) allows identification of arousals temporally linked to the transition while limiting inclusion of events too distant to be plausibly related. Arousals not meeting this criterion were classified as T–. The second criterion considered the concomitant increase in EMG tone (M+) or its absence (M–). We previously showed that using the T+/T– and M+/M– criteria led to significant power differences between arousal types within the beta and theta bands while the alpha band was not significantly different [6]. Although T+ and T– arousals were significantly different, the most important feature was that M– arousals presented, respectively, more and less power in the lower theta and beta bands compared with M+ arousals.

We used the absolute number of arousals rather than arousal density to capture the total arousal burden across the night, which was our primary variable of interest. Normalizing to total sleep time (TST) can obscure meaningful differences when TST varies, specially between young and late middle-aged individuals. By including TST as a covariate, we were able to assess the independent contribution of arousal events to genetic risk for AD and cognitive outcomes, focusing on whether a greater overall arousal load—regardless of sleep duration—was linked to neurodegenerative vulnerability. For completeness, we nevertheless report as supplementary results the analysis outcomes when using arousal density rather than number.

Genotyping, quality control, and imputation

Blood samples and buccal swabs were collected and stored at −20°C within few hours until DNA extraction. The genotyping was performed using the Illumina Infinium OmniExpress-24 BeadChip arrays (Illumina, San Diego, CA) based on Human Build 37 (GRCh37). All the study participants were European ancestry. Established quality control (QC) procedure was performed using PLINK [34] (http://zzz.bwh.harvard.edu/plink/). In brief, the SNPs were excluded as follows: >10% missing genotypes, <95% call rate, minor allele frequency below 0.01, out of Hardy–Weinberg equilibrium (p-value <10-4 for the Hardy–Weinberg test). SNPs on 23rd chromosome as well as ambiguous SNPs (A-T, T-A, C-G, G-C) were excluded as well. The data was matched for deviation with European ancestry using 1000 Genomes Project dataset (1KGP, https://www.internationalgenome.org). Imputation was conducted using the Sanger imputation server (https://imputation.sanger.ac.uk/) based on the Haplotype Reference Consortium (r1.1) as reference panel and using Eagle2.4 pre-phasing algorithm. The detailed data processing and analysis for young and late middle-aged sub-sample is as described previously in [19, 30]. We finally ended with 7 165 614 SNPs common to all participants.

PRS calculation

PRS analyses can be used to assess the genetic liability of an individual for a phenotype by calculating the weighted sum of risk alleles effect size identified in genome-wide association studies. In the current study, we calculated a PRS for AD for each participant using summary statistics from the recent GWAS meta-analysis of European ancestry [35]. This approach differed from the PRS calculation originally applied in part of the young individuals' dataset [19]. The standardization and QC of GWAS summary statistics was performed by MungeSumstats, a Bioconductor R package [36]. In the process, the summary statistics was pruned to align reference alleles to build GRCh37, remove multiallelic variants, and adjust weights for the appropriate reference alleles. The PRS was then generated using SBayesR algorithm implemented in GCTB software. The approach assumes that the SNP effects are drawn from mixtures of distributions with the key metrics defining these genetic architectures estimated through Bayesian frameworks. To derive PRSs from GWAS effect estimates of SNPs, SBayesR essentially uses Bayesian linear mixed model and the reference linkage disequilibrium (LD) correlation matrix. In our analysis, we used banded LD matrix to improve the prediction accuracy as recommended by the authors of GCTB. We used p-value thresholding after PRS computation through PLINK to include only the SNPs reaching stringent GWAS significance (p-value $<1x10^{-8}$) to restrict the number of genetic markers to a minimum.

MRI data

MRI data were used in late middle-aged individuals in order to determine the region of interest used for extraction of Aβ burden value based on PET images, as fully described in [6]. Quantitative

multiparametric MRI acquisition was performed on a 3-Tesla MR scanner (Siemens MAGNETOM Prisma, Siemens Healthineers) to get a magnetization transfer–weighted (MT-weighted) contrast, based on multi-echo 3D fast low angle shot at 1 mm isotropic resolution [37]. MRI multiparameter maps were processed with the hMRI toolbox [38] (http://hmri.info) and SPM12 (Welcome Trust Centre for Neuroimaging, London, United Kingdom) to obtain a quantitative MT map and segmented images (gray matter, white matter, CSF), normalized to the standard MNI space using unified segmentation [39].

PET scan

Aβ PET imaging was performed only in late middle-aged individuals as fully described in [6]. Younger individuals typically have no detectable Aβ accumulation such that it would be unethical to expose them to radiation. Aβ PET imaging was performed using [^{18}F]Flutemetamol, except for three volunteers for which [^{18}F]Florbetapir was used as fully described in [6]. PET scans were performed on an ECAT EXACT+ HR scanner (Siemens). Individual PET average images were manually reoriented according to MT-weighted structural MRI volumes and coregistered to the individual space structural MT map. Flow-field deformation parameters obtained from DARTEL spatial normalization of the MT maps were applied to averaged coregistered PET images [40]. We did not provide correction for partial volume effect, as this type of PET processing was not included in Centiloid scaling pipeline [41]. Volumes of interest were determined using the automated anatomical labeling (AAL) atlas [42]. Standardized uptake value ratio (SUVR) was computed using the whole cerebellum as a reference region[41]. As images were acquired using two different radioligands, their SUVR values were converted into Centiloid units [41]. Aβ burden was averaged over a composite mask covering the previously reported earliest aggregation sites for Aβ pathology [43]—frontal medial cortex and basal part of temporal lobe (fusiform and inferior temporal gyri). According to the study by Krasny et al. [44], the amyloid PET positivity threshold was set at Centiloid 20.

Cognitive assessment

Cognitive assessments were conducted at baseline in part of the younger sample (N = 357, as part of the same unique study) while they were administered at baseline and at 2 years (mean 767 ± 54 days) and 7y (mean 2647 ± 98 days) follow-up in the late middle-aged sample. Since significant associations between arousals and PRS for AD were observed only in the late middle-aged individuals (see Results), the following description focuses on the cognitive data from this group (the tests used for the young individuals were slightly different—for details see Figure S4). At the first time point, a cognitive battery of neuropsychological tasks was carried out in two sessions, while well rested. A first session of ∼1 hour was performed in the afternoon prior to the sleep assessment, approximately 7.5 hours before habitual bedtime, and a second session of ∼1.5 hours was performed on another day (between 12 and 6 hours prior to habitual bedtime). From those two sessions, three domain-specific composites scores were computed for the memory, executive function, and attentional domains, and they consisted of the standardized (z- scores) sum of the standardized domain-specific scores, where higher values indicate better performance.

The first session comprised (a) mnemonic similarity task (MST) [45]; (b) category verbal fluency (letter and animals) [46]; (c) digit symbol substitution task (DSST) [47]; and (d) visual N-back task (1and 3-back variants) [48]. The second session of ∼1.5 hours was performed on another day (between 12 and 6 hours prior to habitual bedtime) and comprised (a) inverse order digit span task [47]; (b) free and cued selective reminding test (FCSRT) [49]; (c) trail making test (TMT) [50], and (d) and logical memory from Wechsler memory test (MEM-III) [51]. The memory score consisted of the FCSRT (sum of all four free recalls), the recognition memory score from the MST, and delayed recall from the logical memory of MEM-III test. The executive function score included verbal fluency tests (letter and animals score for 2 minutes), inverse order digit span, TMT (part B minus part A), and N-back (3-back variant). The attentional score comprised the DSST, TMT (part A), and N-back (1-back variant).

The second assessment took place two years after the first, and the third was conducted seven years after the initial assessment. At these two time points, the same procedure and tests were used to compute the three composite scores. However, all individuals did not participate in the follow-up assessments and only 66 old individuals participated in the second time point assessment and 64 old individuals participated in the third time point assessment (with 48 individuals common to both follow-ups). The substantial dropout observed at the second cognitive assessment was primarily due to the COVID-19 crisis. For each composite score, cognitive change was computed as the baseline performance minus the follow-up performance, divided by the baseline performance, so that a higher score indicates a higher change over time.

Statistics

Statistical analyses were performed in the R environment (version 4.1.3) (R Development Core Team, 2017) using generalized additive models for location scale and shape (GAMLSS) [52, 53] based on the distribution of the dependent variables. GAMLSS offers a wide variety of family of distributions for model fitting [54–56] and provides more flexibility than a generalized linear model (GLM) or generalized additive model (GAM). Outliers among all the assessed metrics lying beyond four times the standard deviation were removed from the analysis (the final number of individuals included in each analysis is reported in each table). Our primary objective was assessed in the first GAMLSS including the total number of arousals as the dependent variable and a four-way interaction among PRS for AD, transition status, EMG status, and age group as the main predictor. Since it included all variables of interest in the same model, it was not corrected for multiple comparisons and significance was set at $p < 0.05$. Additionally, all related three-way and two-way interaction terms were included. Sex and TST were added as covariates in the model and subjects were treated as random factors. This model yielded significant association between the four-way interaction and total number of arousals (see Results). Hence, a subsequent post-hoc GAMLSS analysis was used to specify which type of arousal was associated with the PRS for AD and in which age group this association was observed. This second GAMLSS included the PRS for AD as the dependent variable and four two-way interactions between each type of arousal (T + M+, T + M−, T−M+, T−M− arousal) and age group as independent variables. Sex and TST were added as covariates.

The association between arousal and cognition in each time point was assessed in models including each composite score as a dependent variable together with two types of arousals related to the PRS for AD (T + M+ and T + M− arousals) as independent variables as well as age, sex, education and TST as covariates (i.e. nine models in total, three per time point). Given the limited sample size of the follow up analyses, models yielding p values less than

0.05 were considered significant (i.e. not corrected for multiple comparisons). For completeness, we report whether significant associations would have survived false discovery rate (FDR) correction (following Benjamini–Hochberg procedure; $p < 0.005$ for rank 1; $p < 0.011$ for rank 2; $p < 0.016$ for rank 3, $p < 0.022$ for rank 4, $p < 0.027$ for rank 5, $p < 0.033$ for rank 6, $p < 0.038$ for rank 7, $p < 0.044$ for rank 8, $p < 0.050$ for rank 9). As an exploratory analysis, in similar models, we also considered the association between recognition memory score of MST in all three time points and T + M+ and T + M− arousals as this memory task is highly sensitive to early signs of cognitive change [45, 57].

We computed a priori sensitivity analysis to get an indication of the minimum detectable effect size in our main analyses given our sample size. According to G*Power 3 (version 3.1.9.4) [58], taking into account a power of .8, an error rate α of .05), a sample size of 540 allowed us to detect small effect sizes $f^2 > .023$ (confidence interval: .008 –.063; $R^2 > .022$, R^2 confidence interval: .008 –.059) within a linear multiple regression framework including four predictors (Arousals number, EMG status, transition status, age group) and two covariates (sex and TST). We also computed a similar prior sensitivity analysis for the younger and late middle-aged groups separately, taking into account a power of .8, an error rate α of .05, a sample size of 453 young individuals allowed us to detect small effect sizes $f^2 = .032$ (confidence interval: .02 – .09; $R^2 > .031$, R^2 confidence interval: .015 –.084); while a sample size of 87 late middle-aged adults allowed us to detect medium-large effect sizes $f^2 = .187$ (confidence interval: .09 –.56; $R^2 > .157$, R^2 confidence interval: .082 –.358). There is limited published data on the precise effect sizes for quantitative sleep metrics and genetic associations in late middle-aged adults. However, the recent studies have used similar sample size in the analysis [59] providing further support to the validity of our study results.

Results

Comparing each type of arousals between the two age groups, we observed that the number of arousals associated with a sleep stage transition, i.e. T + M+ and T + M−, significantly increased in the late middle-aged individuals ($\mathbf{p < 0.005}$), while those not associated with a stage transition, i.e. T−M− & T−M+, decreased in this age group ($\mathbf{p < 0.0001}$, Table 1). Besides, age, body mass index (BMI), depression, sleep quality, and chronotype scores were significantly higher in the late middle-aged group compared to the younger group, whereas the younger group had a significantly longer TST.

Arousal heterogeneity reflects different associations with PRS for AD

Prior to seeking association between PRS and sleep metrics, we first assessed whether early PET Aβ burden and PRS for AD were correlated in late middle-aged individuals, since only this group underwent Aβ assessment. We did not find a significant association between Aβ levels and PRS for AD (Figure 2A; Sperman's $r = -.05$; $p = .64$, and Sperman's $r = -.08$; $p = .43$, when including the three Aβ-positive individuals—i.e. with centiloid values >20- of the sample, Figure S1). To assess whether outcome could be different in the older part of our sample, we computed additional correlation analyses between Aβ burden and PRS for AD, separately in individuals below and above the median age within the late middle-aged group. As for the entire sample, this did not lead to significant associations (N = 43; Sperman's $r = -0.03$; $p = .85$ for the older half of the middle-aged group and N = 45; Sperman's $r = -0.07$; $p = .62$ for the younger half of the middle-aged group), further supporting that PRS for AD is relatively independent of Aβ accumulation and grasps, at least in part, a distinct aspect of the AD-related risk as previously suggested by others [16–18].

We then targeted our primary objective in a GAMLSS assessing whether arousal number, as dependent variable, was associated with PRS for AD across the different types of arousals (T+/T− & M+/M−), including age group, sex, and TST as covariates. The model yielded a significant 4-way interaction between PRS for AD, arousal transition and EMG statuses and age group ($\beta = 0.02$; $t = 2.52$; $\mathbf{p = 0.01}$), indicating that the association between arousals and PRS varied based on transition and EMG status as well as age group. Besides, the statistical model yielded significant main effects of transition status, age group, sex, TST, as well as significant two-way interactions between transition and EMG status, transition status and age group, EMG status and age group, and three-way interactions between PRS, and EMG and transition status, and between EMG and transition status and age groups (Table 2). Adding the variables that significantly differed between the two age groups (i.e. BMI, depression, sleep quality and chronotype) as a covariate to this primary GAMLSS did not change the statistical outputs although all these variables were significantly associated with number of arousals, indicating that the observed associations were unlikely to be driven by these group differences (Table S1).

To understand what was driving the 4-way interaction, we computed a post hoc GAMLSS with PRS, as a dependent variable, and the interaction between the number of each arousal type and age group as covariate, including their interactions (4 two-way interactions) regressing out TST and sex. The model yielded a significant link between PRS and the interaction between T + M− arousals and age group ($\beta = -0.32$; $t = -2.40$; $\mathbf{p = .02}$) as well as a nominally significant link with the interaction between T + M+ arousals and age group ($\beta = 0.29$; $t = 1.96$; $\mathbf{p = .05}$) on top of the main effect of T + M+ and T + M− arousals number (Figure 2B-C, Table 3). The other associations, including the association between PRS and interaction between T− M− and T−M+ arousals and age group, were not significant (Figure 2D-E, Table 3; statistical outcomes were the same when using arousal density rather than absolute number Table S2). Post hoc contrasts showed that there is a negative association between PRS and T + M+ arousal number ($\beta = -.30$; $t = -2.57$; $\mathbf{p = .01}$) as well as a positive association between T + M− arousals ($\beta = .29$; $t = 3.09$; $\mathbf{p = .003}$) in the late middle-aged group but not the younger group ($\beta = .01$; $t = .22$; $p = .83$ and $\beta = .02$; $t = .29$; $p = .77$ respectively; Figure 2B, C). Hence, the post hoc analysis indicates that the significant 4-way interaction found in the primary analysis is driven by the association between PRS and both T + M+ arousals and T + M− arousals in the late middle-aged group.

Given the sex imbalance of our samples (particularly in the young sample), we further assessed sex differences and could not find evidence that they were contributing to our finding (Table S3; Figures S2–S3). Besides, we could not find indications that the association between PRS and T + M+ arousals in late middle-aged individuals was driven by arousals detected during REM or NREM sleep (according to ASSM criteria, arousals must be associated with muscle tone increase during REM, such that all M− arousal were only detected during NREM) but rather found that it is the total number of T + M+ in REM and NREM that was associated with the PRS for AD (Table S4).

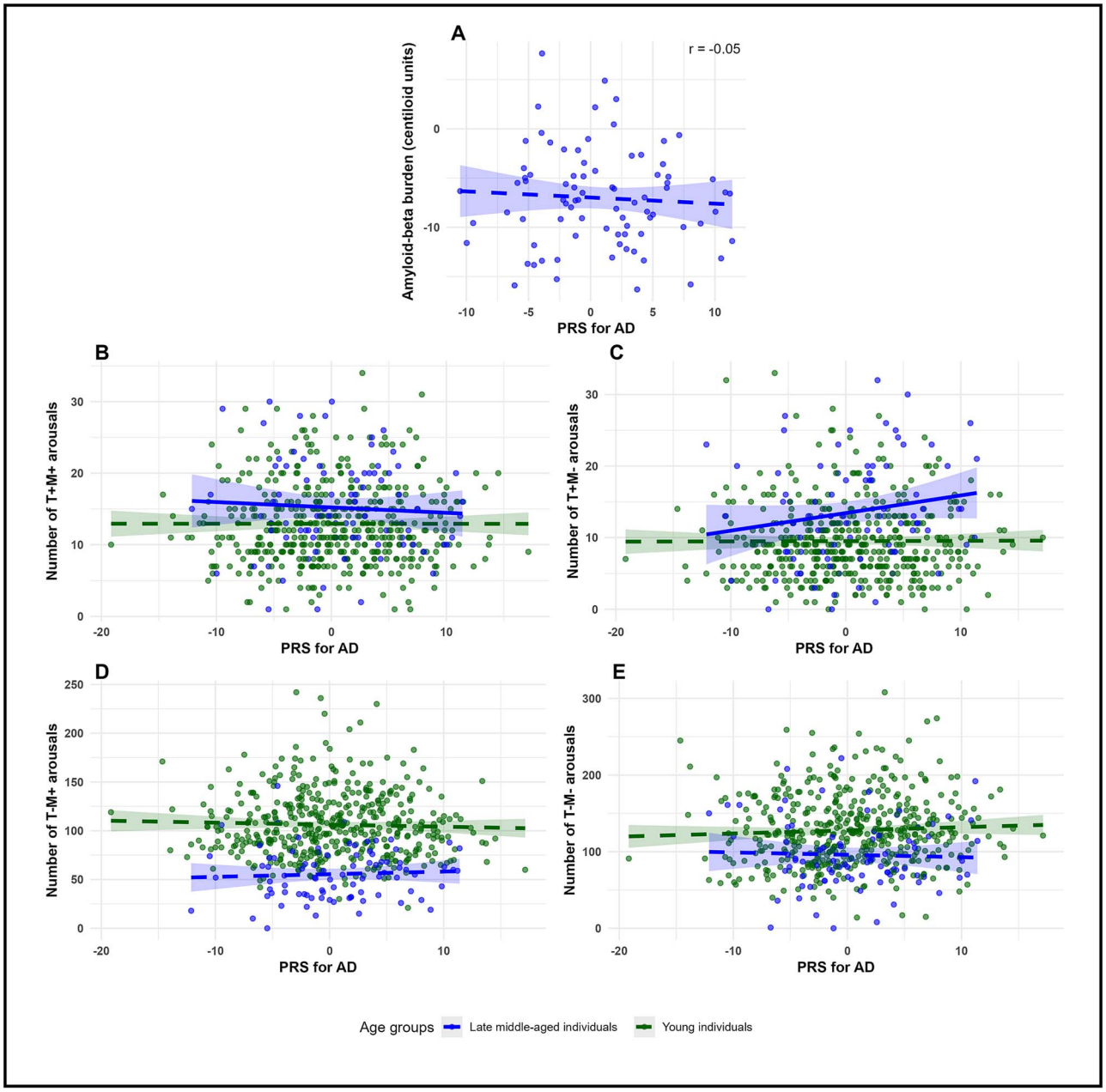


Figure 2. Associations between PRS for AD and sleep arousals. (A) Spearman correlation analysis showed that amyloid-beta burden and PRS for AD were not significantly correlated after excluding Aβ-positive individuals (N = 82; $p = 0.64$). Amyloid PET positivity was defined as a Centiloid value ≥20 (see Figure S1 for the association between PRS for AD and Aβ burden including Aβ-positive individuals). (B) Association between number of T + M+ arousals and the PRS for AD; age group by arousals interaction (N = 540; $p = 0.05$); post hoc subgroup analyses led to a significant negative association for the late middle-aged ($p = 0.01$) but not the young group ($p = 0.83$). (C) Association between number of T + M− arousals and the PRS for AD; age group by arousals interaction (N = 536; $p = 0.02$); post hoc subgroup analyses led to a significant positive association for the late middle-aged ($p = 0.003$) but not the young group ($p = 0.77$). (D) Association between number of T−M+ arousals and the PRS for AD; age group by arousals interaction (N = 539; $p = 0.14$). (E) Association between the number of T−M− arousals and the PRS for AD; age group by arousals interaction (N = 540; $p = 0.13$). Simple regression lines are used for a visual display and do not substitute the GAMLSS outputs (see Table 3 for complete statistical outputs). Solid and dashed regression lines represent significant and non-significant outputs of the GAMLSS, respectively.

Arousals linked with PRS for AD are associated with cognitive performance and cognitive change in late middle-aged individuals

We then tested whether cognitive performance of the late middle-aged individuals was differentially associated with the two arousal types that showed opposite association with PRS for AD. The GAMLSSs for each time point included each cognitive domain as dependent variable (i.e. three GAMLSS at each time point) and T + M+ arousals and T + M− arousals as covariates together with sex, age, education, and TST. Considering the baseline assessment, GAMLSSs yielded a negative association between attentional domain and T + M− arousals ($\beta = -.06$; $t = -2.26$; $\mathbf{p = .02}$) and a positive association between attentional domain and T + M+ arousals ($\beta = .08$; $t = 2.09$; $\mathbf{p = .04}$) on top of the effect of age and education (Table 4). There was no significant association between these arousals and the other cognitive domains (i.e. memory and executive functions; $p > .10$; Table 4, Figure 3A–F). Adding PRS as a covariate to these models did not change

Table 2. Association between arousals number and PRS for AD (N = 540)

Independent variable	t	Estimate	SE	*p*-value
PRS	−0.80	−0.004	0.005	0.42
Transition status	41.43	1.29	0.03	<**0.0001**
EMG status	−1.00	−0.04	0.04	0.32
Age group	−7.15	−0.29	0.03	<**0.0001**
Sex	14.94	0.03	0.02	<**0.0001**
TST	38.02	0.003	0.00006	<**0.0001**
PRS*Transition	1.61	0.009	0.006	0.11
PRS*EMG	1.75	0.01	0.007	0.08
Transition*EMG	13.48	0.59	0.04	<**0.0001**
PRS* age group	0.78	0.004	0.006	0.44
Transition * age group	23.91	0.80	0.03	<**0.0001**
EMG*age group	−5.83	−0.31	0.04	<**0.0001**
PRS*Transition*EMG	−2.54	−0.02	0.008	**0.01**
PRS*Transition*age group	−1.73	0.01	0.006	0.08
PRS*EMG*age group	−1.24	−0.01	0.008	0.22
Transition*EMG*age group	−2.27	−0.08	0.05	**0.02**
PRS*Transition*EMG*age group	2.52	0.02	0.009	**0.01**

Analyses were performed using a generalized additive model for location, scale, and shape (GAMLSS). Results with *p* values less than 0.05 were considered statistically significant (df = 1801.64). Estimates in GAMLSS are reported in log scale. PRS: polygenic risk score; TST: total sleep time; EMG: Electromyography; SE: Standard error.

Table 3. Association between PRS for AD and the Interaction between numbers of each arousal type and age group (N = 540)

Independent variable	t	Estimate	SE	*p*-value
T + M+ arousals number	−1.93	−0.30	0.13	**0.05**
T−M+ arousals number	1.28	0.04	0.03	0.20
T + M− arousals number	2.49	0.30	0.11	**0.01**
T−M− arousals number	−1.19	−0.02	0.02	0.23
T + M+ arousals number *age group	1.96	0.29	0.15	**0.05**
T−M+ arousals number *age group	−1.50	−0.04	0.03	0.14
T + M− arousals number *age group	−2.40	−0.32	0.13	**0.02**
T−M− arousals number *age group	1.52	0.03	0.02	0.13
Age group	−0.22	−2.33	2.59	0.83
Sex	−0.11	−1.23	1.45	0.91
TST	−0.29	0.0009	0.007	0.77

Analyses were performed using a generalized additive model for location, scale, and shape (GAMLSS). Results with *p* values less than 0.05 were considered statistically significant. Estimates in GAMLSS are reported in log scale. TST: total sleep time. SE: Standard error.

the statistical outputs while PRS itself was not significantly associated with the cognitive performance ($p > .93$).

We then focused on our first follow-up at 2 years that was completed in part of the late middle-aged individuals (N = 66). The GAMLSSs led to a significant negative association between T + M+ arousals and performance change over the memory domain, indicating that more T + M+ arousals at baseline are associated with less memory change over 2 years ($\beta = -.12$; t = −3.22; $\mathbf{p = .002}$), on top of a main effect of age (Table 4). The associations with the performance change in the other cognitive domains were not significant ($p > .37$; Table 4, Figure 3G–L). Adding PRS as a covariate to these models did not change the statistical output while PRS itself was not significant ($p > .09$).

We further considered cognitive change after 7 years that was assessed in a subset of the late middle-aged participants (N = 64; 73% overlap with 2y follow up – see methods). GAMLSSs yielded a positive association between memory change and T + M− arousals ($\beta = .03$; t = 2.33; $\mathbf{p = .02}$), on top of the effect of education (Table 4). Again, the associations with other cognitive domains were not significant ($p > .37$; Table 4, Figure 3M–R). Again also, adding PRS as a covariate to this model did not change the results while PRS itself was not significant ($p > .16$). The analyses were repeated using arousal density rather than numbers at baseline and 2- and 7-year follow up, leading to the same statistical outputs—Table S5).

In a final step, we considered changes in the performance to the MST task, as it is a memory test reported to be highly sensitive to early signs of cognitive change [57]. While there was no significant association between any of the two arousal types and MST performance at baseline ($p > 0.07$; Table 5; Figure 4A and B), we found a significant negative association between MST performance change after two years and T + M+ arousals ($\beta = -.007$; t = −2.42; $\mathbf{p = .02}$; Table 5; Figure 4C and D). Adding PRS as a covariate to this model did not change the results while PRS itself was not significant ($p > .69$). A subsequent GAMLSS also yielded a significant negative association between MST performance change after seven years and T + M+ arousals ($\beta = -.006$; t = −2.90; $\mathbf{p = .01}$) and a positive association between MST change after seven years and T + M− arousals ($\beta = .004$; t = 2.11; $\mathbf{p = .04}$) on top of a main effect of age (Table 5, Figure 4E and F). Again, adding PRS as a covariate to this model did not change the results while PRS itself was not significant ($p > .70$). Of note, only the association between T + M+ arousal number and memory change after 2 years remained significant (Pcorrected = 0.018) after FDR

Table 4. Association between cognitive domains at each time point and T + M+ and T + M− arousals in late middle-aged individuals

Cognitive assessment session	Cognitive domain (dependent variable)	T + M+ arousals number	T + M− arousals number	Age	Sex	Education	Total sleep time
Baseline cognition	**Attention (N = 87)**	t(91) = 2.09 Estimate = 0.08 SE = 0.04 ***p* = 0.04**	t(91) = −2.26 Estimate = −0.06 SE = 0.03 ***p* = 0.026**	t(91) = −2.18 Estimate = −0.08 SE = 0.04 ***p* = 0.03**	t(91) = 1.24 Estimate = 0.56 SE = 0.45 *p* = 0.22	t(91) = 2.14 Estimate = 0.14 SE = 0.07 ***p* = 0.04**	t(91) = 1.07 Estimate = 0.005 SE = 0.005 *p* = 0.29
	Memory (N = 87)	t(84) = −1.60 Estimate = −0.06 SE = 0.04 *p* = 0.11	t(84) = 0.59 Estimate = 0.02 SE = 0.03 *p* = 0.56	t(84) = 1.28 Estimate = 0.05 SE = 0.04 *p* = 0.20	t(84) = 2.33 Estimate = 1.00 SE = 0.43 ***p* = 0.02**	t(84) = 2.23 Estimate = 0.16 SE = 0.07 ***p* = 0.03**	t(84) = −0.41 Estimate = −0.002 SE = 0.005 *p* = 0.68
	Executive function (N = 87)	t(89) = 1.56 Estimate = 0.09 SE = 0.05 *p* = 0.12	t(89) = −0.69 Estimate = −0.03 SE = 0.04 *p* = 0.49	t(89) = −1.02 Estimate = −0.06 SE = 0.06 *p* = 0.31	t(89) = 0.19 Estimate = 0.12 SE = 0.67 *p* = 0.85	t(89) = 2.30 Estimate = 0.24 SE = 0.11 ***p* = 0.02**	t(89) = −0.04 Estimate = −0.0003 SE = 0.007 *p* = 0.97
Cognitive change after 2 years	**Attention (N = 64)**	t(54) = 0.06 Estimate = 0.003 SE = 0.05 *p* = 0.95	t(54) = −0.82 Estimate = −0.02 SE = 0.03 *p* = 0.42	t(54) = 2.08 Estimate = 0.08 SE = 0.04 ***p* = 0.04**	t(54) = 0.76 Estimate = 0.36 SE = 0.48 *p* = 0.45	t(54) = 0.55 Estimate = 0.05 SE = 0.09 *p* = 0.58	t(54) = −0.62 Estimate = −0.003 SE = 0.005 *p* = 0.54
	Memory (N = 61)	t(51) = −3.22 Estimate = −0.12 SE = 0.04 ***p* = 0.002***	t(51) = 0.89 Estimate = 0.02 SE = 0.02 *p* = 0.38	t(51) = 2.27 Estimate = 0.07 SE = 0.03 ***p* = 0.03**	t(51) = 0.08 Estimate = 0.03 SE = 0.40 *p* = 0.94	t(51) = 0.44 Estimate = 0.03 SE = 0.06 *p* = 0.66	t(51) = −0.40 Estimate = −0.002 SE = 0.004 *p* = 0.69
	Executive function (N = 65)	t(55) = −0.27 Estimate = −0.01 SE = 0.05 *p* = 0.79	t(55) = 0.12 Estimate = 0.005 SE = 0.04 *p* = 0.91	t(55) = −0.01 Estimate = −0.0004 SE = 0.04 *p* = 0.99	t(55) = −1.11 Estimate = −0.76 SE = 0.69 *p* = 0.27	t(55) = −0.12 Estimate = −0.01 SE = 0.09 *p* = 0.90	t(55) = −1.36 Estimate = −0.008 SE = 0.006 *p* = 0.18
Cognitive change after 7 years	**Attention (N = 64)**	t(54) = 0.46 Estimate = 0.02 SE = 0.04 *p* = 0.65	t(54) = −1.16 Estimate = −0.04 SE = 0.04 *p* = 0.25	t(54) = 0.76 Estimate = 0.04 SE = 0.05 *p* = 0.45	t(54) = 0.30 Estimate = 0.17 SE = 0.58 *p* = 0.77	t(54) = 0.43 Estimate = 0.04 SE = 0.09 *p* = 0.67	t(54) = 0.43 Estimate = 0.002 SE = 0.006 *p* = 0.67
	Memory (N = 60)	t(50) = −0.85 Estimate = −0.02 SE = 0.02 *p* = 0.40	t(50) = 2.33 Estimate = 0.03 SE = 0.01 ***p* = 0.02**	t(50) = 1.89 Estimate = 0.04 SE = 0.02 *p* = 0.06	t(50) = 2.03 Estimate = 0.47 SE = 0.23 *p* = 0.05	t(50) = 2.67 Estimate = 0.08 SE = 0.03 ***p* = 0.01**	t(50) = −0.72 Estimate = −0.002 SE = 0.002 *p* = 0.48
	Executive function (N = 56)	t(46) = −1.59 Estimate = −0.08 SE = 0.05 *p* = 0.12	t(46) = 0.67 Estimate = 0.03 SE = 0.05 *p* = 0.51	t(46) = 1.94 Estimate = 0.10 SE = 0.05 *p* = 0.06	t(46) = 2.12 Estimate = 1.07 SE = 0.50 ***p* = 0.04**	t(46) = 1.86 Estimate = 0.14 SE = 0.07 *p* = 0.07	t(46) = 1.82 Estimate = 0.008 SE = 0.004 *p* = 0.07

*Remained significant after false discovery rate (FDR) correction across the nine tests (3 time points × 3 cognitive domains). Analyses were performed using a generalized additive model for location, scale, and shape (GAMLSS). Results with *p* values less than 0.05 were considered statistically significant. Estimates in GAMLSS are reported in log scale. Prior to the analysis, we removed the outliers among all variables by excluding the samples lying beyond four times the standard deviation (the final number of individuals included in each analysis is reported below each dependent variable). SE: Standard error.

correction (Benjamini–Hochberg procedure) across the nine tests performed for each arousal subtype (3 time points × 3 cognitive domains). All other associations should be interpreted with caution. The analyses with MST were repeated using arousal density rather than arousal numbers leading to the same statistical outputs—Table S6). Finally, we found no significant association between T + M+ or T + M− and baseline performance in young individuals in any cognitive domain, suggesting the associations we uncovered previously are specific to the late middle-aged individuals of our sample (Table S7; Figure S4).

Discussion

Sleep arousals are commonly viewed as disruptive and leading to negative functional outcomes [60]. This likely arises in part from their increased prevalence with aging, their association with external disturbances (e.g. noise) or with hypopnea/apnea in patients with sleep disordered breathing (SDB). Arousals are also triggered spontaneously, i.e. without a concomitant disturbance, and these may serve beneficial purposes for brain functioning [61]. In addition, spontaneous arousal are heterogenous, likely in part because of differential activity of the LC [62, 63], and this heterogeneity is associated with early Aβ burden [6]. In the present study, we determined whether spontaneous arousals are associated with the risk for developing AD and with cognition in healthy younger and late middle-aged individuals. We show that, depending on whether they are associated with a transition of sleep stage and with an increase in muscle tone, arousal can be associated with an increased or decreased polygenic risk for AD in late middle-aged, but not in younger adults. We further show that, in late middle-aged individuals, arousals heterogeneity is associated not only with current attentional performance but also bears predictive value for memory performance 2 and 7 years later. Although the effect sizes we observed were modest, if persistent, these small effects could influence lifelong health trajectories. These findings add to the growing literature showing that alterations in microstructural elements of sleep electrophysiology are associated with early AD neurobiology and precede the onset

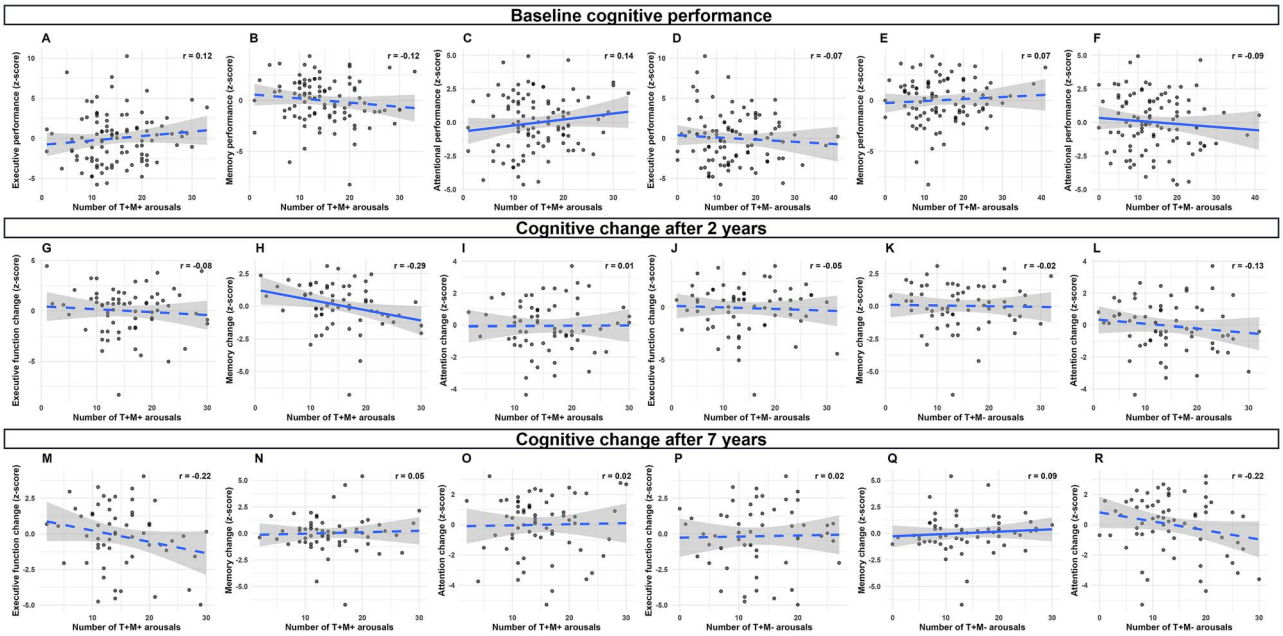


Figure 3. Associations between sleep arousals and cognitive scores at baseline and 2y and 7y follow ups. Associations between the number of T + M+ arousals and baseline executive (A), memory (B) and attention* (C) scores. Associations between the number of T + M− arousals and baseline executive (D), memory (E) and attention (F) scores. Associations between the number of T + M+ arousals and executive (G), memory* (H) and attention (I) score changes after 2 years. Associations between the number of T + M− arousals and executive (J), memory (K) and attention (L) score changes after 2 years. Association between the number of T + M+ arousals and executive (M), memory (N), and attention (O) score changes after 7 years. Association between the number of T + M− arousals and executive (P), memory* (Q), and attention (R) score changes after 7 years. * Significant associations. Simple regression lines are used for a visual display and do not substitute the GAMLSS outputs (see Table 4 for complete statistical outputs). Solid and dashed regression lines represent significant and non-significant outputs of the GAMLSS, respectively.

Table 5. Association between MST scores at each time point and T + M+ and T + M− arousals number in late middle-aged individuals

Cognitive assessment (dependent variable)	T + M+ arousals number	T + M− arousals number	Age	Sex	Education	Total sleep time
MST performance at baseline (N = 87)	t(89) = −1.85 Estimate = −0.003 SE = 0.001 *p* = 0.07	t(89) = 1.28 Estimate = 0.001 SE = 0.001 *p* = 0.20	t(89) = −0.40 Estimate = −0.0007 SE = 0.002 *p* = 0.69	t(89) = 0.51 Estimate = 0.01 SE = 0.02 *p* = 0.61	t(89) = 0.07 Estimate = 0.0003 SE = 0.003 *p* = 0.94	t(89) = −0.06 Estimate = −0.00001 SE = 0.0002 *p* = 0.95
MST change after 2 years (N = 64)	t(56) = −2.42 Estimate = −0.007 SE = 0.003 ***p* = 0.02**	t(56) = 1.29 Estimate = 0.003 SE = 0.002 *p* = 0.20	t(56) = −0.10 Estimate = −0.0003 SE = 0.003 *p* = 0.92	t(56) = 0.01 Estimate = 0.0005 SE = 0.04 *p* = 0.99	t(56) = −0.07 Estimate = −0.0003 SE = 0.005 *p* = 0.95	t(56) = 1.33 Estimate = 0.0005 SE = 0.0003 *p* = 0.19
MST change after 7 years (N = 47)	t(38) = −2.90 Estimate = −0.006 SE = 0.002 ***p* = 0.01**	t(38) = 2.11 Estimate = 0.004 SE = 0.002 ***p* = 0.04**	t(38) = 2.73 Estimate = 0.006 SE = 0.002 ***p* = 0.01**	t(38) = 0.52 Estimate = 0.01 SE = 0.03 *p* = 0.61	t(38) = 0.06 Estimate = 0.0003 SE = 0.004 *p* = 0.95	t(38) = 1.74 Estimate = 0.0005 SE = 0.0003 *p* = 0.09

Analyses were performed using a generalized additive model for location, scale, and shape (GAMLSS). Results with *p* values less than 0.05 were considered statistically significant. Estimates in GAMLSS are reported in log scale. Prior to the analysis, we removed the outliers among all variables by excluding the samples lying beyond four times the standard deviation (the final number of individuals included in each analysis is reported below each dependent variable). MST: mnemonic similarity task. SE: Standard error

of AD symptoms [6, 64–66]. They may contribute to establishing a marker of AD risk in otherwise healthy individuals.

We showed, in the same sample of late middle-aged individuals, that defining arousal based on stage transition and association with muscle tone was meaningful as arousal types differed in their oscillatory compositions, but—in absence of a younger group—could not report age related changes across the different types of arousals [6]. Here, we report that, contrary to the general assumption, not all arousal types increase in the late middle-aged group. Only the number of arousals that are associated with sleep stage transition, and therefore most impinging on sleep continuity, increase with age, while those not associated with these transitions become less common. This constitutes the first important observation that arousal heterogeneity is meaningful. In addition, it is precisely those arousals increasing with age that we find associated with the PRS for AD. In other words, the extent of the age-related difference in T+ is linked to the genetic risk for developing AD.

We find that, in late middle-aged individuals, more T + M− arousals are associated with an increased PRS for AD, which is similar to the association between T + M− arousals and higher early amyloid burden we previously reported in the same sample [6]. Arousals associated with sleep stage transition but not with a detectable muscle tone increase appear therefore to be

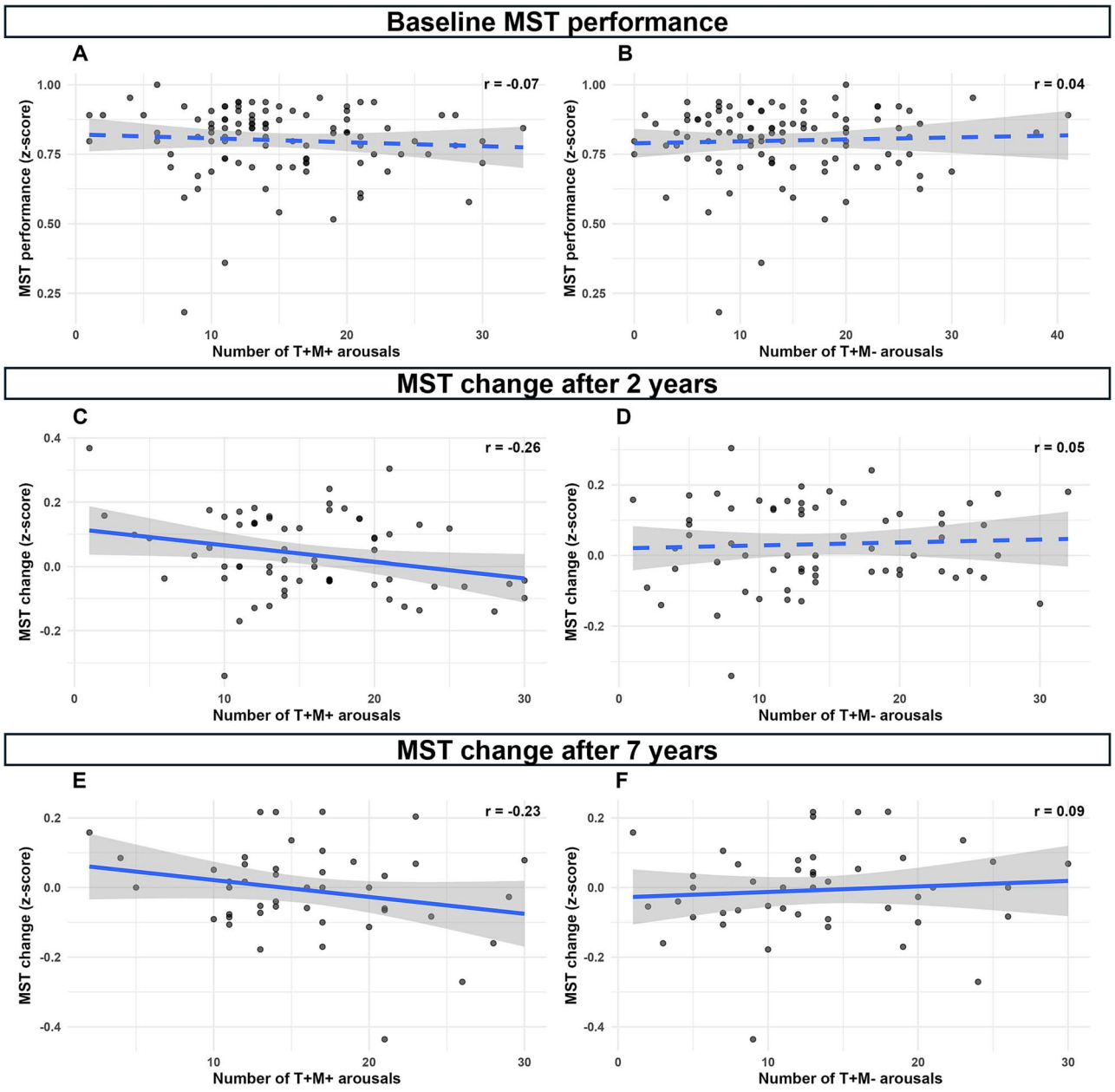


Figure 4. Associations between sleep arousals and MST scores. Association between baseline MST performance and the number of T + M+ **(A)** and T + M− **(B)** arousals. Association between MST performance change after 2 years and the number of T + M + * **(C)** and T + M− **(D)** arousals. Association between MST performance change after 7 years and the number of T + M + * **(E)** and T + M−* **(F)** arousals. * significant associations. MST: Mnemonic similarity task. Simple regression lines are used for a visual display and do not substitute the GAMLSS outputs (see Table 5 for complete statistical outputs). Solid and dashed regression lines represent significant and non-significant outputs of the GAMLSS, respectively.

related to more risk for developing AD both based on genetic and protein burden assessment. T + M− arousals may therefore reflect a chronic sleep disruption that contributes to, or is at least associated with, long-term neural vulnerability to AD-related processes. In line with this negative view, the amount of T + M− arousals is also associated with poorer attentional performance at baseline and memory change at 7y (as shown using the global memory score and the exploration of the MST, a task sensitive to early hippocampal dysfunction [57], which supports the idea that attentional deficits may precede and potentially exacerbate memory deterioration over time. In contrast to T + M− arousals and still in the late middle-aged participants, we find that the amount of T + M+ arousals is associated with a reduced PRS for AD, better attentional performance at baseline and better memory performance at 2 and 7 years (shown through both the global memory and MST scores at 2 years and the MST score at 7 years). Hence, these arousals would represent a better situation in terms of AD risk, associated with better attention processes which could favor subsequent better memory function.

According to our findings, the main feature differentiating negative and positive transition arousals is whether or not they are accompanied by an increase in EMG tone. Since by definition an arousal that would exclusively consist of a muscle activation (without EEG activation) do not exist, arousals with muscle tone increase were proposed to represent stronger brain activation

[67]. A study in mice showed that cortico-hippocampal coherence increases prior to and during arousals accompanied by EMG [68]. It is also shown that stronger arousals that are accompanied by increased muscle tone are associated with the appearance of theta activity in the hippocampus [69], while theta activity of hippocampus has been reported to be important for memory processing [70].

Studies in animals showed that optogenetic activation of the LC, in the midbrain, causes arousals associated with EMG both during REM and NREM sleep, most often leading to full wakefulness [71]. More importantly, spontaneous arousals in rodents were recently reported to align with variations in noradrenaline (arising from LC activity) leading to two types of arousal [62, 63]. The LC appears to regulate arousal intensity, with most LC surges (70%) not leading to full wakefulness. Only stronger surges (30%) cause brief awakenings, while smaller ones induce partial arousal affecting the heart and thalamus but not the cortex [63]. The LC may therefore underlie arousal heterogeneity, particularly those associated with EMG changes. According to our results, these may be the most profitable, whether or not they are strong enough to lead to a sleep stage transition (sleep stage being a somewhat arbitrary classification). In that respect T + M− arousal may represent incomplete arousal events in which the full arousal network is not recruited or arousal arising from a distinct set of brain regions and, according to our findings, would be associated with more deleterious outcomes. We stress that multiple brain regions other than the LC were reported to contribute to the regulation of sleep arousals and may therefore contribute to our findings. In humans, an EEG-fMRI study showed that the midbrain, thalamus, basal ganglia, and cerebellum, were activated during arousal while cortical regions were deactivated [72]. Besides, studies in rodents reported that the medial septum [73], neocortical areas, and the hippocampus [74], were associated with spontaneous arousals during sleep. Importantly, most of the current knowledge on the role of LC in arousals is derived from NREM sleep, and the specific contribution of the LC to REM-related arousals remains insufficiently characterized in humans. Further studies are therefore needed to better delineate the interaction between these regions for arousal generation, particularly during REM sleep, when LC is silent.

We emphasize that in our previous work on arousal heterogeneity, we found that T−M+ arousals were associated with reduced Aβ burden and better attention performance [6], which is only partially in line with our current findings, where T + M+ are associated with lower PRS for AD and better attention. We may have lacked sensitivity to find positive associations with both T + M+ and T−M+ arousals across both analyses. The discrepancy may also arise from the fact that we used two different approaches that do not grasp exactly the same part of the risk for AD. Consistent with previous research [75], we indeed found no association between PRS for AD and Aβ burden. PRS may capture a broader genetic risk for AD and may be more predictive of disease progression than amyloid accumulation itself [75]. In our earlier study, we interpreted the sleep-stage transition component as the key feature linking arousals to AD risk. Taken together, however, the two studies may suggest the following: while T− arousals may be associated with lower Aβ accumulation and T+ arousals with reduced genetic vulnerability, arousals accompanied by EMG activation—regardless of stage transition—consistently mark lower risk for future AD. This pattern supports the idea that motor-tone recruitment during arousals reflects a more complete and coordinated activation of the arousal system, which may have protective or compensatory value in maintaining cognitive function. One could speculate that M+ arousals offer recurring opportunities to transiently synchronize distant brain areas, similarly to sleep spindles [76]. This warrant future investigation in a distinct, and ideally larger, sample of late middle-aged or older individuals.

Despite having a much larger sample, we could not isolate associations between arousal types and PRS in young adults. This could mean that it is only when brain function and sleep become more susceptible to challenges [77] that association between arousal and the biology of AD emerges in those that are more prone to develop AD. Hence, sleep arousals may not represent a promising marker of AD risk in young adults. Yet in late middle-aged individuals, (T + M− and T + M+) arousals, and not the PRS for AD, were associated with cognitive changes over 2 or 7 years. Sleep microstructure could therefore constitute a promising early marker of future cognition and brain ageing trajectory that is more sensitive than risk assessment based on both genetics and amyloid beta burden assessments [6].

We acknowledge several limitations of our study. First, the number of participants who completed the follow-up cognitive assessment was relatively small (N = 66 and N = 64) and the number of participants that completed both follow-up assessment was even smaller (N = 48 – see Methods). Our sensitivity at follow-ups was therefore lower and may have hindered other weaker significant associations such that our results have to be taken mostly in relative terms (stronger vs. weaker effect rather than presence vs. absence of effects). In addition, the memory composite and the MST scores were not consistently associated with T + M+ and T + M− arousals across the two follow-up assessments at 2 and 7 years. It is rather the overall pattern of associations across both scores that is consistent with memory performance changes being associated with arousal types. Whether this precise pattern reflects a lack of sensitivity or actual differences over different aspects of memory function is not known. We stress that the analyses including cognitive measure were not corrected for multiple comparisons and, if FDR correction is applied, only the link between T + M+ arousal number and 2-year memory change remains significant speaking for caution in results interpretation. Besides, the exclusion criteria applied were stringent and do not reflect the variability present in the general population. Although this guarantees that our findings are not biased by common age-related health issues, our participants exhibited minimal Aβ accumulation, with few individuals classified as Aβ-positive (N = 3). In particular, since we did not included patients with SDB, our findings likely do not generalize to the perturbation-induced arousals, which have been associated with adverse behavioral [78, 79] and neurodegenerative outcomes [66]. Furthermore, the predictive validity of PRS remains a subject of ongoing debate [80] and we cannot determine which participants will ultimately develop AD. Moreover, as already stated and even though it was controlled for, sex was not balanced within and across age groups while women exhibit distinct sleep characteristics [81, 82], and are also more susceptible to AD [83]. We could not therefore fully determine sex-differences for our variables of interest. All these aspects limit the generalizability of our finding and warrants future investigations including larger and sex-balanced samples with more lenient exclusion criteria, e.g. including individuals with SDB and additional / more detailed memory performance assessments.

Conclusion

By leveraging EEG data and genotyping across a diverse age range, we provide novel insights into how subtle features of sleep architecture may reflect or interact with underlying genetic

susceptibilities. We report that spontaneous arousals during sleep are not uniform but vary in their association with genetic risk for AD and bears prediction value for future cognitive change depending on their electrophysiological characteristics. These associations emerged only in older individuals, consistent with age-related vulnerability to subtle sleep disruptions. By distinguishing arousal subtypes, our study highlights sleep microstructure as a potential early marker of neurodegenerative risk during early aging. How arousal may interact with other features of sleep microstructure to contribute to AD risk remains to be assessed.

Supplementary material

Supplementary material is available at *SLEEP* online.

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Author contributions

Nasrin Mortazavi (Conceptualization, Methodology, Software, Formal Analysis, Investigation, Data Curation, Writing—Original Draft Preparation, Writing—Review & Editing, Visualization, Project Administration), Gilles Vandewalle (Conceptualization, Validation, Investigation, Resources, Writing—Original Draft Preparation, Writing—Review & Editing, Supervision, Project Administration, Funding Acquisition), Puneet Talwar (Conceptualization, Methodology, Formal Analysis, Investigation, Data Curation, Writing—Original Draft Preparation, Writing—Review & Editing), Fabienne Collette (Validation, Resources, Writing—Review & Editing, Funding Acquisition), Christine Bastin (Validation, Resources, Writing—Review & Editing, Funding Acquisition), Pierre Maquet (Resources, Writing—Review & Editing, Funding Acquisition), and Mikhail Zubkov (Methodology, Writing—Review & Editing). All authors have read and agreed to the published version of the manuscript.

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Data availability

The data and analysis scripts supporting the results included in this manuscript are publicly available via https://gitlab.uliege.be/CyclotronResearchCentre/Public/fasst/sleep_arousals_ad_prs. Researchers willing to access the raw data should send a request to the corresponding author (GV). Data sharing will require evaluation of the request by the local Research Ethics Board and the signature of a data transfer agreement (DTA).

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SLEEP, 2026, **00**, 1–17
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