Brain and cognitive correlates of sleep fragmentation in elderly subjects with and without cognitive deficits

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Abstract:

Introduction: Sleep disturbances are increasingly recognized as a risk factor for Alzheimer's disease (AD). However, no study has assessed the relationships between objective sleep fragmentation (SF), brain and cognitive integrity across different cognitive stages, from cognitively unimpaired elderly subjects to patients with Subjective Cognitive Decline (SCD) and/or Mild Cognitive Impairment (MCI).

Methods: 30 cognitively unimpaired elderly participants and 36 SCD/MCI patients underwent a neuropsychological evaluation, structural MRI, ^{18}F-fluorodeoxyglucose and ^{18}F-Florbetapir PET scans, and an actigraphy recording over a minimum of six consecutive nights. Multiple regression and mediation analyses were performed between SF parameters, neuroimaging data, and cognitive scores.

Results: In cognitively unimpaired elderly participants, SF intensity mediated the association between fronto-hippocampal hypometabolism and lower executive functioning. Moreover, to a lower extent, increased SF variability was related to thalamic atrophy and ventromedial prefrontal amyloid burden. However, in SCD/MCI patients, SF no longer contributed to the expression of cognitive deficits.

Discussion: These findings suggest that SF may directly contribute to lower cognitive performance in cognitively unimpaired elderly subjects. Therefore, treating sleep disturbances before the onset of cognitive deficits may help to cope with brain alterations and maintain cognitive functioning.

<u>Keywords:</u>

Sleep, ageing, Subjective cognitive decline, Mild cognitive Impairment, MRI, amyloid,

glucose metabolism, actigraphy, sleep fragmentation.

<u>Abbreviations:</u>

Aβ, beta-amyloid; AD, Alzheimer's disease; BMI, body mass index; FDG, ^{18}F-

fluorodeoxyglucose; MCI, mild cognitive impairment; MMSE, mini mental state

examination; NREM, non rapid eye movement; SCD, subjective cognitive decline; SF, sleep

fragmentation.

<u>Highlights</u>

- Sleep fragmentation relates to brain and cognitive changes in healthy ageing.

- Sleep fragmentation did not impact the expression of cognitive deficits in patients.

- Treating sleep disturbances may be optimal before the onset of cognitive deficits.

Research in context:

Systematic Review: In the existing literature, sleep disturbances are increasingly recognized as a risk factor for Alzheimer's disease (AD). However, no previous study has assessed the associations between sleep disruption, brain and cognitive integrity across different cognitive stages.

Interpretation: We showed that the disruption of the first hours of sleep is related to the alteration of brain regions typically affected in ageing and AD, and may directly contribute to lower cognitive performance in cognitively unimpaired elderly participants. However, this is no longer the case in SCD/MCI patients.

Future Direction: These findings have important clinical implications, as they suggest that treating sleep disturbances before the onset of cognitive deficits may help to cope with brain changes and maintain cognitive performance. Further studies need to be conducted on larger samples and using polysomnography to assess changes in sleep architecture and microstructure more specifically.

1. Introduction

As no curative treatment for Alzheimer's disease (AD) is currently available, it is particularly important to identify modifiable lifestyle factors that might help prevent, delay the onset, or slow down the progression of the disease. In this context, there is a growing interest in better characterizing age-related sleep changes and their associations with AD. Indeed, ageing is characterized by a progressive decline in sleep quality, including a decrease in non-rapid eye movement (NREM) sleep, paralleled by greater sleep fragmentation (SF) [1]. Moreover, age-related changes in sleep quality have been related to cognitive decline and increased risk of developing dementia [2–4]. Sleep disturbances are also a core symptom of AD [1]. They worsen with dementia severity and are related to patients' cognitive deficits, especially memory impairments [5]. Accumulating evidence indicates that the disruption of slow-wave sleep, the deepest NREM-sleep stage predominating during the first part of the night, is associated with increased beta-amyloid (Aβ) deposition in the brain [6–8], a hallmark of AD known to start decades before the apparition of the first cognitive symptoms [9]. Moreover, several self-reported sleep parameters, including longer sleep latency [10,11] and shorter sleep duration [12], have been associated with increased amyloid deposition in frontal areas and/or in the precuneus. In addition to amyloid deposition, sleep disruption may also be associated with neuronal injury, as measured with grey matter atrophy or hypometabolism, both known to be closely related to cognitive decline [13,14]. Previous studies reported that, in cognitively unimpaired elderly subjects, poor self-reported sleep quality is related to reduced grey matter volume in frontal regions and the insula [10,15], and that increased actigraphy-measured sleep fragmentation is associated with atrophy in the orbitofrontal cortex [16].

However, we lack a comprehensive overview of the relationships between objective measures of sleep quality and amyloid deposition, atrophy and brain glucose metabolism in cognitively

unimpaired elderly participants. Furthermore, we have no information about these links in patients with subjective and/or objective cognitive deficits. Clarifying these associations using complementary neuroimaging modalities that reflect different aspects of brain integrity, and across different cognitive stages, would help determining to what extent and at which stage of the disease the treatment of sleep disturbances would be beneficial to prevent cognitive decline.

Thus, the present study aims at investigating and comparing the relationships between objectively measured SF and cognitive, structural, functional and molecular brain changes, in cognitively unimpaired elderly subjects versus in patients with subjective and/or objective cognitive deficits, including subjective cognitive decline (SCD) and mild cognitive impairment (MCI) patients.

2. Materials & Methods.

2.1. Participants

Sixty-six participants from the IMAP+ study ("Imagerie Multimodale de la Maladie d'Alzheimer à un stade Précoce", Caen, France) were included in the present study. They were all right-handed, native French speakers, with at least 7 years of education, living at home, without history or clinical evidence of neurological or psychiatric disorders, alcohol use disorder or drug abuse, and with a normal somatic examination. The inclusion and group classification of the participants were based on a clinical interview and a standardized neuropsychological assessment, according to internationally agreed criteria, but did not rely on neuroimaging biomarkers, as they were the main outcome measures in our analyses.

Thirty cognitively unimpaired elderly subjects were recruited from the community. They were aged over 60 years, had no memory complaint and never referred to a memory clinic, and performed in the normal range (i.e., within 1.65 standard deviation (SD) of the mean) for their

age and education level in all screening tests. In addition, thirty-six patients aged over 50 years were recruited from local memory clinics, to which they attended for self-reported cognitive concerns. During the clinical interview, the physician ensured i) that the complaint was not related to current medication, psychiatric or neurological diseases (including anxiety or depression), or other medical conditions, ii) that independence in daily-life was preserved, and iii) that they did not fulfil NINCDS-ADRDA criteria for probable AD [17]. Among the 36 patients, 22 had objective cognitive deficits and met clinical criteria for single or multiple domain amnestic mild cognitive impairment (aMCI) [18], with predominant episodic memory deficits (1.5 SD from the normal mean for age and education). Fourteen patients did not show significant objective cognitive impairment and met criteria for subjective cognitive decline (SCD) [19]. Clinical diagnosis for patients was assigned by consensus under the supervision of senior neurologists and neuropsychologists.

Once included in the study, all participants performed a detailed neuropsychological assessment, an actigraphy recording and three neuroimaging scans within a mean interval of 1.9 ± 3.1 months. The IMAP+ study was approved by the local ethics committee (CPP Nord-Ouest III) and registered at http://clinicaltrials.gov (nb. NCT01638949). All participants gave their written informed consent to the study prior to the examinations.

2.2. Cognitive assessment

Participants underwent a detailed neuropsychological assessment, encompassing multiple domains of cognition (verbal and visual episodic memory, semantic memory, working memory, executive functioning, processing speed, visuospatial abilities, language skills and praxis), fully described in previous publications [20,21]. In the present study, analyses focused on episodic memory and executive functioning, as they are particularly sensitive to ageing and AD [22], and closely related to sleep quality [23]. We used composite scores to

reflect cognitive abilities with robust proxies and to minimize the issue of multiple statistical testing (see [24] and **Supplementary Material** for further details). Higher values always indicated better performance. Furthermore, symptoms of depression and anxiety were assessed using the Montgomery-Asberg Depression Rating Scale [25] and the trait version of the State-Trait Anxiety Inventory [26], respectively.

2.3. Actigraphy recording

2.3.1. Actigraphy data collection

The sleep-wake cycle was recorded in all participants using the MotionWatch 8 wrist-worn tri-axial actigraph (CamNTech Ltd, Cambridge, UK), for at least six consecutive nights (range: 6-8 nights; mean ± SD: 7.05 ± 0.51 nights). Participants were instructed to wear continuously the device on their non-dominant wrist until the end of the recording. They had to press the event marker button at lights off and lights on, and to fill in a sleep diary upon awakening each morning [27] to facilitate data analysis.

2.3.2. Actigraphy data analysis

Data were analyzed using the MotionWare software (version 1.1.25, CamNTech Ltd, Cambridge, UK), and sampled using a 5-seconds epoch. A sensitivity threshold of 20 counts was applied to distinguish activity from rest. For each night, the "Lights Off" and "Got Up" markers were placed by the same experimenter in the appropriate position following the event marker put by the participant, and cross-validated with the light sensor data and sleep diary information. Then, "Fell Asleep" and "Woke up" markers were automatically adjusted from the combination of the activity data and the "Lights Off" and "Got Up" markers, respectively, allowing the collection of the assumed sleep time. Then, a fragmentation index was calculated as the sum of the percentages of mobile time and immobile bouts below or equal to one minute in the assumed sleep period. Two distinct parameters of SF were considered: SF

intensity (corresponding to the mean level of fragmentation over the whole recording), and intra-individual SF variability (computed as SF standard deviation across nights) **(Fig. 1)**. These parameters were computed over the first half of the assumed sleep duration for each subject.

2.4. Neuroimaging procedure

Participants underwent a structural T1 MRI, as well as ^{18}F-fluorodeoxyglucose (FDG-) and Florbetapir-PET scans, to measure grey matter volume, brain glucose metabolism and amyloid burden, respectively. All examinations were performed at the Cyceron Center (Caen, France). Details on MRI and PET images acquisition and pre-processing are available in previous publications [28,29] and are fully described in the **Supplementary Material**. Briefly, T1-weighted images were pre-processed in SPM12. FDG-PET and Florbetapir-PET images were pre-processed using MRI data for partial volume effect correction and spatial normalization. PVE-corrected normalized and scaled Florbetapir-PET images were also used to extract the individual global cortical amyloid standard uptake value ratio (SUVr) using a predetermined neocortical mask including the entire grey matter, except the cerebellum, occipital and sensory motor cortices, hippocampi, amygdala and basal nuclei [30].

2.5. Statistical analyses

2.5.1. Group comparisons

Between-group comparisons for demographics, cognitive scores and sleep parameters were assessed using Student t-tests for continuous variables and chi-square tests for categorical variables, with statistical significance set to $P<.05$.

Patients' patterns of atrophy, hypometabolism and amyloid burden were explored using two-sample t-tests in SPM12, adjusted for age, gender and body mass index (BMI).

2.5.2. *Voxel-wise regression analyses*

Within each group, voxel-wise multiple regression analyses were performed between SF parameters and each neuroimaging modality independently in SPM12, controlling for age, gender, BMI and the complementary SF parameter (i.e., we controlled for SF variability when SF intensity was the predictive variable, and conversely). Afterwards, complementary analyses were performed with the addition of several covariates to the main model. Only results obtained with the initial 4-covariates model are presented, as the main results remained essentially unchanged when adding the MMSE, depression score, or sleep medication use as covariates. We report the slight changes observed on these secondary analyses in the **Supplementary Material**. For all neuroimaging analyses, results were evaluated for significance at $P<.001$ (uncorrected) combined with a minimum cluster size determined by Monte-Carlo simulation using the AlphaSim program to achieve a corrected statistical significance of $P<.05$ (**Supplementary Table 1**). Results obtained at the same statistical threshold but with a lower cluster size were considered as trends.

2.5.3. *Multiple regression analyses*

Multiple regression analyses were performed between SF parameters and cognitive scores (i.e., composite scores for executive functioning and episodic memory) within each group. Age and gender were included as covariates in the regression models, and results were considered significant after applying a Bonferroni correction for multiple comparisons: the statistical threshold for significance was set to $P=(.05/\text{number of comparisons})$.

2.5.4. *Mediation analyses*

When the same SF parameter was significantly associated to both a brain and a cognitive variable, then causal mediation analyses were performed in order to assess the directionality of the relationships. For this purpose, brain data (i.e., grey matter volume, glucose metabolism or amyloid values) were extracted in the clusters significantly associated with SF parameters

in the voxel-wise analyses described above. Then, two different models were tested to determine whether the brain variable mediated the relationships between sleep and cognition or whether the sleep variable mediated the relationships between the brain variable and cognition. These analyses were performed using the R package 'mediation' [31], and we report the average direct effects (ADE) and average causal mediation effect (ACME) estimated using non-parametric bootstrapping (5000 bootstrap resamples, $P<.05$) for both models.

3. Results

3.1. Between-group differences

Demographic data, neuropsychological performance, and sleep parameters for each subgroup, as well as between-group differences, are reported in **Table 1**. The two groups did not differ for age, gender, years of education, BMI, state-trait anxiety, and sleep parameters. As expected, SCD/MCI patients presented lower global cognitive functioning (MMSE: t=2.03, P=.046; Mattis dementia rating scale: t=2.88, P=.005), episodic memory (t=2.92, P=.005) and executive performance (t=2.39, P=.020). They also presented higher depressive symptoms (MADRS: t=-3.27, P=.002). Moreover, SCD/MCI patients presented significant brain changes compared to cognitively unimpaired elderly subjects including (i) atrophy within the para-hippocampal and hippocampal region, (ii) hypometabolism in the precuneus and posterior cingulate cortex, (iii) widespread amyloid deposition mainly in frontal regions **(Supplementary Fig. 1)**. Consistently, the global cortical amyloid SUVr was significantly higher in SCD/MCI patients compared to cognitively unimpaired elderly subjects (t=-2.61, P=.011).

3.2. Relationships between SF and neuroimaging

Results of significant voxel-wise regression analyses are presented in **Fig. 2**, and detailed peak statistics and coordinates of significant clusters are reported in **Supplementary Table 2**.

3.2.1. *Cognitively unimpaired elderly subjects*

SF intensity negatively correlated to brain glucose metabolism in the ventromedial prefrontal cortex, hippocampus and parahippocampus, bilaterally. No significant association was found with grey matter volume or amyloid burden.

SF variability negatively correlated to grey matter volume within the thalamus. Moreover, we observed a trend towards a positive correlation with amyloid burden in the left rectus gyrus. When sleep medication was added as a covariate in the model, both associations became trends **(Supplementary Table 3)**. No association was found between SF variability and brain glucose metabolism.

3.2.2. *SCD/MCI patients*

SF intensity negatively correlated with brain glucose metabolism in the left insula while no significant association was found with grey matter volume or amyloid burden. SF variability was not associated with any neuroimaging modality.

3.3. *Relationships between SF and cognition*

In cognitively unimpaired elderly subjects, SF intensity was significantly associated to worse performance in executive functioning (**Fig. 3A**; r=-0.47, *P*=.01), and episodic memory (**Fig. 3B**; r=-0.40, *P*=.03), although this latter did not survive the Bonferroni correction for multiple comparisons. In contrast, SF variability was not related to any cognitive score in this group.

We did not find any significant association between SF parameters and cognitive performance in SCD/MCI patients (see **Supplementary Table 4** for detailed results).

3.4. *Mediation analyses in cognitively unimpaired elderly subjects*

As SF intensity was significantly associated to both executive performance and hippocampal and prefrontal glucose metabolism, these three variables were entered in causal mediation analyses. The results of the two models tested in cognitively unimpaired elderly subjects are summarized in **Fig. 4**. They showed that SF significantly mediated the relationship between total brain glucose metabolism and executive performance (p=0.02; **Table 2)**. No other mediation analysis was conducted, as no other sleep variable was significantly associated to both a brain and a cognitive variable.

4. Discussion

The present study aimed at investigating and comparing the brain and cognitive correlates of SF in healthy ageing versus in a group of SCD/MCI patients. In cognitively unimpaired elderly participants, SF intensity was related to frontal and medial temporal metabolism, and to cognitive performance, especially executive functioning. Moreover, SF variability was related to thalamic atrophy and, to a lesser extent, to frontal amyloid deposition. By contrast, in SCD/MCI patients, we only report a significant association between SF intensity and glucose metabolism in the left insula, while no relationship between sleep and cognition was found.

The brain regions found to be associated with the fragmentation of the first hours of sleep include frontal and medial temporal areas, as well as the thalamus in cognitively unimpaired elderly participants, and the insula in patients. These regions are known to be closely involved in sleep physiology. They are largely involved in the generation of NREM-sleep oscillations, such as sleep spindles [32] and slow waves [33,34], that are notably critical for sleep-dependent memory processes [35,36]. Besides, these regions are also known to be particularly sensitive to ageing and affected in the early stages of AD. Indeed, on the one hand, the medial prefrontal cortex is one of the first brain regions exhibiting amyloid deposition [37], and is

also sensitive to glucose metabolism changes in ageing and AD [38–40]. On the other hand, the medial temporal lobe is early affected by tau pathology [41], with atrophy already detectable in the preclinical and prodromal stages [42,43]. The involvement of this common set of brain regions in both sleep physiology and AD pathophysiology might at least partly underlie the (possibly bidirectional) links between sleep problems and AD.

As a first step towards characterizing the directionality of these associations, we performed causal mediation analyses. We showed that the intensity of the fragmentation of the first hours of sleep mediated the relationship between fronto-hippocampal hypometabolism and lower executive performance. This suggests that sleep disruption resulting from fronto-hippocampal hypometabolism may directly contribute to cognitive deficits in cognitively unimpaired elderly subjects. Therefore, treating sleep disturbances in cognitively unimpaired elderly subjects exhibiting a high level of SF could improve their ability to cope with brain changes and potentially reduce their risk of cognitive decline. Nevertheless, due to a limited statistical power, mediation analyses were restricted to a single set of variables (i.e., sleep fragmentation, brain glucose metabolism in fronto-hippocampal areas and executive functioning), as they were the only ones for which the same sleep parameter was related to both a brain and a cognitive variable. Thus, these analyses are likely to picture only part of the potential associations existing between sleep, brain and cognitive integrity. They do not preclude that some sleep changes could induce brain alterations that may, in turn, underlie cognitive deficits [44]. Moreover, it is also likely that some brain changes are directly underlying cognitive deficits, independently from sleep disturbances.

Besides the associations between metabolic changes and SF intensity, our results reveal that a higher night-to-night variability of SF was rather associated to structural and molecular brain alterations, namely grey matter atrophy and amyloid deposition. Of note, these results should be taken cautiously as they were only trends when taking into account regular use of sleep

medication. This suggests that experiencing inconsistent sleep quality over time might be associated with brain changes that might be less reversible, i.e. more durable and that might conduct to long-term cognitive deficits and dementia. This interpretation is in line with existing data showing that a greater variability in sleep quality is related to a higher risk of cognitive impairment and dementia [4,45], as well as other physical and mental health conditions [46,47]. Further investigations need to be conducted in order to unravel the determinants of this aspect of sleep, such as the use of sleep medication, lifestyle or psycho-affective factors that might vary from one day to another and induce changes in sleep quality. Addressing this question is essential in order to be able to take over sleep problems in their entirety in the elderly population.

By contrast to our results in cognitively unimpaired individuals, the relationships between sleep and brain or cognitive variables were almost non-existent in SCD/MCI patients – only a link between SF intensity and insula glucose metabolism was found. We hypothesize that, while cognitive performances are directly impacted by sleep quality (as probably other lifestyle factors) in asymptomatic individuals, they may be mainly and more directly driven by underlying neuropathological processes (such as amyloid deposition) at a cognitively impaired (SCD/MCI) stage, so that the direct influence of sleep disruption would no longer be detectable. This observation is supported by previous works showing no association between slow-wave sleep fragmentation nor the amount of REM-sleep and cognitive performance in MCI patients [48,49]. Taken together, these results suggest that preserving sleep quality could help to cope with brain alterations and maintain cognitive performance in the normal range, but such interventions may be less efficient once patients experience measurable cognitive deficits.

The present study has some strengths, including an objective sleep assessment over several consecutive nights combined to multimodal neuroimaging data, allowing us to assess for the

first time the relationships between sleep fragmentation intensity versus variability on structural, metabolic and molecular brain alterations in the same sample. Moreover, these associations were evaluated both in cognitively impaired and unimpaired elderly subjects, thus helping to give a more comprehensive picture of their role at different cognitive stages. However, some limitations must be mentioned. First, our study is limited by the small sample size and the cross-sectional nature of the analyses. Further analyses should be conducted using a longitudinal design, together with larger sample sizes in order to complement our mediation analyses and assess the nature and directionality of the relationships between sleep, brain and cognitive integrity more exhaustively. Second, we were not able to characterize the origin of sleep fragmentation (e.g., obstructive sleep apnea, periodic limb movements or restless legs syndrome), as participants did not undergo polysomnography recordings. Further studies should aim at determining the relative contribution of the multiple factors that can cause sleep fragmentation, such as sleep disorders or lifestyle risk factors. In addition, polysomnography will also be necessary to specifically assess changes in NREM-sleep oscillations, and to test the potential impact of other sleep alterations such as REM-sleep disruption [50].

Our interpretation of the data is that the first hours of sleep is associated with an alteration of brain regions typically affected in ageing and AD, and may directly contribute to lower cognitive performance. However, in SCD/MCI patients, sleep disruption may no longer contribute to the expression of cognitive deficits. Therefore, preserving sleep quality in cognitively unimpaired elderly subjects may help to cope with brain changes and maintain cognitive functioning.

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Figure 1: Illustration of a representative actigraphy recording with computation of SF parameters.

Abbreviations: SF, sleep fragmentation.

SF was computed over the first half of the night. SF intensity corresponded to the mean level of SF over all recorded nights, and SF variability corresponded to the standard deviation of SF across recorded nights.

Table 1: Participants characteristics.

Abbreviations: MADRS, Montgomery and Asberg depression rating scale; MCI, mild cognitive impairment; MMSE, mini mental state examination; NS, non-significant; SCD, subjective cognitive decline; SD, standard deviation; SF, sleep fragmentation; STAI, State-Trait Anxiety Inventory; SUVr, standard uptake value ratio.

* Due to missing data in some patients, n=30 for episodic memory, n=33 for the MADRS, n=34 for executive functioning.

[†] Between-groups differences were assessed using Student t-tests for all variables, except for gender for which chi-square statistics were used. Statistical significance was set to P<.05.

[‡] n=26 healthy elderly and 34 patients with valid Florbetapir-PET scan. Amyloid positivity was defined as >0.985, based on mean SUVr + 2 SDs in a group of 41 healthy young individuals (aged <40 years).

Figure 2: Neuroimaging correlates of SF.

Abbreviations: BMI, body mass index; FDG: ^{18}F-fluorodeoxyglucose; MCI, mild cognitive impairment; SCD, subjective cognitive decline; SF, sleep fragmentation.

Results of the voxel-wise regression analyses between SF parameters and neuroimaging data in cognitively unimpaired elderly participants (left part) and SCD/MCI patients (right part). Results are presented at P<.001 (uncorrected), and adjusted for age, gender, BMI and the complementary SF parameter (i.e., SF variability when SF intensity was considered, and conversely).

Figure 3: Significant associations between SF intensity and cognitive performance in cognitively unimpaired elderly subjects.

Abbreviations: SF, sleep fragmentation.

Scatterplots illustrating the associations between SF intensity and **(A)** executive performance,

and **(B)** episodic memory performance. Partial correlation coefficients and p-values adjusted for age and gender are indicated on corresponding graphs. Results were considered significant after applying a Bonferroni correction for multiple testing (alpha = .05/4 = .01).

Figure 4: Causal mediation analyses performed in cognitively unimpaired elderly subjects.

Abbreviations: FDG, ^{18}F-fluorodeoxyglucose; NS, non-significant; SF, sleep fragmentation. Direct effects in filled arrows (simple regressions between variables) are expressed as standardized regression coefficients, and indirect effects in dotted arrows (multiple regressions in which the predictor and the mediator are both added in the model) as partial correlation coefficients. *P<.05, **P<.01, ***P<.001.

Table 2: Detailed statistics of causal mediation analyses in cognitively unimpaired elderly subjects.

Abbreviations: ADE, average direct effect; ACME, average causal mediation effect; CI, confidence interval.

In both models, executive functions were entered as the dependent variable. In model 1, brain glucose metabolism was the independent variable and SF intensity the mediator, whereas in model 2, SF intensity was the independent variable and brain glucose metabolism the mediator.

Table 1: Participants characteristics.

	Cognitively unimpaired elderly subjects (n=30)	SCD/MCI patients (n=36) [1]	Group comparison [2]
Demographics			
Age (years ± SD)	73.3 ± 7	71.5 ± 8.2	NS
Gender (% women)	56.7%	38.9%	NS
Education (years ± SD)	12.1 ± 3.5	11.8 ± 3.2	NS
Body Mass Index (mean ± SD)	24.5 ± 3	24.6 ± 4.5	NS
Florbetapir SUVr (% positive) [3]	1 ± 0.2 (30.8 %)	1.2 ± 0.3 (50 %)	**p=0.011**
MADRS (mean ± SD)	1 ± 1.9	3.4 ± 3.7	**p=0.002**
STAI-B (mean ± SD)	37.4 ± 10	38.8 ± 8.2	NS
Participants on sleep medication (number, (%)) [4]	1 (3.3%)	3 (8.3%)	NS
Cognition			
MMSE (mean ± SD)	28.6 ± 1.2	27.6 ± 2.4	**p=0.046**
Mattis total score (mean ± SD)	140.2 ± 3.3	136.2 ± 6.9	**p=0.005**
Episodic memory composite score (mean ± SD)	0.0 ± 0.7	-0.8 ± 1.3	**p=0.005**
Executive functioning composite score (mean ± SD)	0.0 ± 0.7	-0.8 ± 1.7	**p=0.020**
Sleep parameters			
SF intensity (mean ± SD)	30.3 ± 14.7	28 ± 11.1	NS
SF variability (mean ± SD)	10.9 ± 4.7	11.5 ± 5.2	NS

Abbreviations: MADRS: Montgomery and Asberg Depression Rating Scale, MCI: Mild Cognitive Impairment, MMSE: Mini Mental State Examination, NS: non-significant, SCD: Subjective Cognitive Decline, SD: Standard Deviation, SF: sleep fragmentation, STAI-B: State-Trait Anxiety Inventory form B, SUVr: Standard Uptake Value ratio.

[1] Due to missing data in some patients, n=30 for episodic memory, n=33 for the MADRS, n=34 for executive functioning.

[2] Between-groups differences were assessed using Student t-tests for all variables, except for gender for which chi-square statistics were used. Statistical significance was set to $p < 0.05$.

[3] n=26 healthy elderly and 34 patients with valid Florbetapir-PET scan. Amyloid positivity was defined as >0.985, based on mean SUVr + 2 SDs in a group of 41 healthy young individuals (aged <40 years).

[4] Use of sleep medication on a regular basis (>1/week), excluding phytotherapy and homeopathy.

Table 2: Detailed statistics of causal mediation analyses in cognitively unimpaired elderly subjects.

	ADE			ACME		
	Estimate	$CI_{95\%}$	P-value	Estimate	$CI_{95\%}$	P-value
Model 1	-0.344	[-5.396 ; 3.526]	0.85	**4.051**	**[0.657 ; 8.723]**	**0.02**
Model 2	-0.028	[-0.059; -0.005]	0.02	0.002	[-0.016 ; 0.024]	0.87

Abbreviations: ADE: average direct effect, ACME: average causal mediation effect, CI: confidence interval.

In both models, executive functions were entered as the dependent variable. In model 1, brain glucose metabolism was the independent variable and SF intensity the mediator, whereas in model 2, SF intensity was the independent variable and brain glucose metabolism the mediator.
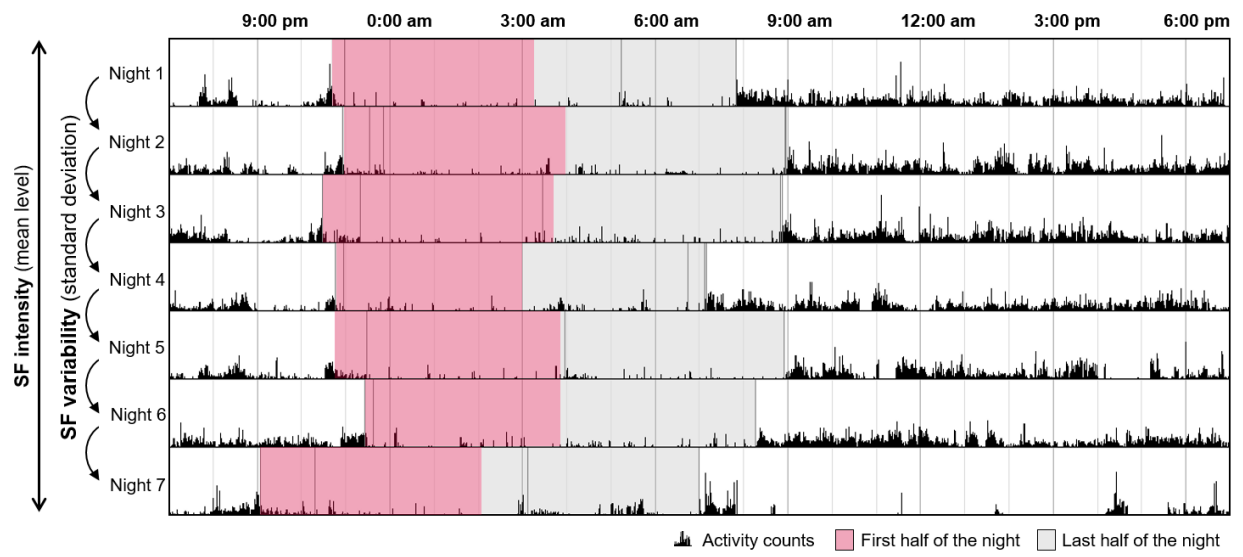
Figure 1

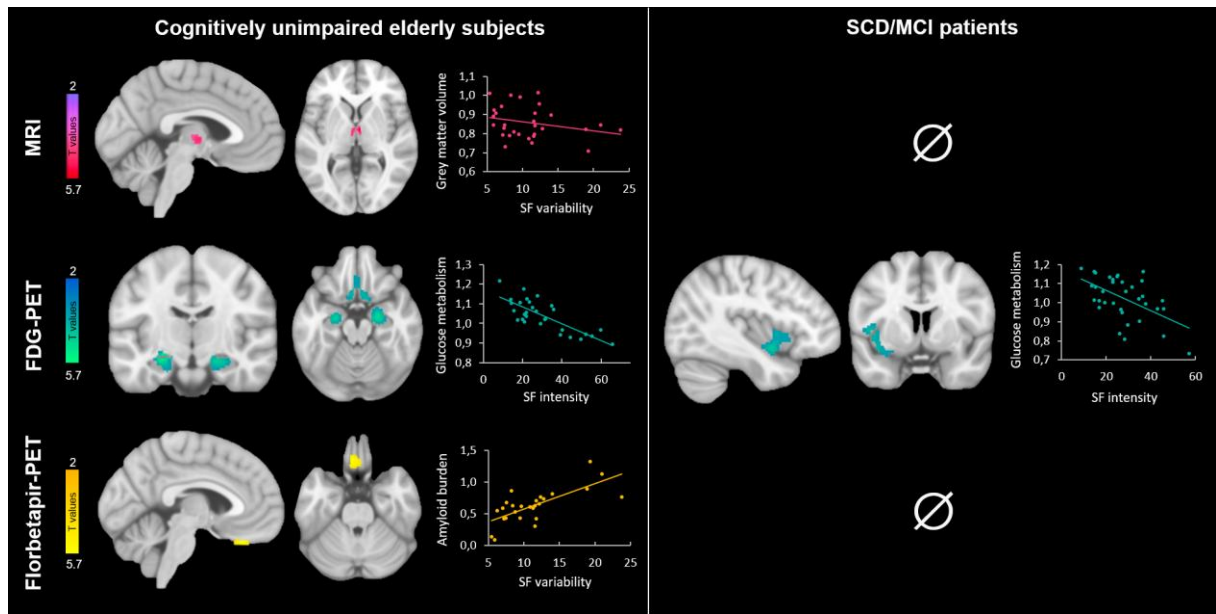**Figure 2**
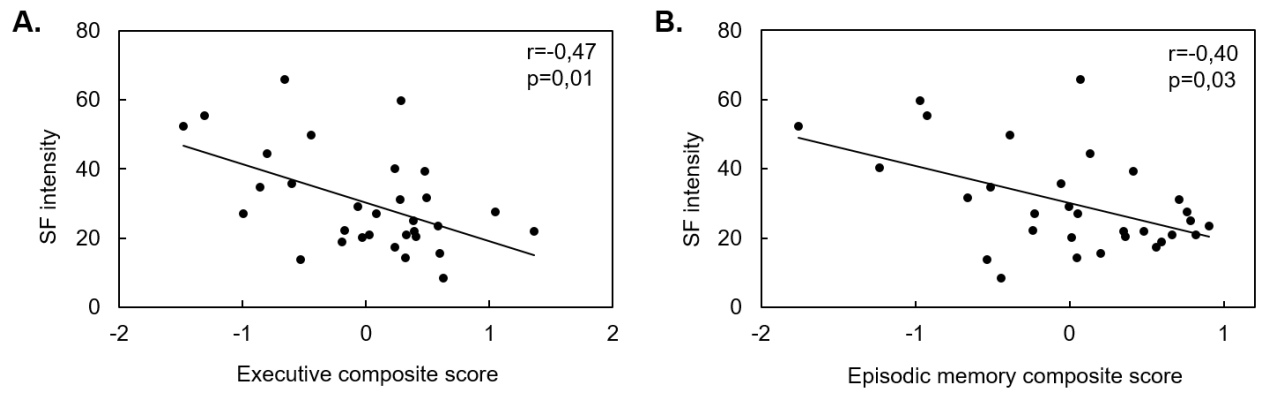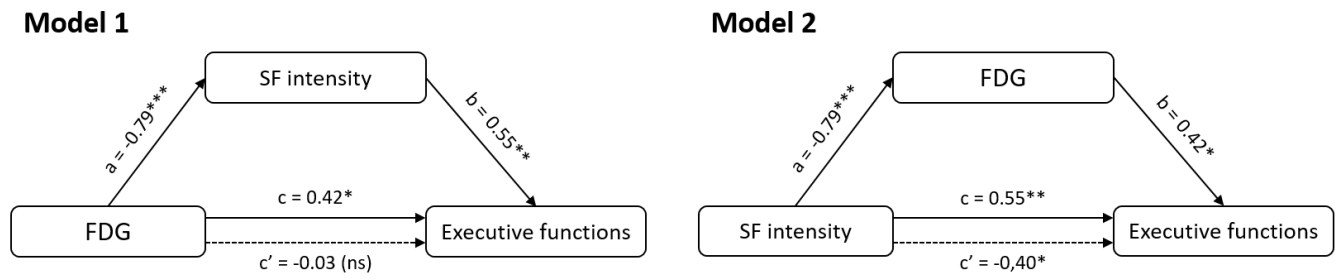
Figure 3

Figure 4

Model 1

SF intensity

a = -0.79***

b = 0.55**

FDG

c = 0.42*

Executive functions

c' = -0.03 (ns)

Model 2

FDG

a = -0.79***

b = 0.42*

SF intensity

c = 0.55**

Executive functions

c' = -0,40*

Supplementary Material

Supplementary methods

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Supplementary Figures

Supplementary Figure 1: Patterns of atrophy, hypometabolism and amyloid de position in SCD/MCI patients compared to cognitively unimpaired elderly subjects.

Supplementary Tables

Supplementary Table 1: Determination of minimal cluster sizes for neuroimaging analyses.

Supplementary Table 2: Result of voxel-wise multiple regressions between SF parameters and neuroimaging data with 4 covariates.

Supplementary Table 3: Result of voxel-wise multiple regressions between SF parameters and neuroimaging data with 5 covariates.

Supplementary Table 4: Relationships between SF parameters and neuropsychological scores in cognitively unimpaired elderly subjects and SCD/MCI patients.

<u>Neuropsychological scores</u>

To obtain more robust proxies of cognitive abilities and minimize the issue of multiple statistical testing, composite cognitive scores were used instead of multiple (sub)tests, as described in [1]. For that purpose, performance from different tasks that showed neither ceiling nor floor effects were z-transformed and averaged as follows:

- **Executive functions**

  - TMT test (time difference between TMT part B and part A)*

  - Stroop test (time difference between the interference and color cards)*

  - Verbal fluency (number of words beginning with ''p'' in 2 min)

- **Episodic memory**

  - 3 consecutive free recalls + delayed free recall from the Free and Cued Selective Reminding Test

  - Free recall of the BEM (*Batterie d'Efficience Mnésique*) figure

  - 2 free recalls from the *Encoding Storage Retrieval* (ESR) paradigm (two 16-word lists, one being encoded incidentally and superficially, the other after deep and intentional encoding)

  - 2 free recalls from a visual version of the ESR paradigm (based on two lists of nonfigurative graphical signs)

* note that before averaging, Z-scores derived from reaction times were reversed so that increasing values always indicated better performances.

Neuroimaging data acquisition and pre-processing

6.1.1. MRI data acquisition and pre-processing

A high-resolution T1-weighted anatomical image was acquired on a 3T Philips Achieva MRI scanner, using a 3D fast-field echo sequence (3D-T1-FFE sagittal; repetition time = 20 ms; echo time = 4.6 ms; flip angle = 10°; 180 slices with no gap, slice thickness = 1 mm, field of view = 256×256 mm²; in-plane resolution = 1×1 mm²). The T1-weighted images were iteratively segmented, spatially normalized to the Montreal Neurological Institute (MNI) space, modulated to correct for non-linear warping effects, and smoothed with a 8-mm full width at half maximum (FWHM) Gaussian kernel using the voxel-based morphometry toolbox (VBM12) implemented in SPM12 software (Statistical Parametric Mapping, www.fil.ion.ucl.ac.uk/spm). Images were then masked to exclude non-grey matter voxels from the analyses.

6.1.2. PET data acquisition and pre-processing

FDG- and Florbetapir-PET scans were acquired in two separate sessions, with a Discovery RX VCT 64 PET-CT scanner (General Electric Healthcare) with a resolution of 3.76 x 3.76 x 4.9 mm (field of view = 157 mm). Forty-seven planes were obtained with a voxel size of 1.95 x 1.95 x 3.2 mm. A transmission scan was performed for attenuation correction before the PET acquisition. For FDG-PET, participants were fasted for at least 6 hours before scanning. After a 30-min resting period in a quiet and dark environment, ≈180 MBq of FDG were intravenously injected as a bolus. A 10-min PET acquisition scan began 50 minutes after injection. For Florbetapir-PET, each participant underwent a 20-min PET scan, beginning 50 min after intravenous injection of ≈ 4 MBq/kg of Florbetapir. FDG- and Florbetapir-PET data were first corrected for partial volume effects (PMOD Technologies Ltd, Adliswil, Switzerland), coregistered onto their corresponding MRI, and then spatially normalized using the deformation parameters derived from the MRI procedure. The resulting images then

underwent quantitative scaling, using cerebellar grey matter as a reference. Finally, both FDG-PET and Florbetapir-PET resulting images were smoothed using a 10-mm FWHM Gaussian kernel and masked to exclude non-grey matter voxels from the voxelwise analyses. PVE-corrected normalized and scaled Florbetapir-PET images were also used to extract the individual global cortical amyloid standard uptake value ratio (SUVr) using a predetermined neocortical mask including the entire grey matter, except the cerebellum, occipital and sensory motor cortices, hippocampi, amygdala and basal nuclei [2].

<u>Supplementary Table 1</u>: Determination of minimal cluster sizes for neuroimaging analyses.

Statistical design	Minimal cluster sizes (k voxels)		
	MRI	FDG-PET	Florbetapir-PET
Group comparisons	141	145	132
Multiple regressions in cognitively unimpaired elderly subjects	127	124	118
Multiple regressions in SCD/MCI patients	130	111	145

<u>Abbreviations</u>: FDG, ^{18}F-fluorodeoxyglucose; MCI, mild cognitive impairment; MRI, magnetic resonance imaging; PET, positron emission tomography; SCD, subjective cognitive decline.

Minimal cluster sizes (k) were determined for each statistical design at the $P<.001$ (uncorrected) level by Monte-Carlo simulation using the Alphasim program, in order to achieve a corrected statistical significance of $P<.05$.

<u>Supplementary Figure 1:</u> Patterns of atrophy, hypometabolism and amyloid deposition in SCD/MCI patients compared to cognitively unimpaired elderly subjects.
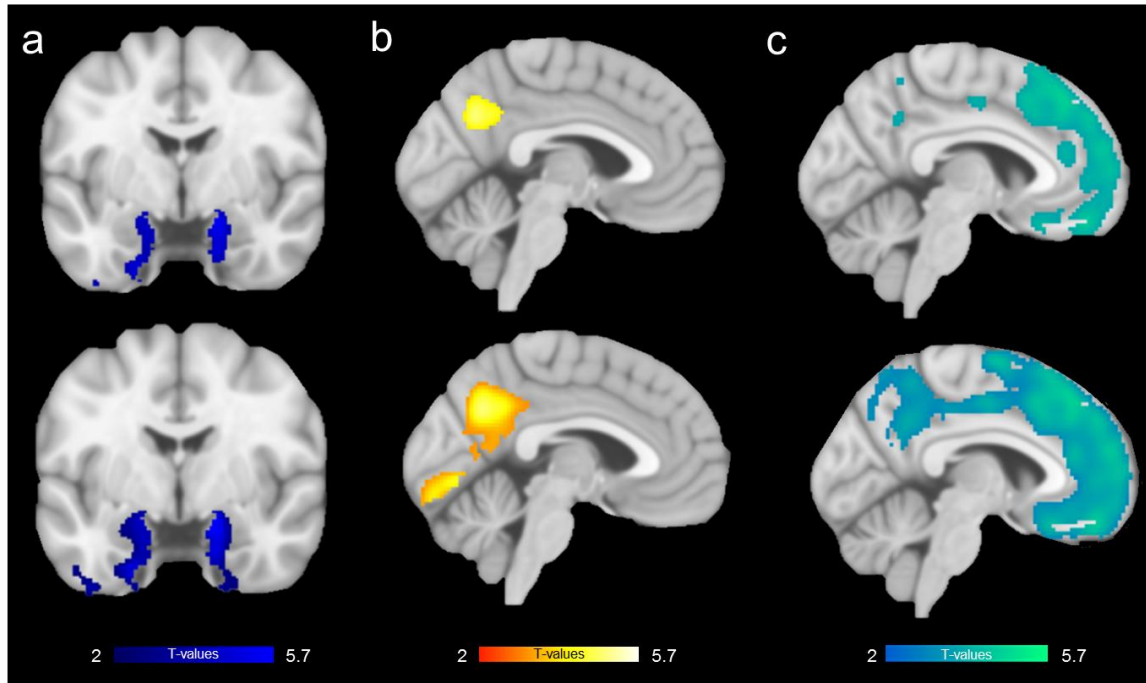
<u>Abbreviations</u>: BMI, body mass index; MCI, mild cognitive impairment; SCD, subjective cognitive decline.

Results of the two-sample t-tests exhibiting patterns of **(a)** atrophy, **(b)** hypometabolism, and **(c)** amyloid deposition, in SCD/MCI patients compared to healthy elderly subjects. Results are presented at the P<.001 uncorrected level in the top line, and the P<.005 uncorrected level in the bottom line, and were adjusted for age, gender and BMI.

Supplementary Table 2: Result of voxel-wise multiple regressions between SF parameters and neuroimaging data with 4 covariates.

Brain areas	Cluster extent		MNI coordinates (mm)			T-value
	Voxels	mm³	x	y	z	
Cognitively Unimpaired Elderly Subjects						
SF intensity and FDG-PET (n=30)						
R caudate, R hippocampus, R parahippocampal gyrus, B rectus gyri, B anterior cingulate gyri, B frontal medial orbital gyri	789	6 312	8	6	-6	5.73
L hippocampus, L amygdala	247	1 976	-24	-14	-16	5.40
SF variability and MRI (n=30)						
B thalamus	149	503	4	-8	0	5.22
SF variability and Florbetapir-PET (n=26)						
L rectus gyrus	80	640	-6	32	-28	4.57
SCD/MCI Patients						
SF intensity and FDG-PET (n=35)						
L insula	301	2 408	-38	0	-12	4.48

Abbreviations: B, bilateral; BMI, body mass index; FDG, ^{18}F-fluorodeoxyglucose; L, left; MCI, mild cognitive impairment; R, right; SCD, subjective cognitive decline; SF, sleep fragmentation.

Voxel-wise multiple regressions were corrected for age, gender, BMI and the complementary SF parameter. Brain regions listed were significant at P<.001 (uncorrected). MNI coordinates are given for the main peak of each significant cluster. In cluster labelling, the first listed region corresponds to the label of the peak voxels, and the following regions are other regions included in the cluster.

Supplementary Table 3: Result of voxel-wise multiple regressions between SF parameters and neuroimaging data with 5 covariates.

Brain areas	Cluster extent		MNI coordinates (mm)			T-value
	Voxels	mm^3	x	y	z	
Cognitively Unimpaired Elderly Subjects						
SF intensity and FDG-PET (n=30)						
R caudate, R hippocampus, R parahippocampal gyrus, B rectus gyri, B anterior cingulate gyri, B frontal medial orbital gyri	901	7 208	8	6	-6	5.89
L hippocampus, L amygdala	248	1 984	-24	-14	-16	5.30
SF variability and MRI (n=30)						
B thalamus	95	321	4	-8	0	4.23
SF variability and Florbetapir-PET (n=26)						
L rectus gyrus	32	256	-6	32	-28	3.94
SCD/MCI Patients						
SF intensity and FDG-PET (n=35)						
L fusiform gyrus, parahippocampus, hippocampus	111	888	-22	-30	-20	4.82
L insula	411	3 288	-38	0	-12	4.62

Abbreviations: B, bilateral; BMI, body mass index; FDG, ^{18}F-fluorodeoxyglucose; L, left; MCI, mild cognitive impairment; R, right; SCD, subjective cognitive decline; SF, sleep fragmentation.

Voxel-wise multiple regressions were corrected for age, gender, BMI, the complementary SF parameter and regular us of sleep medication. Brain regions listed were significant at P<.001 (uncorrected). MNI coordinates are given for the main peak of each significant cluster. In cluster labelling, the first listed region corresponds to the label of the peak voxels, and the following regions are other regions included in the cluster.

<u>Supplementary Table 4</u>: Relationships between SF parameters and neuropsychological scores in cognitively unimpaired elderly subjects and SCD/MCI patients.

Cognitive score	Cognitively unimpaired elderly subjects (n=30)		SCD/MCI patients (n=36)*	
	SF intensity	SF variability	SF intensity	SF variability
Episodic memory composite score	r=-0.40 P=.03	r=-0.11 P=.56	r=-0.04 P=.85	r=0.08 P=.67
Executive functioning composite score	**r=-0.48 P=.01**	r=-0.11 P=.57	r=0.15 P=.46	r=0.144 P=.47

<u>Abbreviations</u>: MCI, mild cognitive impairment; MMSE, mini-mental state examination; SCD, subjective cognitive decline; SF, sleep fragmentation.

R-values correspond to partial correlation coefficients, controlling for age and gender. Results in bold are still significant after applying a Bonferroni correction for multiple testing within each group (alpha = .05/4 = .01).

* Due to missing data, n=33 for the Mattis total score, n=34 for the executive functioning composite score, n=30 for the episodic memory composite score.

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