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# The timing of NREM sleep spindles is modulated by the respiratory cycle in humans

Running title: Respiration-locked timing of sleep spindles

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## **ABSTRACT**

Coupling of sleep spindles with cortical slow waves and hippocampus sharp-waves ripples is crucial for sleep-related memory consolidation. Recent literature evidenced that nasal respiration modulates neural activity in large-scale brain networks. In the rodent, this respiratory drive strongly varies according to vigilance states. Particularly, during sleep, respiration promotes the coupling between hippocampal sharp-wave ripples and cortical DOWN/UP state transitions. However, no study has examined whether sleep spindles could be respiration-modulated in humans. In this work, we aimed to investigate the influence of breathing on brain oscillations during non-rapid-eye-movement (NREM) sleep in humans by examining the coupling between sleep spindles and respiration cycle. Full night polysomnography of ten healthy participants were analysed. Spindles and slow waves were detected during N2 and N3 sleep stages. Spindle-related sigma power as well as spindle and slow waves events were analysed according to the respiratory phase. We found a significant coupling between sleep spindles and respiration cycle, with enhanced sigma activity and spindles during the second part of the expiration phase. No coupling was observed between breathing and slow waves. Breathing cycle influences the dynamics of brain activity during NREM sleep. This may enable sleep spindles to synchronize with other brain rhythms including hippocampus sharp wave ripples, and facilitate information transfer between distributed brain networks.

## **KEYWORDS**

Sigma; slow waves; breathing; coupling

## **INTRODUCTION**

Sleep spindles are brief (typically 0.5-2 s) bursts of sigma (10-16 Hz) oscillations recorded in the electroencephalogram (EEG) during non-rapid-eye-movement (NREM) sleep. They involve oscillations within reticulo-thalamo-cortical loops and have been associated with cognitive functions including learning and memory (Fernandez & Luthi, 2020). Spindles are synchronized with other NREM sleep oscillations. Their coupling with cortical slow waves and hippocampus sharp-wave ripples is crucial for triggering reactivation of learning material and promoting hippocampo-neocortical transfer of information in the context of sleep-related memory consolidation (Klinzing, Niethard, & Born, 2019). Such transfer, which requires a fine temporal alignment between these different activities, could be facilitated by respiration (Heck, Kozma, & Kay, 2019). Indeed, respiration was shown to modulate neuronal activity in large-scale subcortical and cortical networks, during both wake (Kluger & Gross, 2021; Zelano et al., 2016) and sleep states (Girin et al., 2021; Tort, Hammer, Zhang, Brankack, & Draguhn, 2021). In the rodent, the respiratory cycle modulates the timing of sharp-wave ripples, whose occurrence probability increases during the early expiration phase (Liu, McAfee, & Heck, 2017). During sleep, respiration promotes the coupling between hippocampal sharp-wave ripples and cortical DOWN/UP state transitions, a neuronal process involved in memory consolidation (Karalis & Sirota, 2022). Behaviourally, the enhancement of recognition memory in humans by nasal breathing during consolidation strongly supports a role for breathing in the consolidation process (Arshamian, Iravani, Majid, & Lundstrom, 2018). Thus, given the importance of sleep spindles in memory consolidation, and the potential role of respiration in their coupling with other neuronal events, we aimed here at examining whether spindles could be respiration-modulated in humans.

## **METHODS**

### **Participants**

Ten healthy subjects (mean  $\pm$  SD age  $26.7 \pm 7.1$  years; 8 females, 2 males) who had participated in different research projects including a full night polysomnography (PSG) were randomly selected for this study. All participants had benefited from an extended clinical evaluation. Exclusion criteria were: any sleep disorders; any medical or psychiatric conditions; any medication or drug known to influence sleep; shift work; sleep deprivation (ruled out by a 7-day actigraphy prior the PSG). All participants granted informed consent. The study was approved by the Hospices Civils de Lyon Ethics Review Board (N° 22\_5757, 10/06/2022).

### **Sleep recordings**

Night sleep recordings were conducted in the Centre for Sleep Medicine and Respiratory Diseases of Lyon University Hospital between 2017 and 2021. The following signals were recorded with the Deltamed/Natus® acquisition system (sample rate: 256 Hz): EEG (bipolar montage: Fp2-C4 ; C4-T4 ; T4-O2; Fp1-C3 ; C3-T3; T4-O2; Fz-Cz; Cz-Pz), electro-oculogram, chin and tibialis electromyogram, EKG, nasal airflow (nasal pressure and oronasal thermistor), pulse oximetry, microphone, and respiratory efforts (thoracic and abdominal belts).

### **Respiratory recording and processing**

Respiratory cycle detection was performed using an algorithm previously described applied on nasal pressure signal (Roux et al., 2006). Briefly, it consists in performing signal smoothing for noise reduction and detection of zero-crossing points, allowing an accurate definition of the inspiration and

expiration phases (respectively positive and negative deflections). The inspiration (respectively expiration) phase starts at the zero-crossing point of the rising (respectively falling) phase and ends at the zero-crossing point of the falling (respectively rising) phase.

### **EEG data analysis**

Sleep staging was performed in 30 s epochs using YASA toolbox (Vallat & Walker, 2021), an automated sleep scoring algorithm based on the American Academy of Sleep Medicine recommendations. Only N2 and N3 stages were retained for analyses. The same toolbox was used for spindles (12-15 Hz; 0.5-2 s; threshold > 1.5 SD of the root mean square of the sigma-filtered signal) and slow waves (0.2-1.5 Hz, 0.3-1.5 s duration for negative deflection and 0.1-1 s duration for positive deflection, peak-to-peak amplitude 75-350  $\mu$ V) detection. Time-frequency (TF) maps of the NREM sleep signal were obtained using Complex Morlet Wavelet convolutions. Morlet wavelet settings were optimized to capture spindle-related sigma power of the signal as a function of time (frequencies linearly increasing from 10 to 20 Hz and from 7 to 13 cycles in 40 steps, tapering to zero, 4 s duration). TF maps were then sliced into epochs according to onset and offset of each respiratory cycle. TF maps corresponding to each of these epochs were then “stretched” as explained in Roux et al. (2006). Briefly, the time component of these epochs, which can differ from trial to trial, was converted into a phase component defined as  $[0, 2/3\pi]$  and  $[2/3\pi, 2\pi]$  for inspiration and expiration, respectively. As opposed to time representation, the phase representation is common to all epochs. Thus, this phase representation of the respiratory cycle is used as a normalized time basis allowing collecting results in a standardized data format across different participants and providing a way to average oscillatory components of the activity. We then averaged such phase-frequency maps depending on whether they contained spindles or not. Subtraction between both types of phase-frequency maps (with or without spindles) allowed isolating spindle-related sigma power as shown in figure 1. Spindle amplitude-envelope peaks and slow wave negative peaks were considered as timing events timestamped thanks to YASA algorithm. These timestamps were converted to phase angle according to the relative time of occurrence during their corresponding breathing cycle.

### **Statistical analysis**

For each participant, mean direction and vector length of these two sets of phase angles (spindles peaks and slow wave negative peaks) were computed using circular statistics. Rayleigh test was performed to test for non-uniformity of circular distribution of angles and data were considered as non-uniform if p-value returned lower than 0.05. Watson-Williams test was performed between spindle and slow waves phase angles for each participant to test for the equality of the direction means. Non-equality was retained if p-value returned lower than 0.05.

## **RESULTS**

One participant (P8) was excluded from the analysis due to poor quality signals.

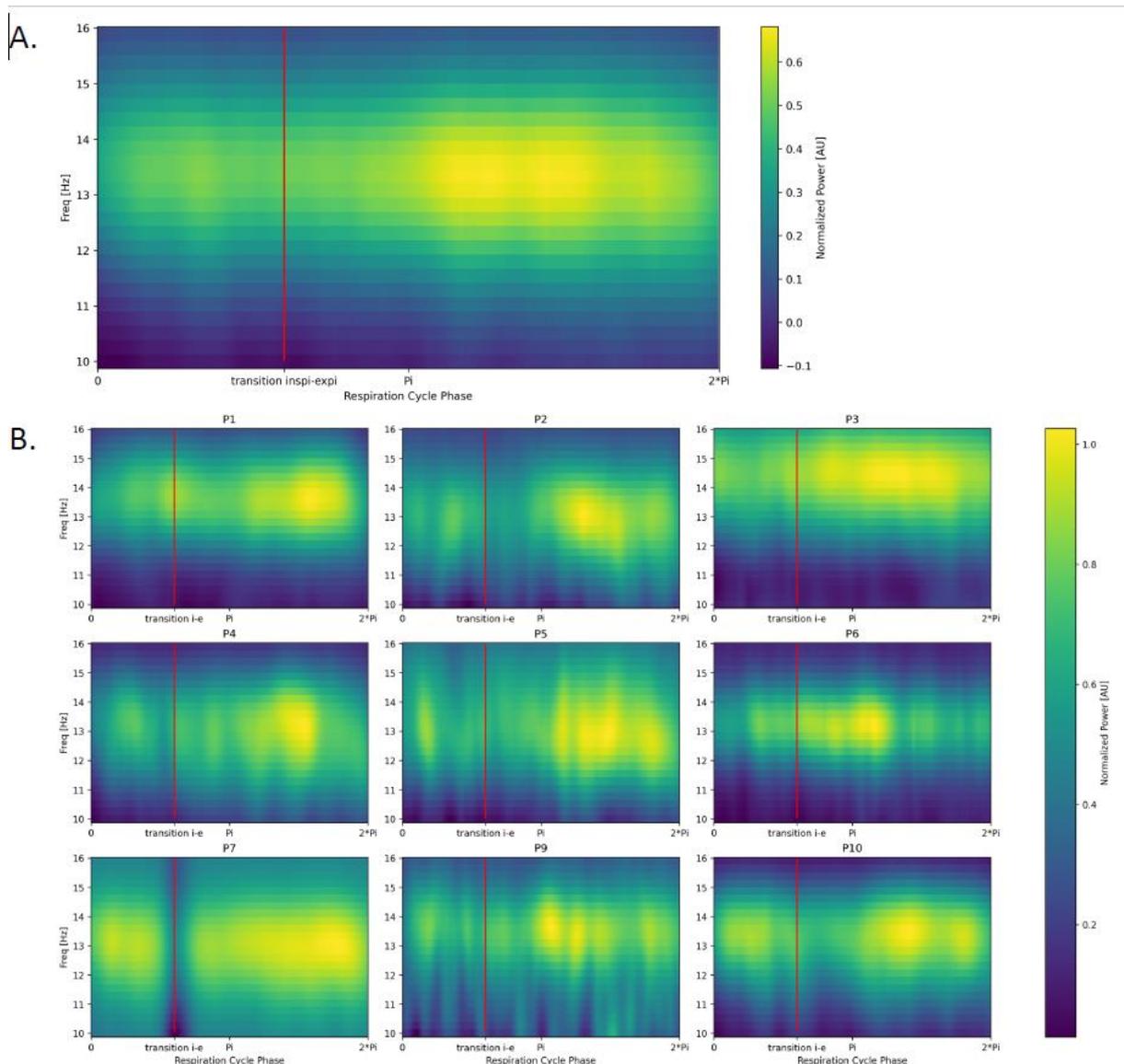
### **Respiration cycles and NREM sleep oscillations features**

On average  $245 \pm 37$  min of NREM sleep N2 + N3 per participant were used for analyses. Participants respiration cycles lasted on average  $3.9 \pm 0.6$  s corresponding to  $1.4 \pm 0.2$  s of inspiration and  $2.5 \pm 0.4$  s of expiration. Considering all bipolar channels ( $n=8$  per subject),  $3400 \pm 1528$  spindles were detected per participant with a 0.84 s mean duration and a 13.5 Hz mean frequency, and  $3803 \pm 2197$

slow waves were detected per participant, with a 1.23 s mean duration, a 0.85 Hz mean frequency, and a negative-to-positive peak mean amplitude of 110  $\mu$ V.

### Spindle-related sigma power coupling with respiration

Spindle related sigma power was extracted using Morlet Wavelet convolutions and computed along respiration cycle phase. As right and left fronto-central bipolar derivations showed the highest power in the sigma band, next analyses related to spindle-related sigma power were performed only on the mean of those signals. As evidenced by the mean respiratory phase-frequency map of sigma band power (Fig.1A), the power was not evenly distributed over the respiratory cycle but was higher during the expiratory phase. Individual maps (Fig.1B) revealed that it was true for all the 9 subjects. Nevertheless, an inter-individual variability appeared in the phase, frequency and/or duration of the episodes of sigma high power.



**Figure 1: Spindle-related sigma power according to respiration phase**

(A) Mean respiratory phase-frequency map of fronto-central sigma band power (n=9 subjects).

(B) Individual phase-frequency maps of fronto-central sigma band power.

Sigma power is higher during the expiratory phase.

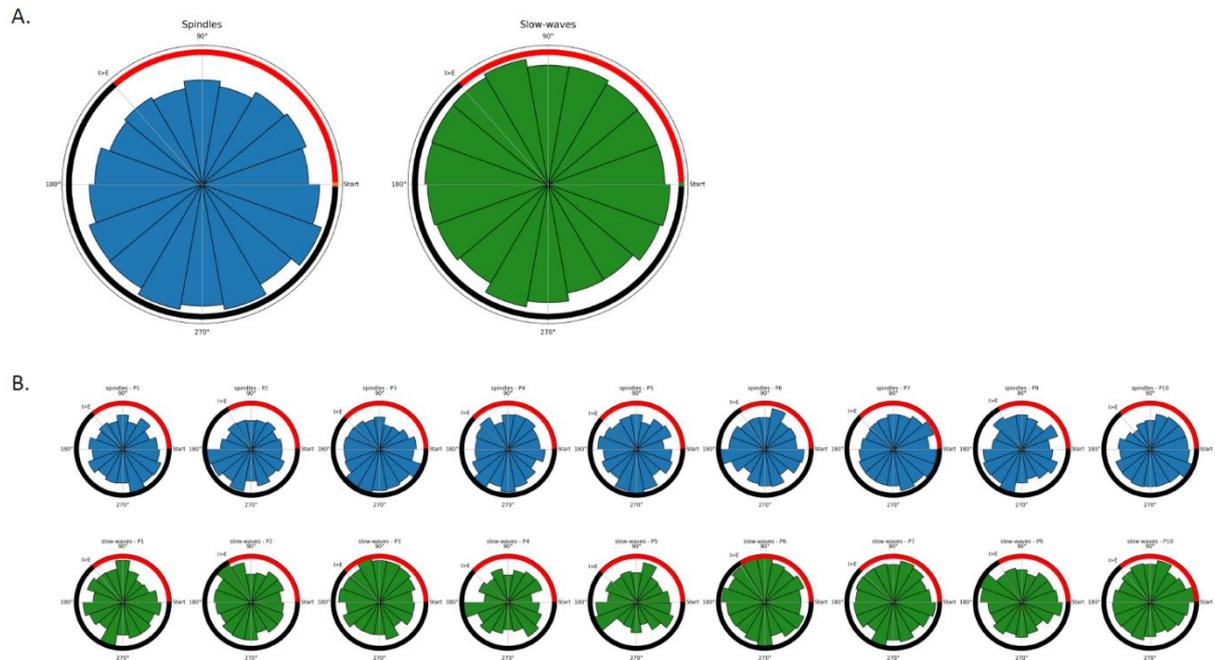
### Spindle and slow waves events coupling with respiration

When considering the probability of occurrence of spindle events detected on all channels as a function of the respiratory cycle, Rayleigh test underlined a non-uniform distribution (Table). The mean direction indicated a significantly increased probability during the expiration phase (Fig.2A, blue polar plots). Individual polar plots (Fig.2B) revealed that it was true for 8 out of 9 subjects (Rayleigh test, Table). Since NREM spindles have been described as phase-locked to sleep slow waves (Clemens et al., 2007), we explored if slow waves were also respiration-phased. Mean (Fig.2A, green) and individual (Fig.2B) polar plots of slow waves maxima revealed that they were not as non-uniformly distributed as spindles. Indeed, only 3 out of 9 subjects exhibited a phase distribution significantly different from a uniform one (P2, P3, P10). In these 3 participants, mean direction indicated an increased probability during the inspiration phase for P3 and P10. For all participants, Watson-Williams test revealed that spindle and slow waves polar distributions were statistically different, as the mean distributions ( $p < 0.05$ ).

**Table. Phase angles of spindle and slow-wave events measured according to respiration cycle in each participant**

Spindle peaks and slow-wave negative peaks were considered as timing events. Rayleigh test was computed to test for uniformity of angles. Only p-values are kept and labelled as significant if lower than 0.05 (ns: non-significant, \*:  $0.01 \leq p < 0.05$ , \*\*:  $0.001 \leq p < 0.01$ , \*\*\*:  $p < 0.001$ ). Circular mean direction in degrees was computed and cells were coloured if significant according to Rayleigh test, in orange if mean direction pointing toward expiration, in green if mean direction pointing toward inspiration.

Participant	Event	p-Rayleigh	Rayleigh Significance	Mean Direction (°)	Mean Vector Length	Event	p-Rayleigh	Rayleigh Significance	Mean Direction (°)	Mean Vector Length
P1	Spindles	5,26694E-06	***	301	0.068	Slow waves	0,14113726	ns	224	0.029
P2	Spindles	2,92275E-10	***	243	0.078	Slow waves	0,000526158	***	178	0.04
P3	Spindles	1,39691E-20	***	263	0.098	Slow waves	7,31438E-06	***	108	0.062
P4	Spindles	0,000217171	***	250	0.051	Slow waves	0,828151868	ns	295	0.015
P5	Spindles	3,40067E-05	***	256	0.053	Slow waves	0,823591514	ns	107	0.018
P6	Spindles	0,946112925	ns	208	0.007	Slow waves	0,063559321	ns	120	0.022
P7	Spindles	8,19476E-14	***	329	0.07	Slow waves	0,968147563	ns	105	0.002
P9	Spindles	0,037578072	*	229	0.048	Slow waves	0,05037914	ns	169	0.026
P10	Spindles	1,70619E-11	***	317	0.099	Slow waves	0,041066984	*	61	0.024



**Figure 2: Spindle and slow waves events occurrence according to respiration phase**

- (A) Mean polar distribution of spindle (blue) and slow wave (green) events according to respiratory cycle (n=9 subjects). Spindle peaks and slow-wave negative peaks were considered as timing events. These events were labelled with the phase angle of occurrence according to breathing cycle and distributed in 18 equal phase bins of respiration ( $20^\circ$  per bin). Inspiration and expiration phases are indicated by the red and black lines respectively.
- (B) Individual polar distribution of spindles (blue) and of slow waves distribution (green) according to respiration cycle.

Spindles occurrence is higher during expiration phase.

## DISCUSSION

We found a significant coupling between sleep spindles and respiration cycle, with enhanced sigma activity and spindle events during the second part of the expiration phase. These results show for the first time that sleep oscillations are modulated by breathing in humans.

During wakefulness, local field potential activity in limbic area including the piriform cortex, the amygdala, and the hippocampus, is entrained by nasal breathing, disappearing with oral breathing (Zelano et al., 2016). This suggests that the respiration-locked effect may be driven by the mechano-sensitivity of olfactory sensory neurons to air pressure, with olfactory bulb responses propagating to other brain area (Girin et al., 2021). During sleep however, a recent study in mice revealed that respiratory modulation of sharp wave ripples occurrence and UP/DOWN states is maintained after olfactory deafferentation, suggesting the existence of an ascending respiratory corollary discharge, likely propagating from the brainstem (Karalis & Sirota, 2022). In line with the extensive data showing the persistence of brain responses to various sensory inputs during sleep (Bastuji & Garcia-Larrea,

1999), our results reveal that breathing is able to influence brain activity during NREM sleep in thalamo-cortical networks in humans. The relative contribution of nasal vs ascending respiratory activity should be further investigated.

We observed that sleep spindles were locked to the expiratory phase with a high within and between subject reproducibility. This oscillatory pacemaker effect of breathing may enable sleep spindles to synchronize with other brain rhythms including hippocampus sharp wave ripples. Respiration-driven enhanced coupling may facilitate information transfer between distributed brain networks, and play a role in sleep-related memory consolidation process (Karalis & Sirota, 2022). Interestingly, we did not find such a breathing effect on slow waves occurrence, suggesting that the oscillation between cortical up and down states may be independent of respiration.

We acknowledge several limitations to the study. First, this was an exploratory study on a small number of participants, with a high proportion of young women (participants were selected from a study on idiopathic hypersomnia). Our results need to be confirmed in larger cohorts of subjects of various ages with more balanced sex ratio. Second, as the number of EEG channels was limited, we were not able to separately study “fast” (13-16 Hz) and “slow” (10-13 Hz) spindles; investigating whether our findings apply to both spindles types would be of high interest given their different functional roles (Fogel & Smith, 2011).

As a conclusion, respiration phase influences the dynamics of brain activity during NREM sleep. The impact of breathing characteristics on sleep-related cognitive functions, as well as the consequences of sleep-related breathing disorders and mechanical ventilation on this breath-to-brain effect remain to be investigated.

#### **Data sharing statement**

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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