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Original Article

ORIGINAL ARTICLE

Relationships between objectives sleep parameters and brain amyloid load in subjects at risk for Alzheimer's disease: the INSIGHT-preAD Study

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Abstract

Study Objectives: Sleep changes have been associated with increased risks of developing cognitive disturbances and Alzheimer's disease (AD). A bidirectional relation is underlined between amyloid-beta (Aß) and sleep disruptions. The sleep profile in participants at risk to develop AD is not fully deciphered. We aim to investigate sleep–wake changes with objective sleep measurements in elderly participants without cognitive impairment depending on their brain amyloid status, positive (Aß+) or negative (Aß–) based on standard absorption ratios (SUVr) positron emission tomography-florbetapir imaging.

Methods: Sixty-eight participants without cognitive impairment who have accepted to be involved in the sleep ancillary study from the InveStIGation of Alzheimer's Predictors in Subjective Memory Complainers (INSIGHT-pre AD) cohort, aiming to record sleep profile based on the analyses of an ambulatory accelerometer-based assessment (seven consecutive 24-hour periods). Neuropsychological tests were performed and sleep parameters have been individualized by actigraph. Participants also underwent a magnetic resonance imaging scan to assess their hippocampal volume. Based on SUVr PET-florbetapir imaging, two groups Aß+ and Aß– were compared.

Results: Participants were divided into two groups: Aß+ ($n = 24$) and Aß– ($n = 44$). Except for the SUVr, the two subgroups were comparable. When looking to sleep parameters, increased sleep latency, sleep fragmentation (wake after sleep onset [WASO] score and awakenings) and worst sleep efficiency were associated with cortical brain amyloid load.

Conclusion: Actigraphic sleep parameters were associated with cortical brain amyloid load in participants at risk to develop AD. The detection of sleep abnormalities in those participants may be of interest to propose some preventive strategies.

Key words: sleep; sleep/wake patterns; brain amyloid load; Alzheimer's disease; actigraphy; PET- amyloid; biomarkers; florbetapir; MRI neuroimaging

Statement of Significance

Sleep disruptions are common in Alzheimer's disease (AD) patients and usually occur in early stage. The goal of the study is to identify a preclinical population of Alzheimer's disease by an early biomarker, PET-CT amyloid and measure its association with an early symptom of the disease, sleep disruptions. Novelty of the research is to propose an objective measurement of sleep disruptions by actigraphy. The future direction of the work would be to reproduce the actigraphic measure and PET-CT amyloid some years later using the INSIGHT longitudinal cohort (INveStIGation of Alzheimer's Predictors in Subjective Memory Complainers) to better understand impact of sleep on brain ß amyliodosis.

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Introduction

Evidence is growing that sleep quality may have an impact on transition between presymptomatic and clinical stage of Alzheimer's disease (AD). Two recent meta-analyses summarized this hypothesis. The first shows that compared to healthy subjects, individuals reporting sleep disturbances had a relative risk (RR) of 1.49 (95% confidence interval [CI]: 1.25 to 1.78) to develop AD. The sleep disorder associated with the highest risk was insomnia [1]. The second reports that individuals with sleep disorders had an RR = 1.55 (95% CI: 1.25 to 1.93); RR = 1.65 (95% CI: 1.45 to 1.86); RR = 3.78 (95% CI: 2.27 to 6.30), respectively to develop AD, cognitive disorders, and preclinical AD compared to healthy subjects. Worst sleep efficiency was associated with higher risk among sleep disorders, followed by sleep latency and nocturnal awakenings [2].

However, associations between brain changes in cognitive decline and AD are not fully understood. Amyloid plaques seemed closely related to sleep–wake cycle, even at an early stage of the disease. Aβ peptide appears to follow a circadian fluctuation [3] with an increase during awakenings and a decrease during sleep. Several mechanisms are possibly underlying this circadian variation. Soluble forms of Aβ peptide could be released during synaptic activity [4] and its night decrease could be explained by low neuronal activity during sleep, especially in stage N3 (SWA) [5–8]. Furthermore, glymphatic system [9] involved in brain metabolites clearance as Aß appears twice faster during sleep compared to awakening [10].

Then, narcoleptic patients studies, showed that absence of orexin could have a protective effect on amyloid plaques genesis [11] and study in the Tg 2576 mouse model of AD obtained the same conclusions after cerebroventricular injection of orexin and almorexant, an orexin receptor antagonist [3].

Finally, nyctemeral cycle perturbations may also increase oxidative stress and alter blood–brain barrier (BBB) functions [12–16]. These two alterations also seem to contribute to the development or progression of AD [17, 18].

In agreement with these pathophysiological hypotheses, several studies report an association between poor quality of sleep and amyloid pathology. A poorer sleep efficiency measured by actigraphy is highlighted in participants with a low level of Aß 42 in the cerebrospinal fluid [19, 20].

Some discrepancies have been underlined regarding the association between a short sleep duration and amyloid deposition. Some authors reported a relationship, in particular in the precuneus [21] and frontal regions [22, 23], although others found no association. A longer sleep latency was also related to brain amyloid load [24].

The present study aimed to investigate sleep–wake changes with objective sleep measurements in elderly participants without cognitive impairment depending of their brain amyloid status, positive (Aß+) or negative (Aß–), based on SUVr PET-florbetapir imaging. We hypothesized that participants with positive brain amyloid load should have more disturbed sleep than participants who did not reach the threshold of PET-florbetapir amyloid.

Method

Participants

Eligible participants should be: aged 70–85 years, with subjective memory complaints, unimpaired cognition (Mini-Mental State Examination [MMSE] [25] score ≥27 and Clinical Dementia Rating score = 0) [26], no evidence of episodic memory deficit (Free and Cued Selective Reminding Test score [FCSRT] total recall score ≥41) [27], visual and auditory acuity adequate for testing, and no systemic or chronic disease that might interfere with follow-up. We excluded people who were under guardianship, were residents in nursing facilities, had presymptomatic monogenic, who could not undergo or refused MRI, had undergone radiopharmaceutical imaging or treatment unrelated to this trial within 2 days before the study imaging session, had neurological diseases (e.g. treated epilepsy, extrapyramidal signs, visual hallucinations, brain tumor, subdural hematoma, and history of head trauma followed by persistent neurological effects), stroke in the previous 3 months, or illiteracy (reading or counting).

The ethics committee of the Pitié-Salpêtrière University Hospital approved the study protocol. All participants signed an informed consent form, given and explained to them 2 weeks before enrolment. Procedures and all testing were done by the same neuropsychologists and physicians.

Three hundred and eighteen participants were recruited in the INveStIGation of Alzheimer's Predictors in Subjective Memory Complainers (INSIGHT)-preAD parent cohort. From them, 81 participants were agreed to participate the sleep ancillary study with actigraphy measurements (Figure 1, flow chart). Thirteen participants were excluded, 6 for actigraphic recording less than 4 days and night and 7 for unusable data due to irregular wear. Thus, 68 participants were analyzed.

Assessment tools

Clinical and cognitive assessment

Each participant had a clinical exam with clinical history and treatment record. History of sleep disorders was specifically sought and participants had to complete sleep diary. All participants had subjective memory complaints and have to complete a behavioral and cognitive battery including memory complaint questionnaire (MCQ) [28], MMSE [25], the Clinical Dementia Rating [26], and the Free and Cue Selective Reminding Tests [27].

Actigraphy

Participants were asked to wear a three-axis accelerometer (GT3X+, Actigraph Corp, Pensacola, FL) on the nondominant wrist for seven consecutive 24-hour periods. The accelerometer was set to record in 60-second epochs and recorded parameters were analyzed with ActiLife software (Actigraph Corp.). Accelerometer can also measure light presence and posture using the embedded inclinometer.

Actigraphy has been shown that this sleep measurement method is comparable to polysomnography [29].

Sleep was assessed using Cole-Kripke algorithm [30, 31].

Additionally, participants were asked to fill a sleep diary.

For each participant, the objective sleep parameters were interpreted in comparison with these sleep diary to get more accurate data.

Several parameters were recorded: sleep onset latency (SOL, period in minutes between bedtime and sleep beginning), total time in bed (TTB, period in minutes between « in bed » time and « out bed » time), total sleep time (TST, period in minutes of sleep during TTB), wake after sleep onset (WASO, period ≥ 5 minutes of awakenings during TTB excluding SL [32], awakenings (number

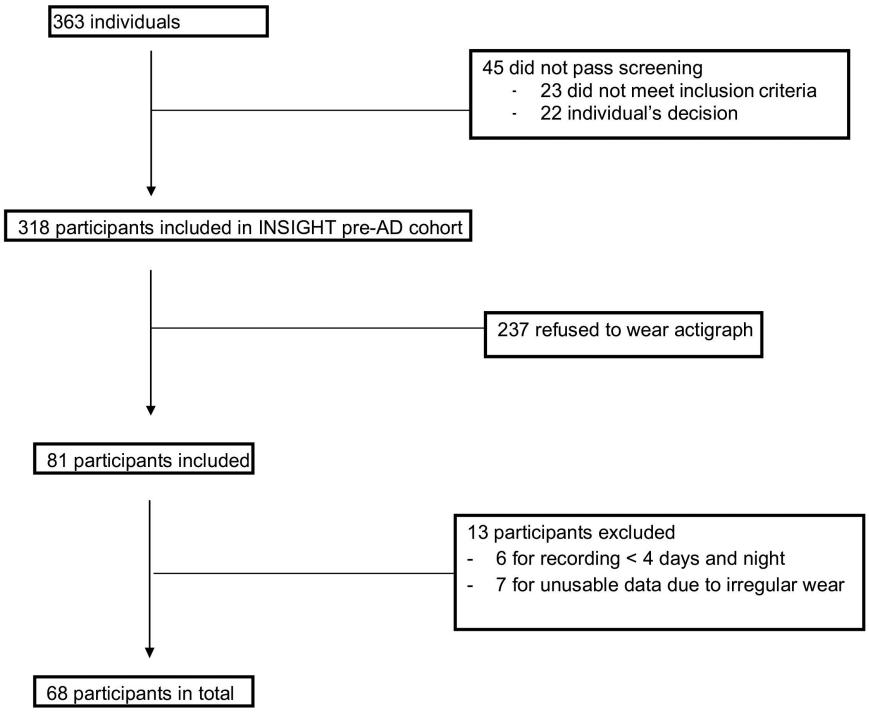

Figure 1. Flow chart.

of awakenings), average of awakenings (AA, average duration in minutes of awakenings), sleep efficiency (SE, percentage ratio of TST divided by TTB), and the nap (sleep period >15 minutes during the day, according to sleep diary) [33].

Brain imaging

MRI

Participants also performed a 3-Tesla magnetic resonance imaging (MRI) (Magnetom VERIO system [Siemens Medical Solutions, Erlangen, Germany]). The images were acquired after standardization of imaging processes by a team specialized in neuroimaging (CATI for "Center for Image Acquisition and Processing", http://cati-neuroimaging.com).

This team centralized the MRIs and verified and processed their qualities to obtain standardized data. The MRI protocol included 3D-T1 sequences that were used to assess gray and white matter volumes through parametric statistical mapping [34]. The right and left hippocampal volumes and their averages were also measured using the SACHA software [35, 36]. Other MRI sequences have been performed but are not detailed in this study [37].

Amyloid PET

The PET-CT was performed 50 minutes after an injection of 370MBq (10mCi) of 18-F-florbetapir [38]. The images were also reconstructed and analyzed by the CATI.

We calculated the standard absorption ratios (SUVr) by averaging the cortical activity of the following regions of interest: posterior cingulum, anterior cingulum, orbito-frontal cortex, precuneus, temporal, and parietal lobe. A PET-Aβ-positive imaging is defined based on the SUVr threshold previously determined (>0.7918) [39–41].

Statistical analysis

Qualitative variables were described using frequency and percentage, and quantitative variables were described using the mean, standard deviation of the median, and the first and third quartiles.

We used the Shapiro test to verify that our cohort came from a normally distributed population. Then, we used Student parametric *t*-test and Wilcoxon nonparametric test for quantitative variables and Chi^2 test for qualitative variables to compare the two groups. Finally, we performed statistical correlation to evaluate the association between two variables, through the correlations of Spearman and Pearson. The results will be presented with their standard deviations in parentheses.

Finally, a logistic regression was performed for each variable of interest, adjusting the results for age, sex, MMSE, and depression. 95% confidence intervals were reported. A p value less than or equal to 0.05 is considered significant. All analyzes were performed using the software R, version 3.3.0.

Results

Participants characteristics

The mean age of participants was 76.7 (SD 3.52) years with a predominance of women (70.6%) (Table 1). Participants frequently had cardiovascular risk factors, especially HBP (47%); these risk factors were predominant in Aß-negative group. Depression was also predominant in Aß-negative group. Then, individuals with OSA or sleep disorders were rare (Table 1). The right hippocampal volume was significantly higher than the mean normalized total (left and right) hippocampal volume (Table 1). Among all 68 participants, 24 (35.3%) presented a positive PET-florbetapir (PET Aß+). Mean MMSE and FCSRT scores were in the

Table 1. Participants demographic, clinical, brain imaging characteristics and test results

	All participants (*n* = 68)	Participants without amyloid ß deposition (*n* = 44)	Participants with amyloid ß deposition (*n* = 24)
Characteristics			
Age (years)	76.661 (3.52)	76.659 (3.55)	76.667 (3.61)
Men (%)	20 (29.4%)	16 (36.4%)	4 (16.7%)
Women (%)	48 (70.6%)	28 (63.6%)	20 (83.3%)
Medical history			
OSA with CPAP	1 (1.4%)	1 (2%)	0
OSA without treatment	1 (1.4%)	1 (2%)	0
Subjective insomnia	6 (8.9%)	5 (11.3%)	1 (4%)
Depression	17 (25%)	13 (29.5%)	4 (16.6%)
HBP	32 (47%)	21 (47.7%)	11 (45.8%)
Dyslipidemia	25 (36.7%)	13 (29.5%)	12 (50%)
Coronary disease	6 (8.8%)	4 (9%)	2 (8.3%)
Subjective feelings about memory and cognition			
MCQ	13.41 (6.27)	13.43 (7.24)	13.37 (4.08)
Cognitive functions			
MMSE	28.6 (0.94)	28.7 (0.95)	28.5 (0.93)
CDR	0	0	0
FCSRT			
Immediate Free Recall	30.56 (5.10)	31.54 (5.00)	28.75 (4.86)
Total Score	46.25 (1.74)	46.29 (1.76)	46.17 (1.74)
Volumetric MRI (cm3)			
Total hippocampal volume	2.68 (0.26)	2.69 (0.26)	2.66 (0.27)
Left hippocampal volume	2.62 (0.30)	2.63 (0.32)	2.61 (0.27)
Right hippocampal volume	2.74 (0.26)	2.75 (0.26)	2.70 (0.28)
Florbetapir PET-imaging			
Cortical SUVr	0.80 (0.20)	0.68 (0.05)	1.01 (0.19)
Precuneus right SUVr	0.77 (0.21)	0.64 (0.06)	0.99 (0.21)
Precuneus left SUVr	0.78 (0.21)	0.66 (0.06)	0.99 (0.23)
Temporal right SUVr	0.84 (0.21)	0.73 (0.06)	1.04 (0.22)
Temporal left SUVr	0.82 (0.19)	0.72 (0.06)	1.01 (0.21)
Parietal right SUVr	0.81 (0.23)	0.68 (0.07)	1.04 (0.23)
Parietal left SUVr	0.80 (0.23)	0.68 (0.07)	1.02 (0.26)
Orbitofrontal right SUVr	0.87 (0.20)	0.75 (0 .08)	1.08 (0.18)
Orbitofrontal left SUVr	0.90 (0.21)	0.78 (0 .08)	1.11 (0.19)
Cingulum antérieur right SUVr	0.77 (0.19)	0.66 (0.06)	0.99 (0.16)
Cingulum antérieur left SUVr	0.74 (0.19)	0.62 (0.06)	0.95 (0.17)
Cingulum postérieur right SUVr	0.75 (0.21)	0.62 (0.06)	0.97 (0.19)
Cingulum postérieur left SUVr	0.76 (0.21)	0.64 (0.07)	0.97 (0.22)

Data are mean (standard derivation) or number (%).
CDR, clinical dementia rating; CPAP, continuous positive airway pressure; FCSRT, Free end Cued Selective Reminding Test; HBP, high blood pressure; MCQ, Memory Complaints Questionnaire; MMSE, Mini-Mental State Examination; OSA, obstructive sleep apnea; SUVr, standard absorption ratio.

normal range (Table 1). The two groups Aß positive and Aß negative did not significantly differ for questionnaire assessing subjective cognitive feelings (MCQ) [28] and there was no difference for the other cognitive tests, including the FCSRT total recall and MMSE (Table 1).

Association between sleep parameters with Aß burden and hippocampal volume

The comparison between participants with PET Aβ– and the PET Aβ+ showed difference for sleep quality during 7 days 24-hour consecutive periods (Table 2).

The PET Aß+ participants had significant worst sleep efficiency ($p < 0.001$) with longer sleep latency ($p < 0.001$) and WASO score ($p = 0.006$). Awakenings were also significantly higher ($p = 0.005$). There was no significant difference for TST between the two groups. Daytime sleep was lower in the PET Aß– group where 90.5% had fewer than three naps per week.

The differences remained significant even after adjusting for sex, age, depression, and MMSE. Several sleep parameters were associated with Aß load: Sleep latency: OR = 1.36 (1.18; 1.72), $p = 0.008$, numbers of awakenings: OR = 1.64 (1.16; 2.41), $p = 0.007$ and WASO score: OR = 1.05 (1.02; 1.08), $p = 0.005$.

We found no significant association between positive amyloid status and total time in bed or total sleep time. In contrast, sleep efficiency was a "protective" factor for positive amyloid status: OR = 0.59 (0.44; 0.72), $p < 0.001$ (Table 3).

There was no significant correlation between regional brain amyloid deposition and sleep parameters. Furthermore, correlation analysis revealed no significant association between sleep patterns and the hippocampal volumes (Tables 4–6).

Discussion

In the present study comparing sleep parameters, objectively assessed using ambulatory accelerometer, and the brain amyloid load among elderly individuals with subjective cognitive

Table 2. Comparisons between sleep parameters and amyloid status

	Amyloid status			
	Negative (n = 44)	Positive (n = 24)	P^*	P^{**}
SL (min)	21.58 (8.23)	49.04 (15)	<0.001	<0.001
Sleep efficiency (%)	90.72 (4.04)	83.49 (3.59)	<0.001	<0.001
WASO (min)	26.76 (18.08)	39.88 (18.61)	0.006	0.006
Awakening	2.73 (1.74)	3.71 (1.66)	0.026	0.005
AA (min)	9.23 (2.71)	10.62 (3.53)	0.074	0.173
TST (min)	463.07 (47.72)	447.69 (57.22)	0.241	0.256
TTB (min)	510.86 (53.06)	536.72 (65.17)	0.081	0.158
Nap >3/week (%)	23.1	76.9	<0.001	
Nap <3/week (%)	90.5	9.5	<0.001	
TST ≤ 420 min (%)	37.5	62.5	0.009	
TST > 420 min (%)	73.1	26.9	0.009	

AA, average awakenings; SE, sleep efficiency; SL, sleep latency; TST, total sleep time; TTB, total time in bed; WASO, wake after sleep onset.
Values in parentheses correspond to standard deviation.
P^*= Student t-test. P^{**}= Wilcoxon Test.

Table 3. Associated factors with a positive amyloid status

	Amyloid status		
	Adjusted OR	(95% CI)	P
SL	1.36	(1.18; 1.72)	0.008
Sleep Efficiency	0.59	(0.44; 0.72)	<0.001
WASO	1.05	(1.02; 1.08)	0.005
Awakening	1.64	(1.16; 2.41)	0.007
AA	1.17	(0.98; 1.43)	0.091
TST	0.99	(0.98; 1.00)	0.067
TTB	1.01	(0.98;1.02)	0.157
TST≤420 min	6.08	(1.67; 26.51)	0.009
Nap ≥3/week	35.66	(9.04; 189.6)	<0.001

AA, average awakenings; CI, confidence interval; SE, sleep efficiency; SL, sleep latency; TST, total sleep time; TTB, total time in bed; WASO, wake after sleep onset.
P: results obtained after logistic regression. Variable adjustment on age, sex, depression, and MMSE score. Values in parentheses correspond to the 95% confidence interval.

complaint, we highlighted that participant considering as positive in the PET-amyloid imaging prior to cognitive impairments had more sleep disturbances such as a longer sleep latency, more frequent awakenings, and an increase of the WASO score. In addition, they presented a worst sleep efficiency and a trend for more daytime sleep and a shorter nighttime sleep.

Our study confirms a previous actigraphy study, which reported that sleep efficiency was worst in cognitive healthy elderly subject with low level of Aß in cerebrospinal fluid (CSF) [19]. Results also show more frequent daytime sleep with amyloid deposition but no difference in sleep duration [19]. Our results are also in agreement with amyloid PET-scan studies assessing sleep using questionnaires [21, 23, 24, 42]. These studies showed that a poor sleep quality is associated with higher cortical amyloid burden. Nevertheless, there was a predominance of amyloid deposition in the precuneus [21] but also in other areas of interest such as the angular gyrus, the cingulate gyrus, and the frontal regions [42]. In our study, we did not find such specific predominance. Spira et al. reveal a strong association between amyloid deposition in precuneus and short duration of sleep. We also found a trend with lower sleep duration (<7 hours) and cerebral amyloid burden, but a limited proportion of short sleepers in our study and a large standard deviation make our results unclear. Finally, Branger et al. show a specific link between a longer sleep latency and prefrontal amyloid deposition but does not find any link with the other parameters involved in sleep quality. However, sleep parameters were subjectively recorded [23].

Their results also support our results, finding no association between sleep disturbances and cortical thickness of areas at risk of early atrophy in AD such as the internal temporal lobe. Brown et al. also found specific association, between sleep latency and amyloid deposition. However, amyloid deposit was more global and not specific to a region of interest, as shown in our study [24].

Finally, recent study in preclinical AD performed several circadian rhythm analysis on actigraphy as rest-activity rhythm fragmentation, amplitude, phase, or robustness. Indeed, intradaily variability (who represents how consolidated the rest-activity rhythm is whithin each 24-hour period) indicates, when higher, more fragmentation of the rest-activity pattern. Results showed that preclinical AD could be associated with rest-activity rhythm fragmentation, especially, the presence of amyloid plaque pathology was associated with increased intradaily variability [43].

Unlike amyloid deposition, we did not find any correlation between hippocampal volumes and sleep quality. However, a trend was found, sleep efficiency was positively correlated (Pearson coefficient = 0.240, p Pearson value = 0.048) with the right hippocampal volume, when we defined WASO as an awakening ≥1 minute and no more as an awakening ≥ 5 minutes. We done both analysis and kept mainly WASO as an awakening ≥ 5 minutes because this parameter seems to be overestimated by wrist actigraphy [32, 44] however, this overestimation is not always found [45] and other's studies measuring sleep in subjective cognitive decline have used WASO as an awakening ≥1 minute [19, 46].

Our results are interesting because a link between sleep–wake disturbances, accumulation of Aß peptide and alteration of hippocampal neurogenesis is possible [47]. In fact, sleep fragmentation seems to induce suppression of hippocampal neurogenesis via activation of glucocorticoids pathways [48–51], and by a proinflammatory state mediated by cytokines [52–58]. Second, theses sleep disturbances could also induce neuronal hyperexcitability via N-Méthyl D-Aspartique Acid (NMDA) receptors and glutamate [59]. It could also suppress the proliferation of cellular neurogenesis [47, 60]. Studies in Drosophila and

Table 4. Correlation analysis between actigraphic sleep parameters and right hippocampal volume

	Right hippocampal volume			
	Coeff. Pearson	*P*-Pearson	Rho Spearman	*P*-Spearman
SL (min)	−0.125	0.311	**−0.098**	**0.426**
Sleep efficiency (%)	0.249	0.04	**0.186**	**0.128**
WASO (min)	−0.223	0.068	**−0.089**	**0.469**
AA (min)	−0.032	0.797	**−0.043**	**0.727**
Awakenings	−0.250	0.040	**−0.076**	**0.535**
TST (min)	**0.142**	**0.246**	0.142	0.248
TTB (min)	**0.014**	**0.911**	0.060	0.624

Shapiro test was used to define normal or non-normal data distribution. According to these results, Pearson test and Spearman test were respectively used for correlation analysis. Data in bold indicates the test used.
AA, average duration of awakenings in minutes with awakening defined as ≥ 5 min; SE, sleep efficiency with awakening defined as ≥ 5 min = TST/TTB; SL, sleep latency; TTB, total time in bed; TST, total sleep time with awakening defined as ≥ 5 min = TTB-SL-WASO; WASO, wake after sleep onset ≥ 5 min.
Awakening: Number of awakening with awakening defined as ≥ 5 min.

Table 5. Correlation analysis between actigraphic sleep parameters and left hippocampal volume

	Left hippocampal volume			
	Coeff. Pearson	*P*-Pearson	Rho Spearman	*P*-Spearman
SL (min)	−0.040	0.745	**0.024**	**0.846**
SE (%)	0.130	0.290	**0.077**	**0.531**
WASO (min)	−0.163	0.185	**−0.093**	**0.452**
AA (min)	−0.095	0.441	**−0.134**	**0.275**
Awakenings	−0.129	0.296	**−0.034**	**0.781**
TST (min)	**−0.004**	**0.972**	0.004	0.976
TTB (min)	**−0.071**	**0.568**	−0.017	0.890

Shapiro test was used to define normal or non-normal data distribution. According to these results, Pearson test and Spearman test were respectively used for correlation analysis. Data in bold indicates the test used.
AA, average duration of awakenings in minutes with awakening defined as ≥ 5 min; SE, sleep efficiency with awakening defined as ≥ 5 min = TST/TTB; SL, sleep latency; TST, total sleep time with awakening defined as ≥ 5 min = TTB-SL-WASO; TTB, total time in bed; WASO, wake after sleep onset ≥ 5 min.
Awakening: Number of awakening with awakening defined as ≥ 5 min.

rodents have shown that sleep deprivation upregulates NMDA receptors in neurons, which increase neuronal excitability and could lead to neuronal death [61–63].

However, excess of neuronal activation may increase the accumulation of Aβ peptide [4–8]. A vicious circle could happen because Aβ peptide could also induce a chronic activation of NMDA receptors and lead again to excitotoxicity and cell death [64]. In addition, these mechanisms could be amplified by the accumulation of Aβ peptide induced by other pathways such as the glymphatic system. Thus, there seems to be a quite direct link between sleep fragmentation and Aβ peptide accumulation, whereas the links between hippocampal neurogenesis and sleep disturbances would be more complex, involving Aβ peptide.

Nevertheless, a study in community-dwelling middle-aged adults found that sleep quality was widely correlated with longitudinal measures of cortical atrophy, but not hippocampal atrophy [65]. The study however assessed only subjective sleep quality using Pittsburgh Sleep Quality Index, but no objective measure of the quality of sleep–wake rhythm.

Table 6. Correlation analysis between actigraphic sleep parameters and mean hippocampal volume

	Mean hippocampal volume			
	Coeff. Pearson	*P*-Pearson	Rho Spearman	*P*-Spearman
SL (min)	−0.086	0.486	**−0.035**	**0.774**
SE (%)	0.201	0.101	**0.132**	**0.284**
WASO (min)	−0.206	0.092	**−0.090**	**0.466**
AA (min)	−0.070	0.569	**−0.105**	**0.394**
Awakenings	−0.200	0.102	**−0.053**	**0.670**
TST (min)	**0.070**	**0.572**	0.074	0.550
TTB (min)	**−0.033**	**0.788**	0.022	0.86

Shapiro test was used to define normal or non-normal data distribution. According to these results, Pearson test and Spearman test were respectively used for correlation analysis. Data in bold indicates the test used.
AA, average duration of awakenings in minutes with awakening defined as ≥ 5 min; SE, sleep efficiency with awakening defined as ≥ 5 min = TST/TTB; SL, sleep latency; TST, total sleep time with awakening defined as ≥ 5 min = TTB-SL-WASO; TTB, total time in bed; WASO, wake after sleep onset ≥ 5 min.
Awakening: Number of awakening with awakening defined as ≥ 5 min.

Furthermore, Lauriola et al. measured sleep by actigraphy in elderly with subjective cognitive decline and shown that objective sleep resulted disrupted compared to controls, without any MRI cortical change even they defined awakenings as ≥ 1minute [46]. Nonetheless, they have found a significant difference only in left medial orbitofrontal thickness, with subjective cognitive decline showing smaller values compared to controls, but this group difference was not maintained accounting for apnea risk, Geriatric Depression Scale, age, and sex. Explanations could be that some participants may have only recently disrupted sleep or because of lack of statistical power.

However, a recent study including 138 participants revealed strong positive correlation of medial temporal lobe atrophy with the fragmentation of the sleep–wake rhythm measured by actigraphy [66]. Authors hypothesized that sleep fragmentation could induce a selective loss of the 2B subunit of NMDA receptors from hippocampal synaptic membranes.

Our study is interested in having tried to correlate objectively both sleep by actigraphy, and the presymptomatic phase of AD by amyloid PET scan. An additional strength of the results is that the INSIGHT-preAD study is a single center cohort, meaning that all participants are assessed by the same team and with the same neuroimaging scanners, which keep variance of data and results to a minimum.

Our study also has several limitations. First, we did not have clinical information on excessive daytime sleepiness (EDS) and we did not assess this clinical parameter with standardized questionnaire. In fact, EDS could influence Aß accumulation in elderly persons without dementia as longitudinal study has shown [67, 68].

Afterwards, we did not consider cardiovascular risk factors. Indeed, it can induce subcortical ischemia and cause vascular cognitive impairment, which could be a patients' selection bias [69]. In addition, an interaction between sleep and vascular cognitive disorders seems exist. Effectively, a positive correlation has been shown between sleep disturbances and severity of white matter hyperintensities in brain MRI of vascular cognitive disorders subjects [70]. In our study, 45 subjects had at least one of three cardiovascular risk factors among high blood pressure, dyslipidemia, and coronary artery disease. In Aß+ group,

17 subjects had one of these risk factors (70%) and in Aß–, 28 subjects (63%). It seems necessary to consider these risk factors, especially since they are also known to be risk factors for AD [69].

Likewise, we did not consider obstructive sleep apnea (OSA) in our analysis. Several studies have shown association between OSA and markers of increased amyloid burden. Sleep quality and/or intermittent hypoxia from OSA are likely candidate mechanisms [71, 72]. However, in our study, in Aß-negative group, there was only one participant with untreated OSA and another one with OSA treated by CPAP. There was no OSA in Aß-positive group.

In our study design, we also did not consider the neural structure protein, Tau that could interact with sleep quality. Indeed, an increased amount of phosphorylated Tau in the CSF appears to be associated with poorer sleep quality in healthy elderly subjects [73].

A recent study has shown that in Aβ-positive subjects, elevated levels of YKL-40 and total Tau and phosphorylated Tau in CSF were predictive of poor sleep quality reported by questionnaires [74]. Other studies measuring the link between these different actors and sleep would be interesting to better understand the complexity of the interaction between Aβ peptide and sleep.

In addition, the results of our study did not provide a response about causality between sleep disturbances and amyloid deposition. In the literature, depending on the method and the question asked, one or the other of these hypotheses could be confirmed. In fact, amyloid plaques seem to appear in brain pathway controlling the sleep–wake cycle, which could induce sleep disturbances [75, 76]. Inversely, poor sleep quality could enhance Aß aggregation [3, 77, 78].

However, a bidirectional relationship could exist. Sleep quality impairment would induce a higher risk of amyloid plaque formation and, in a positive feedback, accumulation of amyloid plaques in brain regions involved in the sleep–wake cycle, would accentuate sleep disturbances [77].

Circadian rhythms are optimal when suprachiasmatic nuclei are synchronized on a 24-hour period of time. However, it has recently been shown that, as the luminous environment, physical activity can act as a "synchronizer." It is interesting to underline this link since several studies have shown the association between regular physical activity and low amyloid burden [79, 80].

Treatment repercussions are interesting. We could recommend rehabilitation and training programs for regular physical activity associated with optimizing sleep quality in presymptomatic AD subjects. Prospective studies assessing the impact of this type of program on amyloid burden would be interesting.

In conclusion, our study reported an association between sleep–wake disturbances as longest sleep latency, more awakenings, increased WASO score, and worst sleep efficiency with Aß burden but without specific brain region localization.

No significant association was found between sleep parameters and hippocampal volumes. Only a trend was found between sleep efficiency, awakenings, and right hippocampal thickness.

Conflict of interest statement

B.D. has received consultancy fees from Biogen, Boehringer Ingelheim, Eli Lilly, and MedAvante and grants for his institution from Merck, Pfizer, and Roche. H.B. has received speaker fees from Roche. M-O.H. has received consultant fees from Eli Lilly and speaker fees from Piramal. E.E., M.S., M.L.N., A.G., P.R., and R.D. have conflict of interest that data collection and sharing for this project were funded by the the INveStIGation of AlzHeimer's PredicTors in Subjective Memory Complainers study (INSIGHT). INSIGHT is funded by generous contributions from the following sponsors: IHU/ICM1, PFIZER2, AVID3, MEMENTO study4, Fondation Plan Alzheimer5, Institute of Memory and Alzheimer's Disease, Paris, France6

[1]IHU-A-ICM - Institut de recherche translationnelle en Neurosciences | Paris Institute of Translational Neurosciences, Paris, France (http://icm-institute.org/menu/fondation/ihua-icm) ICM - Institut du Cerveau et de la Moelle épinière|Brain & Spine Institute, Paris, France (http://icm-institute.org)

[2]Pfizer Pharmaceuticals Inc. (https://www.pfizer.fr|http://www.pfizer.com)

[3]Avid Pharmaceuticals Inc. (http://www.avidrp.com)

[4]MEMENTO (DeterMinants and Evolution of AlzheiMer's disEase aNd relaTed disOrders) Study - Centre Hospitalier Universitaire de Bordeaux, Bordeaux, France (www.chu-bordeaux.fr, https://clinicaltrials.gov/show/NCT01926249)

[5]Fondation Plan Alzheimer, France (http://www.fondation-alzheimer.org)

[6]IM2A - Institut de la Mémoire et de la Maladie d'Alzheimer|Institute of Memory and Alzheimer's Disease, Paris, France.

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