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Age-related changes in fast spindle clustering during NREM sleep and their relevance for memory consolidation

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Abstract

Sleep plays a crucial role in memory consolidation. Recent data in rodents and young adults revealed that fast spindle band power fluctuates at a 0.02-Hz infraslow scale during non-rapid eye movement (NREM) sleep. These fluctuations result from a periodic temporal clustering of spindles and may modulate sleep maintenance and memory consolidation. With age, sleep undergoes substantial changes but age-related changes in spindle clustering have never been investigated. Polysomnography data were collected in 147 older (mean age \pm SD: 69.3 ± 4.1 years) and 32 young-middle aged (34.5 ± 10.9 years) adults. Sleep-dependent memory consolidation was assessed in a subsample of 57 older adults using a visuospatial memory task. We analyzed power fluctuations in fast spindle frequency band, detected fast spindles and quantified their clustering during the night separating encoding and retrieval. Fast spindle band power fluctuated at a 0.02-Hz infraslow scale in young-middle aged and older adults. However, the proportion of clustered fast spindles decreased non-linearly with age ($p < 0.001$). This effect was not mediated by NREM sleep fragmentation. The clustering level of fast spindles modulated their characteristics ($p < 0.001$). Finally, the mean size of spindle clusters was positively associated with memory consolidation ($p = 0.036$) and negatively with NREM sleep micro-arousals density ($p = 0.033$). These results suggest that clusters of fast spindles may constitute stable sleep periods promoting off-line processes such as memory consolidation. We emphasize the relevance of considering spindle dynamics, obviously impaired during ageing, to understand the impact of age-related sleep changes on memory.

Keywords

Sleep, sleep spindles, ageing, memory consolidation, sleep fragmentation, non-rapid eye movement sleep

Clinical Trial Information

Name: Study in Cognitively Intact Seniors Aiming to Assess the Effects of Meditation Training (Age-Well)

URL: <https://clinicaltrials.gov/ct2/show/NCT02977819?term=Age-Well&draw=2&rank=1>

Registration: EudraCT: 2016-002441-36; IDRCB: 2016-A01767-44; ClinicalTrials.gov Identifier: NCT02977819

Statement of Significance

Fast spindles are known to play a key role in sleep-dependent memory consolidation. Several parameters of spindles (density, amplitude, duration) are impaired with ageing. Recent studies have revealed dynamic aspects of fast spindles (power fluctuation and spindle clustering) in rodents and young adults that may influence sleep stability and memory consolidation. For the first time, we investigated these dynamic aspects of fast spindles in older adults, and observed a decrease in fast spindle clustering with age. In addition, a lower degree of spindle clustering was associated with poorer sleep-dependent memory consolidation and greater sleep fragmentation in older adults. Future studies should consider dynamic parameters of spindles to better understand their contribution to sleep and memory functions.

Introduction

Maintaining optimal sleep quality is essential for health and well-being. During sleep, the brain fulfills major functions such as memory consolidation (see [1] for review). However, it still has to monitor the surroundings to wake up if necessary. Evolutionary mechanisms shaped our sleep through these opposite selective pressures [2]. A balance between off-line processes and alertness towards the environment is therefore crucial. Recent evidence on animal and young adults suggests a key role of sigma power fluctuation and non-homogeneous sleep spindle distribution in this equilibrium [3].

Sleep spindles are transient (0.5–2 s) thalamo-cortical oscillations in the sigma frequency band (~ 11–15 Hz) typically observed in scalp-EEG of polysomnography. These discrete events are generated by the thalamic reticular nucleus (TRN) and are a hallmark of NREM sleep (see [4] for review). Several spindle parameters (e.g. number, density, activity) increase overnight after learning a new task and have been associated with consolidation in declarative [5–8] and procedural [9,10] memory. Sleep spindles may promote the reactivation of memories [11,12] and the reorganization of memory traces within neural networks [13,14]. Besides, sleep spindles are also thought to be an index of sleep stability by modulating the effect of external stimuli on brain activity and the propensity to arouse [3,15–18].

Two subtypes of sleep spindles, namely slow (< 13 Hz) and fast (> 13 Hz) spindles, have been described [19–21]. Fast spindles, mainly recorded over centro-parietal regions [22–24], are assumed to be particularly involved in sleep-dependent memory consolidation [11,13,20,25,26]. Some recent studies have focused on the non-homogenous distribution of spindles during NREM sleep and on the fluctuation of spindle frequency power (sigma) [3,10,27,28]. Lecci et al. [3] reported that sigma power (and more specifically power in the fast spindle frequency band) fluctuates throughout NREM sleep at an infraslow scale of around 0.02 Hz (i.e., periodicity of about 50 s) in both mice and young adults. This means that during NREM sleep, periods of low sigma power of about 25 seconds are repeatedly followed by equivalent periods (~25-s) of high sigma power. This fluctuation results from

a periodic clustering of fast spindle activity. Spindles tend to be separated by 3 to 6 seconds, suggesting a refractory period following a spindle and a rhythmicity gathering spindles into clusters [12]. Based on these observations, Boutin and Doyon [29] proposed the concept of “spindle trains” (or clusters) defined as a group of at least 2 temporally clustered spindles interspaced by less than 6 seconds. These clusters occur in periods of high sigma power, while periods of low sigma power mainly contain sparse isolated spindles. Importantly, this 0.02-Hz infraslow oscillation in sigma power has been associated with behavioral arousability change in mice and with episodic memory consolidation in humans. Auditory stimuli have a higher propensity to wake up the animal when they occur in a period of declining sigma power [3]. Besides, higher strength of 0.02-Hz sigma oscillation was associated with better overnight memory consolidation [3]. Moreover, the proportion of clustered spindles appears to increase overnight in the hemisphere that was activated while performing a motor task [30], suggesting that learning specifically promotes spindle clustering in the network recruited during encoding. Finally, several spindle cluster parameters (such as the mean number of spindle per cluster or the inter-hemispheric difference in the proportion of clustered spindles the night following a one-handed motor task) have been associated with procedural memory consolidation [29,30]. This 0.02-Hz infraslow oscillation may allow the brain to switch every ~25 s between an environment monitoring state (with scarce fast spindles) and an off-line state dedicated to internal processes like memory consolidation (with high fast spindle density). Any impairment of this switching may hinder sleep-dependent memory consolidation and increase sleep fragility.

Interestingly, sleep fragmentation increases with age and many studies report that sleep-dependent memory consolidation is less efficient in ageing (see [31,32] for reviews). However, the debate is still open as other studies reported that this process is preserved in older adults [33,34].

Although it is well-established that sleep spindle density, amplitude and duration decrease with age [35–37], the dynamic aspects of fast spindles (i.e., temporal clustering) and of their related power

(fluctuation at a 0.02-Hz infraslow scale) have never been investigated in older adults. This may be particularly relevant as we know that subtle changes in spindle dynamic occur with age. While spindle density and duration increase across sleep cycles in young adults, older adults do not appear to exhibit such a temporal pattern [37,38]. Besides, the U-shaped dynamics of spindle frequency overnight recently observed in young adults (with highest values in the first and last sleep cycles and lowest values in the middle ones) is attenuated in older adults [39].

In this context, our first aim was to determine whether power in the fast spindle band fluctuates in older adults at a 0.02-Hz infraslow scale. Second, to better understand the spindle clustering phenomenon, we compared the amplitude, duration and frequency of clustered and isolated fast spindles. Third, we investigated the impact of age on fast spindle clustering and its dynamics across the night. Finally, we also assessed the links between fast spindle clustering, episodic memory consolidation and NREM sleep fragmentation.

Methods

Participants

Thirty-two young-middle aged adults (age range: [20–52 years], mean \pm SD: 34.5 \pm 10.9 years, 10 women) and 147 older adults cognitively unimpaired ([65–83 years], 69.3 \pm 4.1 years, 89 women) were included. To do so, the data of three cohorts were gathered. Data of 134 older adults were acquired during the baseline visit of the Age-Well randomized clinical trial (RCT) of the Medit-Ageing European project sponsored by the French National Institute of Health and Medical Research (“Institut National de la Santé et de la Recherche Médicale”, Inserm) [40]. All participants gave their written informed consent prior to the examinations, and the Age-Well RCT was approved by the ethics committee (CPP Nord-Ouest III; trial registration number: EudraCT: 2016-002441-36; IDRCB: 2016-A01767-44; ClinicalTrials.gov Identifier: NCT02977819). Eighteen participants of the young-

middle aged group were included as healthy controls in a study investigating the effects of alcohol use disorder on sleep, cognitive and brain alterations (CPP Nord Ouest III, no. IDRCB: 2011-A00495-36). The other participants (14 young-middle aged and 13 older adults) were included as healthy volunteers in a third study investigating sleep changes in ageing and Alzheimer's disease and their impact on memory performance (CPP Caen, 2001-46). All participants presented no evidence of major neurological, vascular or psychiatric disorders. Sleep medication use was an exclusion criteria. None of the older participants had a Mini-Mental State Examination [41] score below 26. These different protocols were approved by ethics committees and participants gave their written consent prior to the examinations after detailed information was provided to them. The use of data from these two latter protocols was approved by the ethics committee (Comité Local d'Ethique de la Recherche en Santé) of Caen University Hospital and University of Caen (approval n° 3381 – 05/16/2022). In accordance with this authorization, we ensured that participants did not object to the analysis of their data for a purpose other than the original study in which they were included.

Sleep recording and analysis

Sleep was monitored with polysomnography (PSG). For 152 participants (134 older and 18 young-middle aged adults), PSG was performed at home using a portable recording device (Siesta®, Compumedics, Australia). Among them, 90 older participants underwent two recordings but only the second one was included in the analyses. Twenty EEG electrodes were placed over the scalp (Fp1, Fp2, F3, F4, F7, F8, FZ, C3, C4, CZ, T3, T4, P3, P4, PZ, O1, O2, vertex ground and a bi-mastoid reference) according to the international 10–20 system, with impedances kept below 5 k Ω . Respiratory movements, airflow, and oxygen saturation were recorded respectively with thoracic and abdominal belts, nasal and oral thermistors, and a finger pulse oximeter. The EEG signal was digitalized at a sampling rate of 256 Hz. For the 27 remaining participants (13 older and 14 young-middle aged adults), PSG data were acquired in the sleep department of Caen University Hospital

using the Nicolet Acquisition System. They underwent two polysomnography nights, the first one being an adaptation night. Eight EEG electrodes were placed over the scalp (C3, C4, T3, T4, O1, O2, vertex ground and a bi-mastoid reference) according to the international 10–20 system, with impedances kept below 10 k Ω . The EEG signal was digitalized at a sampling rate of 125 Hz. For all participants, high-pass and low-pass filters were applied, respectively at 0.3 Hz and 35 Hz.

All recordings were visually scored in 30-second epochs following the recommended standard criteria of the American Academy of Sleep Medicine [42]. Standard parameters of sleep architecture were obtained such as sleep latency, total sleep time (TST, in minutes), wake after sleep onset, sleep efficiency (100 x time asleep / time in bed), each sleep stage proportion (N1, N2, N3 and REM sleep, in %) and number of micro-arousals. NREM sleep fragmentation was estimated by the number of micro-arousals per hour of NREM sleep.

Sleep microstructure analyses were carried out using Matlab and the free and open-source toolbox Sleeptrip (<https://www.sleeptrip.org>; RRID: SCR_017318) based on FieldTrip functions (<http://fieldtriptoolbox.org>; RRID: SCR_004849) [43]. All epochs were visually inspected. An independent component analysis (ICA) was performed for 2 subjects to remove the ECG artifacts on EEG signals. The EEG data of these 2 subjects were visually checked after the procedure. The data were then epoched into 5-s bins. The 5-s epochs containing manually identified EEG artifacts or movement arousals were rejected. Channels with artifacts affecting a majority of the recording were removed. In line with the latest sleep research recommendations in older adults (see [44] for review), we considered N2 and N3 sleep stages as a continuum and performed our analyses on all artifact-free N2 and N3 sleep epochs.

One hundred and thirty four older adults and 18 young-middle aged adults also filled sleep questionnaires (Pittsburgh Sleep Quality Index (PSQI [45]) and Insomnia Severity Index (ISI [46])) and rated their sleep quality and depth during the polysomnography night using the Saint Mary's Hospital questionnaire [47].

Half of the night, sleep cycles and pre-REM periods identification

We also investigated the dynamics of fast spindle clustering across the night. First, the half of the night was defined as the epoch equally distant from the first and last epochs of sleep for each participant. Second, sleep cycles were automatically identified using SleepTrip and then manually checked. As 81 subjects did not exhibit 5 or more sleep cycles, we only considered the first four sleep cycles. A similar procedure has already been applied in studies analyzing spindle dynamics across NREM sleep cycles [48]. To exclude sleep cycles that have just started, we removed from the analysis sleep cycles with less than 10 min of N2-N3 (13 sleep cycles among 704). Third, we defined the pre-REM periods as the last five minutes of N2-N3 epochs before each transition to REM sleep. The rest of N2-N3 epochs was classified as non pre-REM periods. Three older adults had less than 10 minutes of pre-REM periods over the whole night (due to incomplete sleep cycles caused by sleep fragmentation) and were excluded from this analysis.

Fast spindle detection and cluster identification

For each participant, fast spindles were automatically detected in artifact-free N2 and N3 sleep segments using a validated algorithm used in previous publications [49–51] and based on the study by Mölle et al. [52]. As spindle frequency can differ between participants [19,23,53], we used a subject-specific frequency band. NREM sleep power spectra were calculated for all the electrodes of each subject. The individual peak frequency in fast spindle range was visually identified on centro-parietal channels (mean \pm SD: 13.66 \pm 0.5 Hz) and taken as the fast spindle center frequency (Figure 1). Fast spindle peaks were clearly identifiable, except for 3 older adults which were removed from the analyses. The analysis of slow spindles raised several methodological issues. As described above, 14 among the 32 participants of the young-middle aged group and 13 older adults had no frontal EEG channel. Besides, no clear power peak of slow spindles could be identified in a substantial part of the

remaining participants with frontal EEG channels. Previous studies using subject-specific frequency band for spindle detection reported similar issues and focused their analyses on fast spindles [50,54]. We decided to also limit our analyses to fast spindles. For each participant, the EEG signal was filtered with a band-pass width of 2 Hz centered on the fast spindle center frequency (two-pass FIR filter). The root mean square (RMS) signal was calculated using 0.2-s windows for the entire recording and smoothed by a moving average (0.2-s window). A fast spindle was detected if the smoothed RMS signal exceeded an individual amplitude threshold (1.5 times the standard deviation of the filtered signal) for 0.5–2 s. Fast spindles with an amplitude higher than 120 μV were excluded. A visual inspection was performed in a subgroup of participants to check the validity of fast spindle detection. Fast spindle mean peak-to-trough amplitude, duration, average oscillatory frequency, and density (number of spindles per artifact-free N2 and N3 sleep minute) were calculated for each participant and channel.

Fast spindle clustering (reoccurring spindles within the same channel) was investigated using the criteria proposed by Boutin and Doyon [29]. We considered fast spindles as clustered (i.e., temporally clustered into trains) if they were interspaced by less than 6 s. The size of each cluster was defined as the number of fast spindles clustered. A clustering level was associated to each fast spindle. The clustering level corresponded to the number of fast spindles in the potential cluster associated (1 for isolated spindles, ≥ 2 for clustered spindles). Then, for clustered fast spindles, the clustering level corresponded to the size of the cluster to which they belong. To test the effect of fast spindle clustering on their characteristics, we computed the percent difference in amplitude, duration and frequency of clustered fast spindles relative to that of isolated fast spindles. This was performed for each clustering level on all channels of all participants. It allowed us to take into account the inter-channel and inter-individual variability of fast spindle characteristics. As some subjects did not have fast spindles in every clustering levels ≥ 6 , we gathered all fast spindles with a clustering level ≥ 5 .

As fast spindles mainly occur in the centro-parietal region [22-24], we focused our analyses on the channels covering this area. Fast spindle measures were calculated separately on the 2 centro-parietal channels available for all participants (i.e., C3 and C4) and were then averaged. For the memory consolidation analysis specifically, the same procedure was applied on C3, CZ, C4, P3, PZ and P4 as all these centro-parietal channels were available for all subjects who performed the memory task.

0.02-Hz infraslow oscillation analyses

The procedure of Lecci et al. [3] was applied to study fast spindle band power fluctuations. Briefly, all consecutive 120-s periods of artifact-free N2 and N3 sleep were selected for each participant. Here again, a subject-specific frequency band (2 Hz width and centered on individual fast spindle peak frequency) was used to calculate the time-frequency power. The latter was computed for consecutive 100-ms bins in steps of 0.2 Hz with a continuous wavelet transform using Morlet wavelets (4 cycle length). The resulting power time courses were then smoothed (using a symmetric moving average in a 4-s window) and normalized (to the average absolute power in the subject specific fast spindle frequency band across all NREM sleep epochs). Another Morlet wavelet analysis was performed to calculate the spectral profile of the fast spindle band power time courses at a frequency resolution of 0.001 Hz in the 0.001-0.12 Hz range. The resulting signal was averaged across all N2 and N3 sleep epochs of the participant, weighted by their duration. The spectral profile obtained for each subject was normalized to its own mean. Finally, the grand average across subjects was calculated. Individual and group frequency peaks were determined after applying a Gaussian fit (three terms) on each subject and grand average spectra. As the 0.02-Hz sigma oscillation has been reported to be maximal on centro-parietal channels [3,27], we focused our analyses on C3 and C4 electrodes that were available for all subjects.

Memory task

A subgroup of 77 older participants performed a computerized two-dimensional visuospatial object-location task. This task, developed by Rasch et al. [55], is known to be hippocampus-dependent and reinforced by sleep [55], and was successfully used in older adults [56]. Throughout the task, all 24 possible spatial locations were shown as grey squares on the computer screen and corresponded to “the back of the cards”. The locations were displayed in a 6×4 matrix. During the learning phase, performed at the end of the afternoon before the polysomnography night, the first card of each pair was presented alone for one second, followed by the presentation of both cards for three seconds. After an inter-stimulus interval of three seconds, the next pair of cards was presented in the same way. The 12 card-pairs were presented twice in the same order. Immediately after these two runs, recall of the spatial locations was tested using a cued recall procedure. The first card of a pair was displayed and the participant had to indicate, using the computer mouse, the location of the second card. A visual feedback was given in each case by presenting the second card at the correct location for two seconds, regardless of whether the response was correct or not. After the 12 pairs of cards were tested, the percentage of correct answers in the trial was displayed. The cued recall procedure was repeated until the participant reached a criterion of 66.7 % correct responses in a trial (i.e., 8 card-pairs correctly located). The learning score was defined as the score at the last trial of the learning phase (first trial with a score $\geq 8/12$).

The next morning, after removing electrodes, memory performance was probed in a single trial using the same cued recall procedure as during the learning phase. We evaluated memory consolidation by calculating an overnight change in memory performance as follows: $100 \times (\text{recall score} - \text{learning score}) / \text{learning score}$. Of note, 11 participants did not reach the learning criterion and were removed from analyses. To allow a minimum level of practice and repetition during the learning phase, the 6 participants who reached the learning criterion in less than 4 trials were also removed. Finally, after removing 3 participants who did not exhibit a clear fast spindle power peak, the data of 57 older subjects were analyzed.

Statistical analyses

All statistical analyses were performed using R (version 4.1.0; R Project for Statistical Computing; <http://www.r-project.org>). The threshold for statistical significance was set at $p < 0.05$ and was two-tailed. Group comparisons for demographic variables were assessed using a χ^2 test for sex (categorical variable) and a non-parametric Wilcoxon signed-rank test for age (continuous variable with non-normal distribution revealed using Shapiro-Wilk test). Between group differences for sleep architecture and fast spindle characteristics were tested using two-way type-II ANOVAs to account for sex ratio disparity.

Linear mixed models were used to assess the effect of clustering level on fast spindle characteristics, the effect of age group on these latter and on fast spindle proportion for each clustering level. The dynamics of fast spindle clustering across the night (effects of the half of the night, sleep cycles and pre-REM periods) were also tested using linear mixed models. Models were fitted to the data with restricted maximum likelihood using the lme4 package. The significance of fixed effects was assessed with type III F-tests with Satterthwaite's method. Clustering level was considered as a categorical variable in the models as fast spindles with a clustering level ≥ 5 were gathered. To further investigate the interaction between age group and fast spindle proportion in the different clustering levels, post-hoc pairwise Wilcoxon tests with Bonferroni correction were used to assess between-group differences. A Bonferroni correction was also applied in the analyses investigating the different fast spindle characteristics (amplitude, duration and frequency), setting the significance level at $0.05/3 = 0.016$.

Linear and non-linear (2nd order polynomial) regression models were used to evaluate the relationship between age, the proportion of clustered fast spindles and the mean cluster size. Linear and non-linear models were compared using several methods: ANOVA, likelihood ratio test (LRT), Akaike Information Criterion (AIC) and Bayesian Information Criterion (BIC). In the 2nd order

polynomial models, the overall significance of age was assessed using type II F-test, and the overall variance proportion of spindle clustering explained by age was calculated by subtracting the R^2 of the complete model to the R^2 of the model with only sex as covariate. The variances of clustered fast spindle proportion in young-middle aged and older adults were compared using a Fisher test. Linear regressions were also used to test the associations between fast spindles, overnight change in memory performance and NREM sleep fragmentation (i.e., density of micro-arousals during NREM sleep). All analyses were controlled for age (except for group comparisons) and sex. Association between fast spindle clustering and subjective sleep reports were tested using non parametric Spearman's rank correlations.

Mediation analyses were performed to test the direct and indirect effects of age on the proportion of clustered fast spindles and on the mean cluster size using nonparametric bootstrapping based on 5,000 bootstrap samples. These analyses were controlled for sex.

Results

Participant characteristics

Demographic data, sleep architecture and spindle parameters of participants are summarized in Table 1. Sex ratio was statistically different between both groups ($p = 0.0047$) with a higher proportion of men in the young-middle aged group and a higher proportion of women in the older group. Sleep architecture and fast spindle characteristics differed between groups. Thus, compared to young-middle aged individuals, older adults had lower total sleep time ($p < 0.001$), lighter and more fragmented sleep with an increase in %N1, wake after sleep onset (WASO) and in NREM sleep micro-arousal density (all p -values < 0.001). Older adults also exhibited a decrease in %N3 ($p = 0.022$) and in sleep efficiency ($p < 0.001$). Concerning specifically sleep spindles, fast spindle density,

amplitude and duration were reduced in older adults (all p -values < 0.001), whereas there was no effect of age on fast spindle frequency ($p = 0.49$).

Fast spindle band power fluctuation in young-middle aged and older adults

Fast spindle band power fluctuated in older adults at an infraslow scale close to 0.02 Hz. The averaged fast Fourier transform (FFT) of fast spindle band power peaked around 0.02 Hz in both young-middle aged and older groups (Figure 2A). After applying a Gaussian fit on grand average spectra, the peaks were respectively at 0.021 Hz for young-middle aged adults and 0.022 Hz for older adults. The individual FFT peaks did not differ between groups ($F(1,175) = 0.164$, $p = 0.69$; Figure 2B).

To relate the frequency of the infraslow oscillation to fast spindle clustering, we calculated the mean cluster size (mean number of fast spindles per cluster). A slower infraslow oscillation was associated with a higher number of fast spindles per cluster with or without controlling for age and sex ($p < 0.001$, $\beta = -0.37$; Figure 3). The frequency of the infraslow oscillation was also negatively associated with the proportion of clustered fast spindles with or without controlling for sex ($p < 0.001$, $\beta = -0.35$).

Fast spindles characteristics are modulated by their clustering level

To better understand the phenomenon of fast spindle clustering, we analyzed fast spindle characteristics (i.e., amplitude, duration, frequency) for each clustering level, using the data of both young-middle aged and older adults. The clustering level of fast spindles modulated their characteristics (Figure 4). Fast spindle amplitude ($F(4,712) = 190.4$, $p < 0.001$) and duration ($F(4,712) = 710.0$, $p < 0.001$) increased with their clustering level while, to a lesser extent, their frequency ($F(4,712) = 9.6$, $p < 0.001$) decreased. Fast spindles in clusters of at least 5 spindles showed a 6%

increase in amplitude and a 14% increase in duration compared to isolated fast spindles. In contrast, their frequency decreased by 0.2%.

We also investigated whether ageing impacted the effect of clustering level on fast spindle characteristics. After correction for multiple comparisons, a significant interaction between age group and clustering level was observed only for the relative difference in duration ($p = 0.012$), but not for amplitude ($p > 0.49$) and frequency ($p > 0.21$) of fast spindles. Thus, a greater increase in fast spindle duration was observed in young-middle aged compared to old participants, even after considering sex as a confounder (Figure 4).

Age reduces fast spindle clustering

We next investigated the impact of age on fast spindle clustering. Compared to young-middle aged participants, the proportion of clustered fast spindles was reduced in older adults (Table 1) ($p < 0.001$). Both linear and non-linear (2nd order polynomial) adjustments significantly predicted the relationship between the proportion of clustered fast spindles and age, even after controlling for sex (all p -values $p < 0.001$). However, all approaches used to compare the two models (ANOVA, LRT, AIC and BIC) confirmed that the 2nd order polynomial model fitted better our data. This non-linear relationship between age and the proportion of clustered fast spindles showed that this proportion remained relatively stable from 20 to about 50 years but decreased drastically at older ages (Figure 5A). Accordingly, a linear relationship between age and the proportion of clustered fast spindles was found in the older group ($p < 0.001$) while no relationship was found in the young-middle aged group ($p = 0.47$). Of note, greater inter-individual variability in the proportion of clustered fast spindles was observed in older adults compared to young-middle aged ones ($p = 0.023$). To better characterize the impact of age on fast spindle clustering, we compared the proportion of fast spindles between young-middle aged and older adults for each clustering level (Figure 5B). Age affected the clustering level ($F(4,884) = 52.3$, $p < 0.001$). Post-hoc tests revealed that older adults had mostly less clustered

fast spindles compared to young-middle aged participants, that is more isolated ($p < 0.001$) or short clusters of 2 fast spindles ($p < 0.001$) but fewer long clusters of 4 ($p < 0.05$) or of more than 5 spindles ($p < 0.001$). To ensure that this result was not due to the unbalanced sex-ratio of our groups, we replicated our analyses using a subset of the older participants sex-balanced with the young-middle aged participants and obtained similar results to those with the entire cohort.

As sleep fragmentation increases with age, mediation analyses were performed to assess whether the negative association between age and the proportion of clustered fast spindles was mediated by NREM sleep fragmentation (number of micro-arousals per hour of NREM sleep). This relationship was not mediated by NREM sleep fragmentation, age had only a direct effect on the proportion of clustered fast spindles (Figure 6, model 1). Besides, the non-linear association between the proportion of clustered fast spindles and age remained significant after adding NREM sleep fragmentation to the covariates ($p < 0.001$). Linear and non-linear analyses were also performed with the mean cluster size. A similar non-linear association between age and the mean cluster size was found ($p < 0.001$). However, the relationship between age and the mean cluster size was fully mediated by fast spindle density (Figure 6, model 2), the proportion of clustered fast spindles (Figure 6, model 3) and partially mediated by NREM sleep fragmentation (Figure 6, model 4). Direct and indirect effects of these models are reported in Table 2.

Dynamics of fast spindle clustering across the night

We examined the dynamics of fast spindle clustering across the night. We first compared the proportion of clustered fast spindles in the first and last half of the night in young-middle aged and older adults (Figure 7A). We found a significant effect of age group ($F(1,173) = 41.1, p < 0.001$) and of the half of the night ($F(1,174) = 15.8, p = 0.001$). Moreover, we found a significant age group x half of the night interaction ($F(1,174) = 21.9, p < 0.001$), such that the proportion of clustered fast spindles increased in the last half of the night compared to the first one in young-middle aged adults, while it

remained constant in older adults. Similar results were found when considering the different sleep cycles (Figure 7B), with significant main effects of age group ($F(1,172) = 33.6, p < 0.001$) and of sleep cycle number ($F(3,501) = 12.2, p < 0.001$), and a significant age group \times sleep cycle number interaction ($F(3,501) = 12.7, p < 0.001$).

We also analyzed the effect of transitions from NREM sleep to REM in both groups (Figure 7C). We compared fast spindle clustering in the last five minutes of N2-N3 before each transition to REM sleep (pre-REM periods) to the rest of N2-N3 (non pre-REM periods). We found no significant effect of the pre-REM period ($F(1,171) = 0.11, p = 0.74$) and no significant age group \times pre-REM period interaction ($F(1,171) = 0.04, p = 0.84$).

Association between size of fast spindle clusters and memory consolidation

We investigated the link between fast spindle clustering and the overnight change in memory performance. Memory performance is summarised in Table 3. We focused our analyses on the mean cluster size and the three parameters that mediate the effects of age on the mean cluster size (fast spindle density, proportion of clustered fast spindles and NREM sleep fragmentation). Overnight change in memory performance was not associated with fast spindle density ($p = 0.24$), proportion of clustered fast spindles ($p = 0.13$) or NREM sleep fragmentation ($p = 0.84$). However, better overnight consolidation was associated with a higher number of fast spindles per cluster ($\beta = 0.29, p = 0.036$; Figure 8). Importantly, this correlation remained significant after controlling for age and sex. To ensure the robustness of the results, we performed sensitivity analyses with different minimal number of fast spindles to define spindle clusters. The association remained significant for all the size of spindle clusters tested (Table 4). Of note, when considering the first and last half of the night, the association was only significant for the last half of the night ($\beta = 0.33, p = 0.017$).

Association between size of fast spindle clusters and NREM sleep stability

As sleep spindles are also an index of sleep stability, we assessed the link between fast spindle clustering and NREM sleep fragmentation, estimated by the number of micro-arousals per hour of NREM sleep. Following the procedure used for the memory analysis, we focused our analyses on fast spindle density, proportion of clustered fast spindles and the mean cluster size. NREM sleep fragmentation was not associated with fast spindle density ($p = 0.21$) or the proportion of clustered fast spindles ($p = 0.36$) after controlling for age and sex. However, we found that greater NREM sleep fragmentation was significantly associated with fewer fast spindles per cluster ($\beta = -0.16$, $p = 0.033$; Figure 9). Importantly, this result remained significant after controlling for age and sex and was independent of the number of spindles considered to define the clusters (Table 4).

Besides, we also tested the association between subjective sleep quality variables (PSQI, ISI and subjective sleep quality and depth during the polysomnography night) and fast spindle clustering parameters (proportion of clustered fast spindles and mean cluster size). We found no association between subjective reports of sleep quality and fast spindle clustering (all p -values > 0.05).

Discussion

Recent findings in rodents and young adults have brought to light a fluctuation of the fast spindle band power at an infraslow scale of ~ 0.02 Hz during NREM sleep, due to periodic alternation between high sigma power periods (dense in spindles) and low sigma power periods (with scarce spindles). In the present study, we replicated these findings in young-middle aged adults and expanded them to cognitively normal older adults. We also provided the first evidence of a non-linear decrease of the proportion of clustered fast spindles with age. Our data suggest that this proportion remains stable from 20 to approximately 50 years, before dropping at more advanced ages. Importantly, this effect of age on the proportion of clustered fast spindles was not mediated by

NREM sleep fragmentation. Interestingly, young-middle aged adults exhibited an increase of the proportion of clustered fast spindles across the night, but this temporal dynamics was not present in older adults. Besides, fast spindle characteristics were modulated by their clustering level, such that isolated fast spindles exhibited the lowest duration and amplitude while those organized in clusters of at least 5 fast spindles had the highest duration and amplitude. This modulation of fast spindle duration appeared to be slightly attenuated in older adults. Finally, we found that the mean number of fast spindles within a cluster was positively associated with overnight change in memory performance and negatively associated with NREM sleep fragmentation.

Fast spindle clusters, windows for stable sleep and efficient memory consolidation

We found a positive association between the mean cluster size and the overnight change in visuospatial memory performance, while fast spindle density and the proportion of clustered fast spindles were not significantly correlated with memory performance. This result suggests that spindle quantity and clustering (i.e., their temporal organization) may together constitute key factors for memory consolidation. According to the active system consolidation view [57], reactivations of recent, labile memory traces during hippocampal ripples, coordinated with sleep spindles, play a key role in sleep memory consolidation [1]. Indeed, ripples coincide with memory trace reactivations and allow the reorganization of brain regions subserving memory retrieval, with a greater involvement of neocortical areas over time [1]. Besides, hippocampo-neocortical functional connectivity increases during sleep spindles in humans [13] and some cortical regions activated during declarative learning are reactivated during spindles, especially during fast spindles [58]. Sleep spindles are thought to promote synaptic plasticity [4], potentially through changes in calcium activity [59]. Interestingly, it has been shown that calcium activity in dendrites [60] and the occurrence of hippocampal ripples [3] also fluctuate during sleep in rodents, all these events occurring in synchrony with spindle band power variations. Moreover, according to the sequential hypothesis, the succession of NREM and

REM sleep would be essential for memory consolidation ([61], see [62] for review). As the cycling organization of sleep stages is considered to optimize information and memory processing [63,64], repetitive and synchronized fluctuations of spindles-ripples-calcium activity may structure and optimize neuronal plasticity and memory consolidation. In a consistent manner, it has been shown that the effect of NREM sleep disruptions on memory consolidation depends on the periodicity of the disruptions. Using optogenetics to activate arousal-related nuclei, Rolls et al. [65] were able to fragment mice sleep every 60s, 120s or 240s without affecting its overall quantity and quality. Only disruptions every 60s (i.e., approximately at the infraslow time scale) appear to reduce sleep benefit on memory consolidation [65]. Such disruptions would systematically interrupt periods with high spindle power, impairing memory consolidation, while sleep disruptions at longer intervals would still leave some high spindle power periods undisturbed. In this perspective, the correlation observed between mean spindle cluster size and the overnight change in memory performance suggests that the efficiency of memory consolidation requires sufficiently spindle-dense uninterrupted periods. By periodically offering ideal neural conditions for synaptic plasticity, the organization of sleep spindles into clusters would be a major component of the memory consolidation process.

Modulation of fast spindle characteristics contributes to the 0.02-Hz infraslow oscillation

We also found that the clustering of fast spindles (*inter-spindle* parameter) is associated with fine modulations of *intra-spindle* characteristics (amplitude, duration and frequency). The greater the temporary spindle density (i.e., high clustering level), the higher the amplitude and duration of each fast spindles within this cluster. Similar results were reported in a recent study conducted in a small sample of 10 young adults [30]. Despite a slight attenuation of fast spindle duration in older adults, this modulation was present in both young-middle aged and older adults in our study and adds a new degree of complexity to the model proposed by Lecci et al. [3]. First, NREM sleep periods with high

fast spindle band power would constitute a local increase of fast spindle density (*inter-spindle* modulation). Second, the clustered fast spindles in these periods would have a greater amplitude and longer duration (*intra-spindle* modulation). These two levels (inter and intra-spindle parameters) may both participate to a clear distinction between environment monitoring and off-line processing periods that would alternate during NREM sleep.

Fast spindle declustering in ageing

Our results showed a decrease in the proportion of clustered fast spindles with age. As sleep fragmentation increases with age, it could be argued that more fragmented sleep would necessarily lead to reduced proportion of clustered spindles in older adults. However, mediation analyses revealed that age had a *direct* effect on the proportion of clustered fast spindles: NREM sleep fragmentation did not mediate the relationship between these two variables (model 1). Spindle cluster size integrates several aspects as it depends on *spindle density* (more spindles during the night favor longer spindle clusters), *proportion of clustered spindles* (a reduced proportion of isolated spindles favors longer clusters at a fixed spindle density) and *NREM sleep fragmentation* (micro-arousals could interrupt and shorten spindle clusters). Mediation analyses revealed that these 3 parameters mediated the effects of age on mean cluster size (models 2 to 4). Fast spindle clusters become shorter with age because older adults have less fast spindles, their spindles are more often isolated and they have more micro-arousals that can potentially interrupt the ongoing spindle activity. This age-related reduction of fast spindle cluster size could shorten the windows allowing efficient memory consolidation in older adults. However, it may be less relevant to explain the decrease in subjective sleep quality during ageing as no association was found with sleep questionnaires.

Besides, while the proportion of clustered fast spindles was high for almost all young-middle aged adults, substantial inter-individual variability in older adults was observed. This suggests that brain

ageing may affect the clustering of fast spindles with a different magnitude for each participant. This result also raises the question of the neural underpinnings of fast spindle declustering. Fast sleep spindles are known to be generated by the thalamic reticular nucleus (TRN) (see [4] for review). Recent data suggest that their clustering may be orchestrated by the locus coeruleus (LC) [66]. The LC is a small nucleus located in the brainstem and a key structure of the noradrenergic system (see [67] for review). Osorio-Forero et al. [66] found that thalamic norepinephrine (NE) level during NREM sleep in mice fluctuates in an anticorrelated manner with spindle clustering, at a periodicity of about 50 s (i.e., at a 0.02-Hz infraslow scale). Using an optogenetic modulation of LC activity, they could inhibit or favor spindle clustering and causally demonstrated a role of noradrenergic thalamic modulation by LC in spindle clustering. Furthermore, it has been shown that micro-arousals also follow NE oscillations, and are more frequent during the peaks of LC activity [68]. Then, LC periodic activity appears to be crucial for shaping sleep micro-architecture, with spindle-enriched sleep periods when LC is silent and spindle-scarce sleep periods more prone to micro-arousals when LC releases NE.

Interestingly, MRI studies in humans have shown that LC integrity decreases in ageing [69,70]. Lower LC integrity has been associated with a higher number of self-reported nocturnal awakenings in cognitively unimpaired older adults [71]. Therefore, age-related atrophy of the LC may explain the spindle declustering observed in older adults and result in greater sleep fragmentation and poorer memory consolidation, but this hypothesis requires further investigation.

It is noteworthy that LC alterations (neuronal loss, neurofibrillary tangles) (see [72] for review) and spindle changes (density, amplitude and duration reduction) [73,74] have also been described in some neurodegenerative pathologies such as Alzheimer's disease. Further research focusing on spindle dynamics is needed to better characterize the specific spindle alterations in these disorders and understand their impact on memory.

Dynamics of fast spindle clustering across the night

Previous studies have reported an increase in spindle activity throughout sleep cycles which appears to be attenuated with age [37,38,75,76]. More specifically, Purcell et al. [37] found, in a large cohort of 11,630 individuals, that fast spindle density increases throughout the night in adults under 40 years old while it remains constant in adults above 40 years. We provide evidence that fast spindle clustering follows similar dynamics across the night. The proportion of clustered fast spindles increases across the night in young-middle aged adults but remains quite stable in older adults. Interestingly, the link between memory consolidation and fast spindle cluster size was driven by clusters occurring in the last half of the night. Then, the increase of fast spindle clustering across the night may be relevant for memory consolidation but is impaired with age.

Besides, previous studies had also reported an increase in spindle density in the last minutes of NREM sleep before the transitions to REM sleep in both animals [77,78] and humans [37]. More precisely, fast spindle density appears to increase in the last five minutes before the NREM-REM transition in humans [37]. Our results suggest that fast spindle clustering may not be affected by these transitions as the proportion of clustered fast spindles remained stable in the last five minutes before transitions to REM sleep in both age groups.

Strengths and limitations

To the best of our knowledge, this study is the first to investigate infraslow oscillation in fast spindle band and fast spindle clustering in older adults. One of the strengths of this work is the wide age range of participants, allowing to assess age-related changes in spindle clustering during most of the adult lifespan, i.e. from 20 to more than 80 years old. Another asset of the study is the homogeneity of the manual artifact rejection which was performed by a single sleep specialist for all participants.

However, some limitations must be acknowledged. First, as we used polysomnography data collected in different cohorts, acquisition parameters were not strictly the same for all participants (acquisition

device, presence or not of an adaptation night and of sleep questionnaires, number of EEG electrodes, sampling rate). Although all the centro-parietal region was well-covered in the memory consolidation analysis (C3, C4, CZ, P3, P4, PZ), we had to restrict the analyses of age-related changes to C3 and C4 channels. Besides, we had to limit our analyses to fast spindles as almost half of the young-middle aged adults did not have frontal EEG channels and slow spindle power peak was not clearly visible in the remaining participants. The sample size of the young-middle aged group was also moderate. Further studies are needed to replicate our findings and to investigate age-related changes in slow spindle clustering. Moreover, the lack of participants between 52 and 65 years old limits investigations in the first decade following the start of the decrease in fast spindle clustering. However, the large size of the group of older adults allowed us to confirm the negative association between age and the proportion of clustered fast spindle in the 65-83 years old range. Finally, sex ratio was different between the groups of young-middle aged and older adults. Given the evidence for sex differences in spindle characteristics (see [4] for review) and although we have statistically addressed this issue, we cannot rule out residual influences on group differences arising from these biases. However, we replicated our results about age-related changes in fast spindle clustering in sex-balanced subgroups. Besides, given that women tend to show higher spindle density and EEG power, and that the proportion of women was higher in the older adults group, sex bias seems an unlikely explanation for the effects of age on spindle characteristics and clustering reported here.

Conclusion

Overall, our results support the idea that fast spindle organization at an infraslow scale may be a mechanism allowing to maintain a balance between environment monitoring and off-line processing states during NREM sleep. This balance appears to be impaired in older adults, although the switching state periodicity is preserved. Age-related reduction of fast spindle clustering was associated with NREM sleep fragmentation and may affect sleep-dependent memory consolidation.

Further studies are needed to better understand the dynamic aspects of sleep stages, their importance for sleep functions and potential dysregulation in ageing and in various pathological conditions such as neurodegenerative diseases.

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Table 1. Participant characteristics and between-group differences.

	Young-middle aged adults (n = 32)	Older adults (n = 147)	P value	Effect size
Demographics				
Age (year)	34.5 ± 10.9	69.3 ± 4.1	< 0.001	5.9^a
Age range (year)	20-52	65-83	N/A	N/A
Sex ratio (M/F)	22/10	58/89	0.0047	3.38^b
MMSE	N/A	29.1 ± 1.0	N/A	N/A
Sleep architecture				
TST (min)	419.6 ± 56.2	360.5 ± 63.7	< 0.001	0.16
Sleep latency (min)	20.8 ± 18.1	20.7 ± 14.3	0.96	< 0.01
WASO (min)	40.8 ± 30.4	88.1 ± 49.1	< 0.001	0.16
Sleep efficiency (%)	87.1 ± 8.0	77.0 ± 10.4	< 0.001	0.16
% N1	10.6 ± 4.4	14.0 ± 7.5	< 0.001	0.062
% N2	45.8 ± 8.9	47.6 ± 9.1	0.22	< 0.01
% N3	23.4 ± 7.8	20.3 ± 9.8	0.022	0.029
% REM	20.2 ± 5.6	18.0 ± 5.8	0.025	0.028
NREM sleep fragmentation (number of micro-arousals/h)	10.5 ± 6.1	27.6 ± 13.8	< 0.001	0.21
Fast spindle characteristics (C3-C4)				
Density (number per min N2 and N3)	5.3 ± 0.6	4.1 ± 0.7	< 0.001	0.29
Amplitude (µV)	24.9 ± 7.4	18.1 ± 5.5	< 0.001	0.23
Duration (s)	0.82 ± 0.05	0.74 ± 0.04	< 0.001	0.29
Frequency (Hz)	13.26 ± 0.55	13.20 ± 0.51	0.49	0.003
Clustering (% of clustered spindles)	75.2 ± 4.1	67.5 ± 5.9	< 0.001	0.20

Data are presented as mean ± SD. Between-group differences were evaluated using non-parametric Wilcoxon signed-rank test for age and with Chi² test for sex. Between group differences for sleep architecture and fast spindle characteristics were tested using two-way ANOVA to account for sex ratio disparity. Effect sizes were estimated with partial eta squared, except for age (^a Cohen's d) and sex (^b odd-ratio). Abbreviations: F (female), M (male), MMSE (Mini-Mental State Examination), N/A (not applicable), SD (standard deviation), TST (total sleep time), WASO (wake after sleep onset).

Table 2. Detailed statistics of mediation analyses.

Model	ADE			ACME		
	Estimate	95% CI	p	Estimate	95% CI	p
1	-0.195	-0.256; -0.140	< 0.001	-0.013	-0.039; 0.017	0.38
2	0.001	-0.0008; 0.0035	0.21	-0.011	-0.014; -0.010	< 0.001
3	-0.0001	-0.0019; 0.0015	0.89	-0.009	-0.012; -0.007	< 0.001
4	-0.008	-0.012; -0.005	< 0.001	-0.002	-0.003; 0.001	0.019

The independent variable was age in all models. The dependent variables were the proportion of clustered fast spindles (model 1) and the mean cluster size (models 2, 3 and 4). The mediators were NREM sleep fragmentation (models 1 and 4), fast spindle density (model 2) and the proportion of clustered fast spindles (model 3). Abbreviations: ACME (Average Causal Mediation Effect), ADE (Average Direct Effect), CI (Confidence Interval).

Table 3. Learning and recall performance at the memory task.

	Older adults (n = 77)
Number of subjects achieving the learning criterion	66
Number of trials needed to reach the learning criterion	7.6 ± 2.8
Learning score (% correct answers)	71.8 ± 7.3
Recall score (% correct answers)	52.5 ± 13.6
Overnight change in performance (%)	-26.3 ± 20.7

Data are presented as mean ± SD. The task was performed by a subgroup of 77 older adults (see Material and methods section). The overnight change in performance was calculated as follows: $100 \times (\text{recall score} - \text{learning score}) / \text{learning score}$.

Table 4. Replication of the associations between mean fast spindle cluster size, memory consolidation and NREM sleep fragmentation using different minimum spindle cluster sizes.

Spindle cluster sizes	Mean spindle cluster size ~ memory consolidation	Mean spindle cluster size ~ NREM sleep fragmentation
≥ 2 spindles	$\beta = 0.29$; $p = 0.036$	$\beta = -0.16$; $p = 0.033$
≥ 3 spindles	$\beta = 0.28$; $p = 0.043$	$\beta = -0.21$; $p = 0.0052$
≥ 4 spindles	$\beta = 0.28$; $p = 0.049$	$\beta = -0.22$; $p = 0.0036$
≥ 5 spindles	$\beta = 0.31$; $p = 0.030$	$\beta = -0.20$; $p = 0.0060$

Sensitivity analyses were performed to evaluate the robustness of the associations between mean cluster size, memory consolidation and NREM sleep fragmentation obtained using the criteria proposed by Boutin and Doyon [29] (≥ 2 spindles). Different minimum spindle cluster sizes were tested. Analyses were controlled for age and sex, and the standardized regression coefficients β are reported.

Figure 1

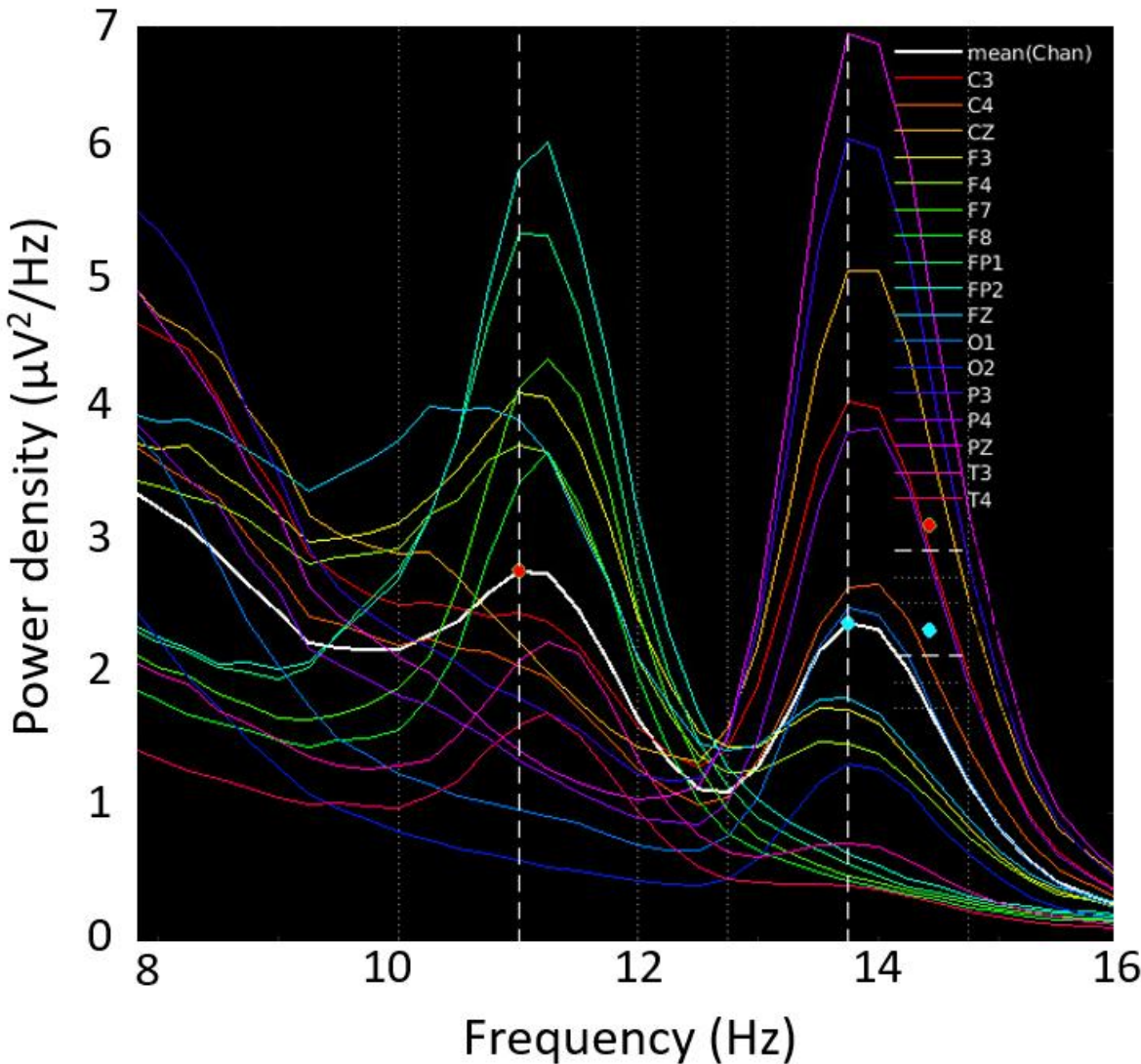


Figure 2

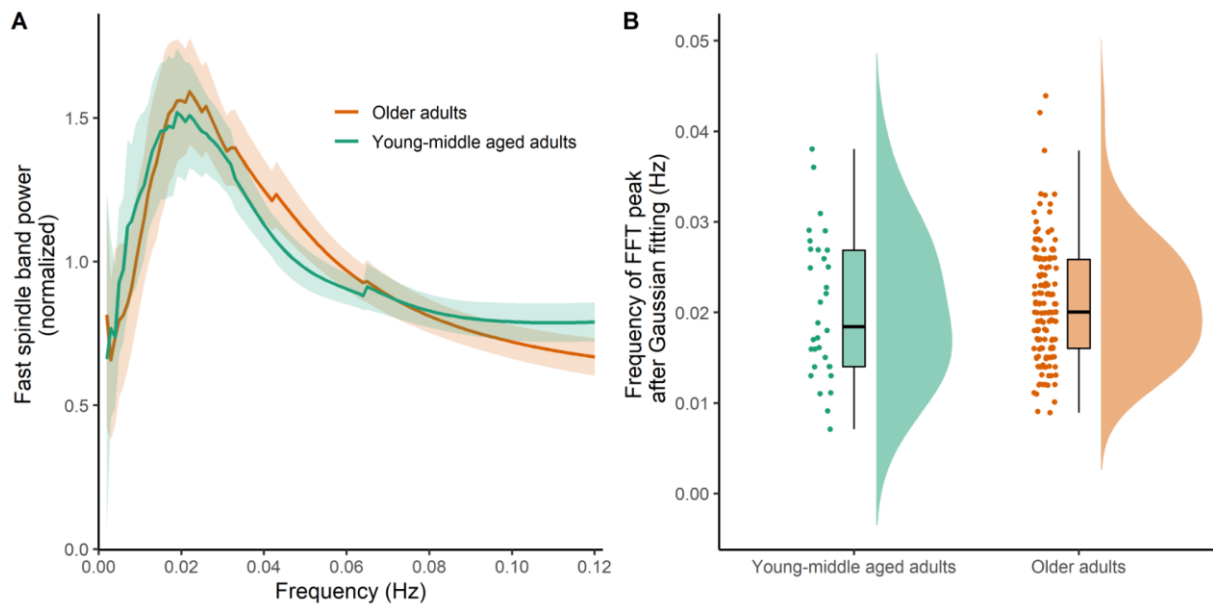


Figure 3

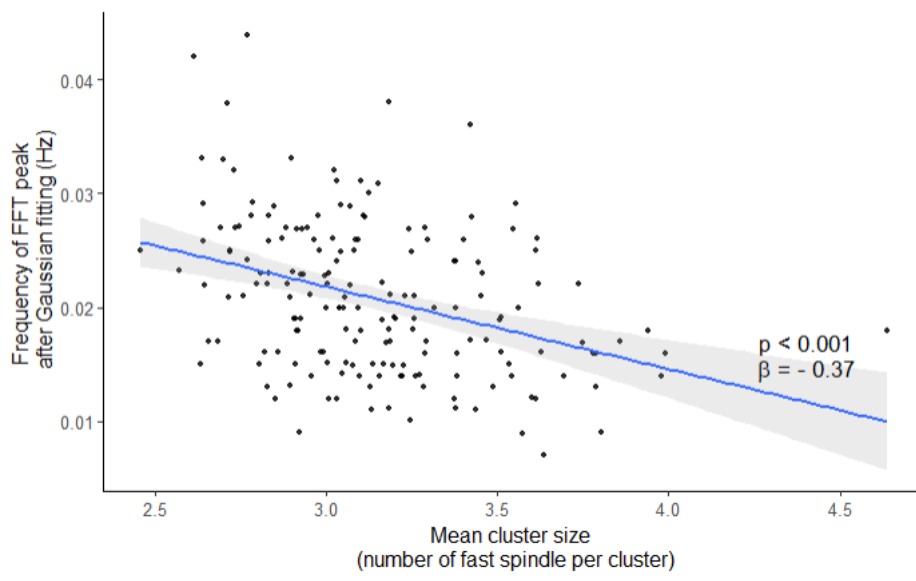


Figure 4

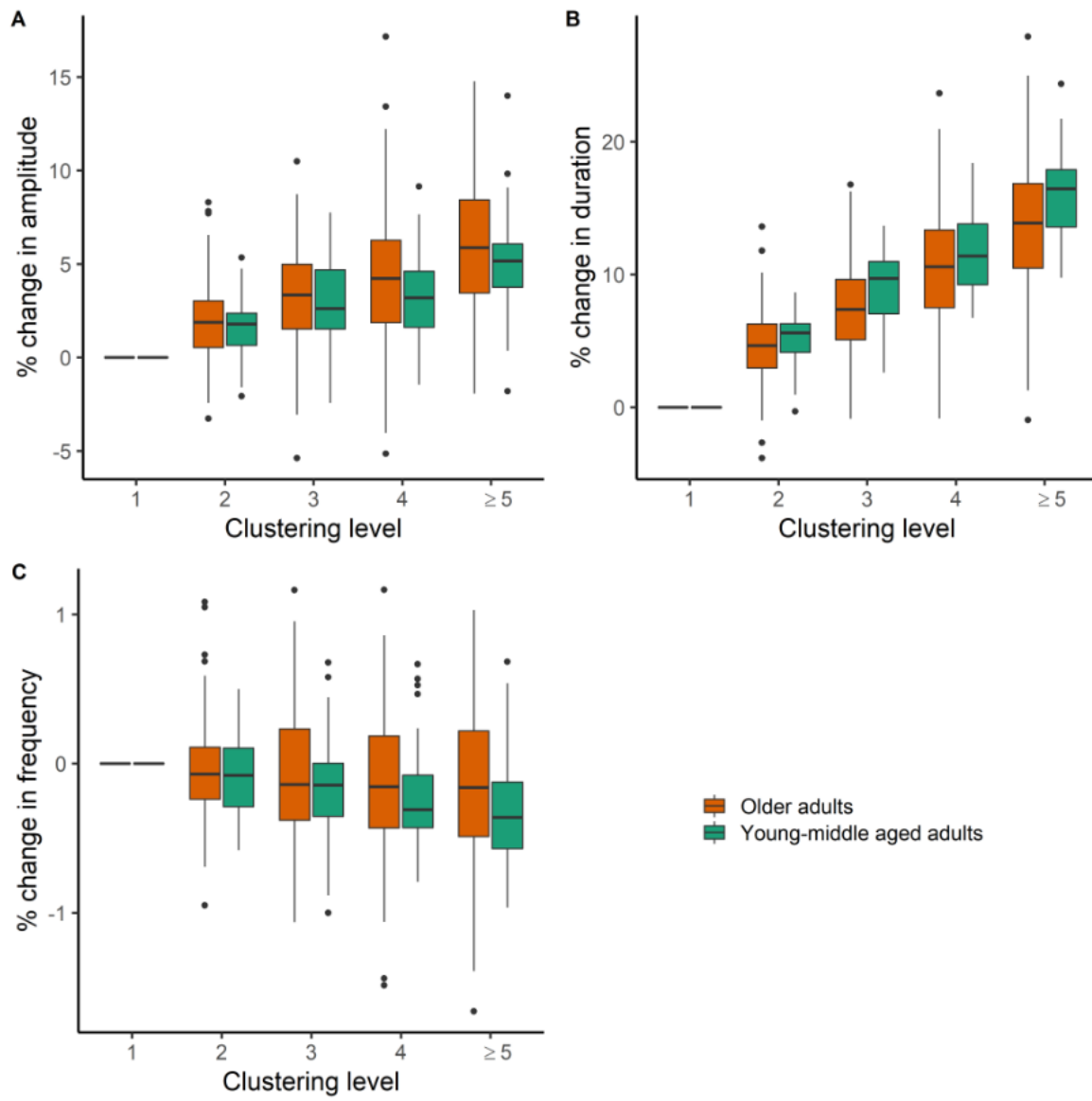


Figure 5

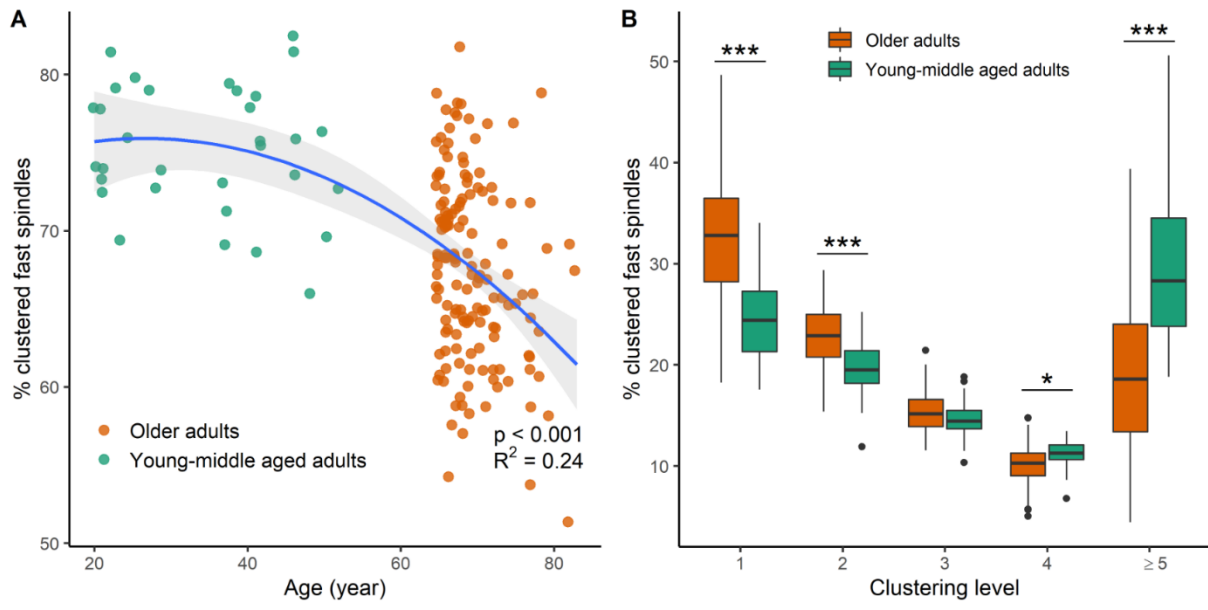


Figure 6

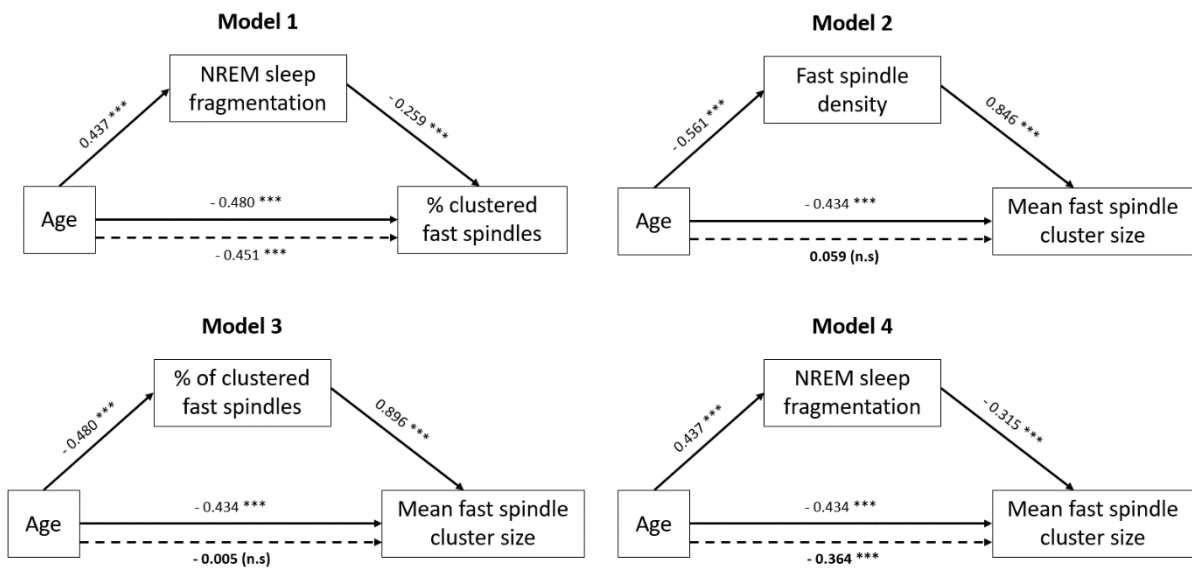


Figure 7

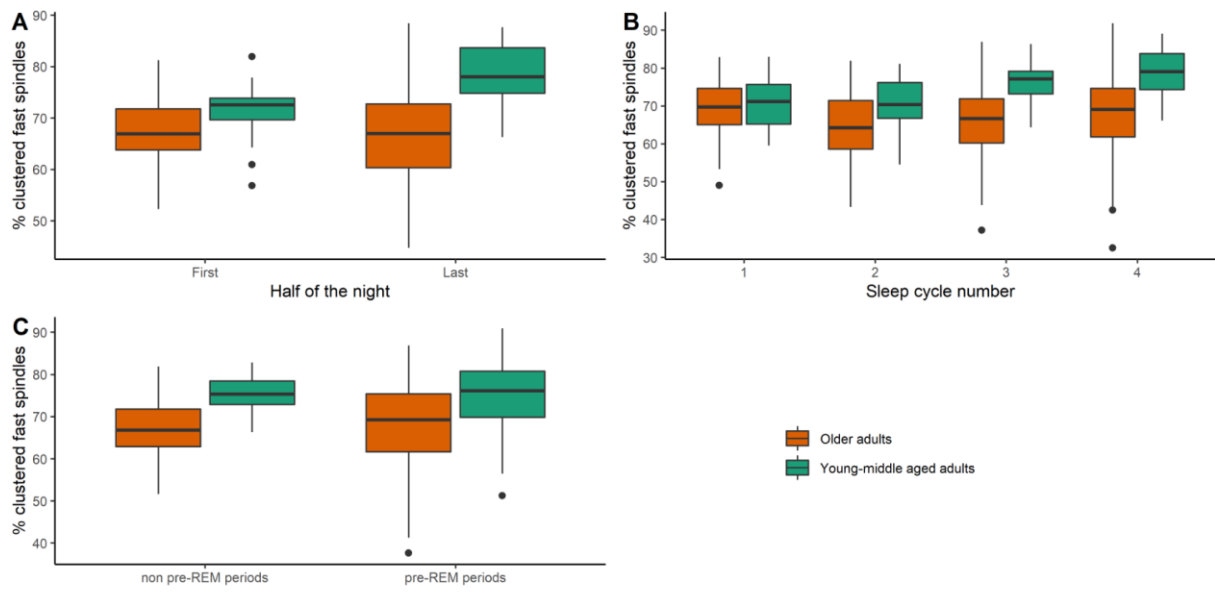


Figure 8

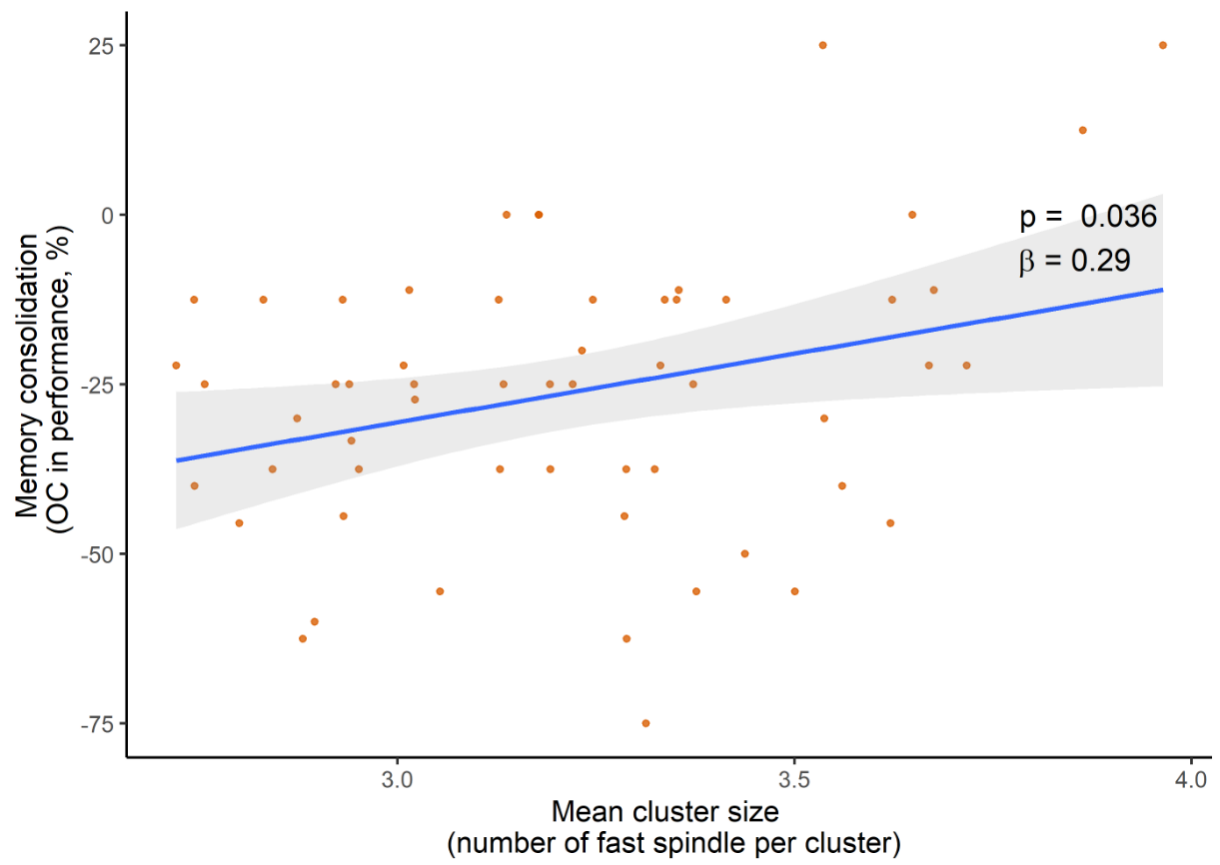
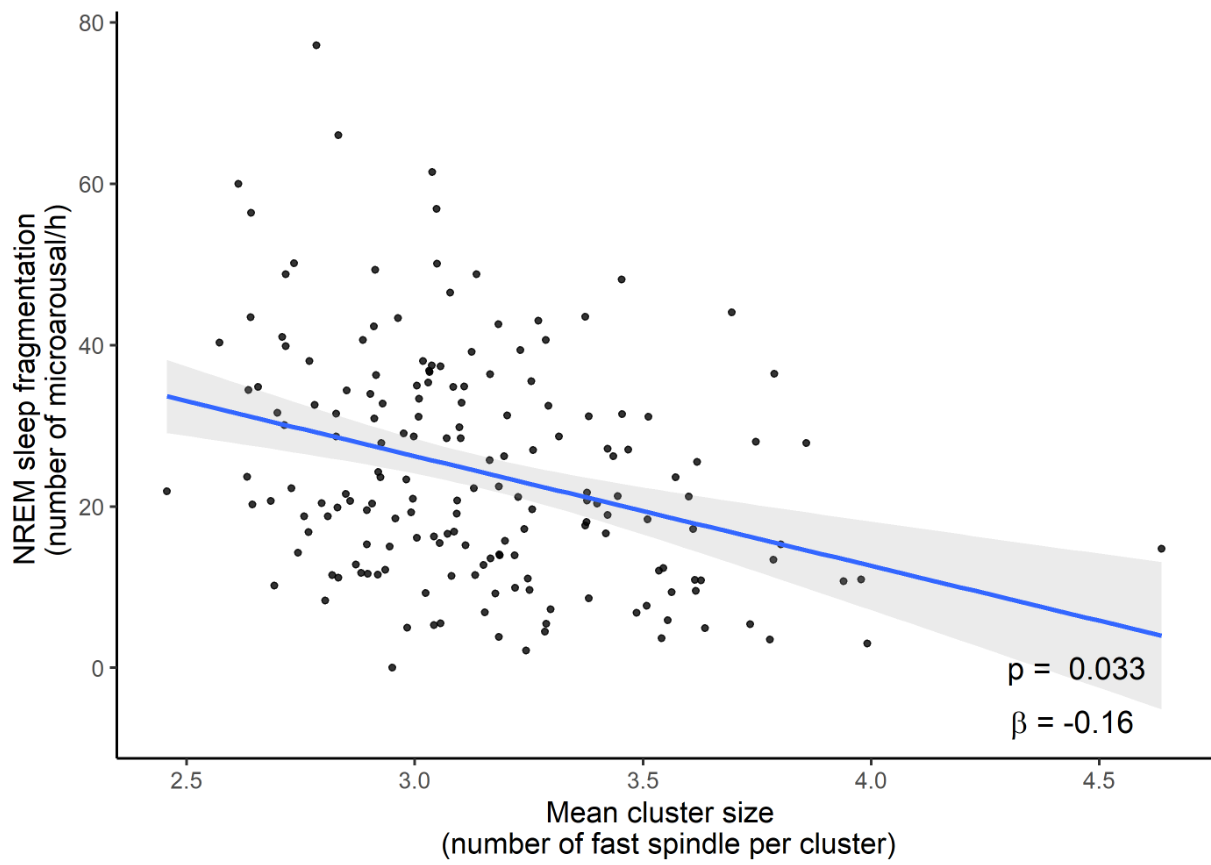


Figure 9

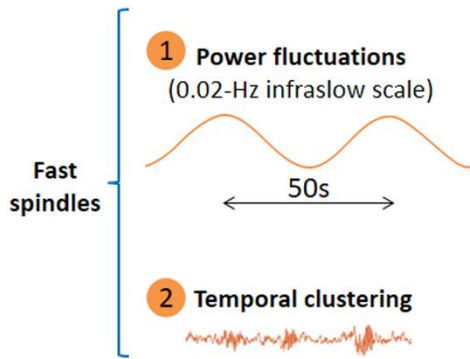


Graphical abstract

Study question:
Does age impact fast spindle temporal dynamics?

 32 young-middle aged adults

 147 older adults



+++

Reduced



Poorer memory consolidation



Increased NREM sleep fragmentation

Main findings:

- Age reduces fast spindle clustering
- These changes are associated with poorer memory consolidation and increased NREM sleep fragmentation.

Images: Freepik (pch.vector); Noun Project (ImageCatalog, Jayson Lim, Yannik Wölfel and Adrien Coquet)