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Loss of sleep spindle frequency deceleration in Obstructive Sleep Apnea

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HIGHLIGHTS

- This study makes use of a novel approach to systematically address non-stationarity in sleep spindle oscillatory frequency: quantification of internal frequency modulation or chirp rate.
- Study suggests that Obstructive Sleep Apnea (OSA) not only slows down sleep spindle frequency, but also disrupts spindle internal frequency modulation.
- Loss of physiological sleep spindle deceleration may signal disruption of the thalamo-cortical loops involved in learning and memory.

ABSTRACT

Objective: Sleep spindles have been suggested as surrogates of thalamo-cortical activity. Internal frequency modulation within a spindle's time frame has been demonstrated in healthy subjects, showing that spindles tend to decelerate their frequency before termination. We investigated internal frequency modulation of slow and fast spindles according to Obstructive Sleep Apnea (OSA) severity and brain topography.
Methods: Seven non-OSA subjects and 21 patients with OSA contributed with 30 min of Non-REM sleep stage 2, subjected to a Matching pursuit procedure with Gabor chirplet functions for automatic detection of sleep spindles and quantification of sleep spindle internal frequency modulation (chirp rate).
Results: Moderate OSA patients showed an inferior percentage of slow spindles with deceleration when compared to Mild and Non-OSA groups in frontal and parietal regions. In parietal regions, the percentage of slow spindles with deceleration was negatively correlated with global apnea-hypopnea index ($r_s = -0.519$, $p = 0.005$).
Discussion: Loss of physiological sleep spindle deceleration may either represent a disruption of thalamo-cortical loops generating spindle oscillations or some compensatory mechanism, an interesting venue for future research in the context of cognitive dysfunction in OSA.
Significance: Quantification of internal frequency modulation (chirp rate) is proposed as a promising approach to advance description of sleep spindle dynamics in brain pathology.

1. Introduction

Sleep spindles have been suggested as neurophysiological markers of sleep homeostasis (Aeschbach et al., 1997; Wei et al., 1999; Himanen et al., 2002). They were found to play a role in important brain functions, such as memory consolidation and learning processes, with significant implications also in brain pathology (Schabus et al., 2004; Born et al., 2006; Ktonas et al., 2009; Urakami, 2009; Barakat et al., 2011; Fogel and Smith, 2011). Traditionally, they have been described through three parameters: voltage, duration and central frequency. Central frequency is usually considered stationary within a spindle's short time frame. Human sleep spindles are often divided into slow

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and fast types. Slow spindles are more prevalent in frontal regions, whereas fast spindles occur more often in parietal locations (Jobert et al., 1992; Broughton and Hasan, 1995; Aeschbach et al., 1997; Werth et al., 1997; Zeitlhofer et al., 1997; Huupponen et al., 2008; Barakat et al., 2011).

Recently, however, internal (within-spindle) frequency variation (chirp) has been demonstrated in rats (Sitnikova et al., 2009) as well as in humans (Andrillon et al., 2011), and systematically measured in humans (Ktonas et al., 2009; Schönwald et al., 2011). The train of discharges that generates the sleep spindle can increase (“accelerate”), decrease (“decelerate”) or maintain a stable frequency over time. In a previous study, we have found that sleep spindles, as measured over central scalp regions of healthy volunteers, preferentially decelerate (Schönwald et al., 2011). In other words, negative chirping (deceleration) is more prevalent than positive chirping (acceleration). This possibly represents physiological modulatory mechanisms related to termination of spindle oscillations at the thalamic reticular level (Destexhe et al., 1994; Steriade, 2000; Destexhe and Sejnowski, 2009) and to its interplay with cortical projections (Bonjean et al., 2011; Caporro et al., 2012).

Sleep spindles have been proposed as sensitive electrophysiological markers of brain dysfunction in Obstructive Sleep Apnea (OSA) Schönwald et al., 2012. In OSA, repetitive episodes of complete or partial upper airway obstruction result in reduced blood oxygenation, sleep fragmentation and numerous consequences to health and quality of life, including memory impairment and increased accident and cardiovascular risk (AASM, 2005). Sleep spindles have been shown to become slower in OSA (Himanen et al., 2003; Ondze et al., 2003; Schönwald et al., 2012), especially in frontal but also in parietal regions. These findings corroborate neuropsychological and imaging studies showing diffuse, predominantly frontal cortical dysfunction in OSA (Naëgelé et al., 1995; Décary et al., 2000; Morrell et al., 2003; Mallat and Zhang, 2002; Alchanatis et al., 2004; O'Donoghue et al., 2005; Thomas et al., 2005).

Considering that sleep spindle oscillations represent thalamocortical activity, a subtle modulation of sleep spindle frequency characteristics, such as chirping, could bear relevant information in the context of neuropathophysiology in OSA. We hereby evaluate, for the first time, slow and fast sleep spindle chirping rate and negative chirp percentage in different brain regions, in patients with and without OSA.

2. Methods

2.1. Subjects and EEG recordings

This study was approved by the institutional review board and ethical committee. All participants provided informed written consent. The present sample has been used in a previous work on general spindle characteristics in OSA (Schönwald et al., 2012), whereby consecutive patients with clinically suspected OSA (AASM, 2005) were prospectively enrolled for polysomnography (PSG) at a university hospital-based sleep clinic between April 2007 and July 2009. On the basis of Apnea-Hypopnea Index (AHI) AASM, 2005, study groups were defined as Non-OSA (AHI < 5), Mild (AHI 5–14) and Moderate (AHI 15–29) OSA. Twenty-one patients with Mild (11) and Moderate (10) OSA and 7 Non-OSA subjects participated in the study. They had no significant inter-group differences in age, gender, body mass index, subjective sleepiness (Johns, 1991; Bertolazi et al., 2009), sleep architecture or mean Non-REM sleep O_2% saturation. Arousal index was higher in moderate OSA when compared to non-OSA subjects. Tricyclic antidepressants were the only medications present in patient regimens well-known to change sleep architecture; however, they were similarly expressed among groups (1 in Non-OSA, 2 in Mild and 2 in Moderate OSA). The other medications were non-psychotropic drugs for non-neurological co-morbidities. For additional details refer to (Schönwald et al., 2012).

Continuous recordings were performed on a 64-channel, 16 bit resolution digital system (Deltamed, Racia-Alvar, France). The recording protocol followed standard guidelines (Iber et al., 2007). Silver electrodes were placed over standard 10–20 IS EEG positions with initial impedances below 10 Kohms. The signal was acquired with 256 Hz sampling rate, filtered at 0.5–35 Hz and analyzed off-line using Coherence 3NT software version 4.4 (Deltamed, France). Sleep stages, arousals and respiratory events were visually scored by a trained rater in accordance with standard recommendations, applying obstructive hypopnea rule 4B (Iber et al., 2007).

2.2. EEG sample

Each subject contributed with 30 min of Non-REM sleep stage 2 (N2) from initial, middle and final (10 min each) portions of the PSG study, to better account for within-night variability. Study epochs were sequential, but not necessarily consecutive, as 30 s epochs containing excessive white noise or any arousals, apnea or hypopnea events were filtered out from the analysis. This measure, which excluded severe OSA subjects from the study, was proposed to minimize potential confounding effect caused by alpha activity in the automatic detection of slow spindles, since respiratory events have been shown to affect EEG frequency even in the absence of visually detected arousals (Dingli et al., 2002), and faster alpha and lower sigma activity (typical of slow spindles) lie in the same frequency range (11–13 Hz). Signal analysis was performed on left and right frontal (F3, F4), central (C3, C4) and parietal (P3, P4) regions. Spindles are known to peak over the midline (Jobert et al., 1992), so that Fz, Cz, and Pz electrodes might be considered better suited to capture spindle frequency behavior. However, the montage used for signal analysis aimed to approximate current AASM guidelines for sleep scoring (Iber et al., 2007) where F3/F4, C3/C4 and O1/O2 electrode positions are the standard recommendation. EEG channels were referenced to (A1 + A2)/2. After a preliminary analysis that did not show significant interhemispheric differences in spindle number, frequency, duration and chirping rate characteristics, results were pooled together for frontal ($F3 \cup F4$), central ($C3 \cup C4$) and parietal ($P3 \cup P4$) regions, allowing a reliable description of sleep spindle behavior over these regions while decreasing family-wise error rate resulting from multiple channel comparisons.

2.3. Automatic spindle detection and chirp quantification

In order to obtain satisfactory time vs. frequency resolution for the automatic detection of sleep spindles and chirp rate quantification, signal analysis was carried out with a Matching pursuit procedure (MP) with Gabor chirplet functions, as described in better detail in Schönwald et al. (2011). Briefly, MP is not a transform, it is an adaptive approximation procedure, whereby the original signal is decomposed into waveforms corresponding to a set of fundamental functions from a large dictionary, which can be represented as atoms in a time–frequency plane. If a signal structure does not correlate well with any particular dictionary function, decomposition will result into a number of non-relevant elements and information will be diluted. MP has been previously described in detail (Mallat and Zhang, 2002; Mallat, 1999) and shown to be suitable for sleep spindle representation (Durka et al., 2001; Durka et al., 2002; Schönwald et al., 2006). To allow chirp quantification, the MP procedure presented in http://eeg.pl (Durka et al., 2001)

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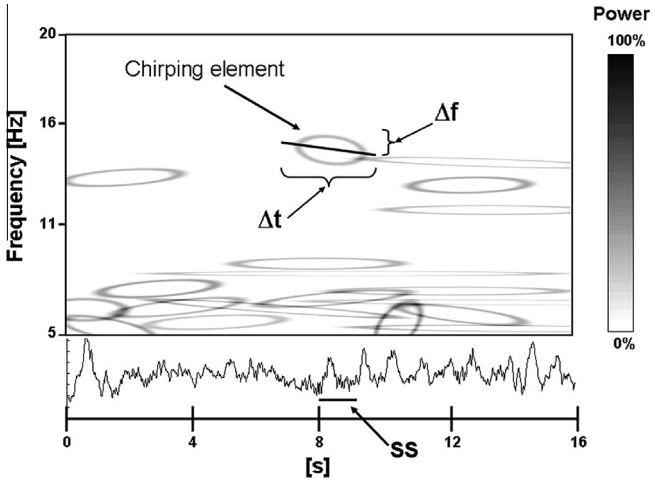

Fig. 1. Sketch of the procedure employed for spindle description by matching pursuit. A 16s NREM sleep EEG segment is shown with the corresponding time–frequency representation in terms of Gabor chirplet atoms in a Wigner plane. Each MP atom is represented here as a hollow ellipse (in order to facilitate visualization) corresponding to its (time, frequency) HW. Relative amplitude is indicated by a gray palette. An example of one atom with negative chirp factor (chirping element) fulfilling automated spindle criteria is highlighted. Notice that in the EEG series, it corresponds to a spindle superimposed with slow activity that partially affects visual spindle recognition. Two robust sigma frequency atoms at the start and end of the EEG segment also fulfill all selection criteria and were included in the analysis. Chirp factor is represented by frequency variation (Δf) in a time period (Δt). MP: matching pursuit; HW: half-width (see text); *f*: frequency; *t*: time; SS: sleep spindle.

has been modified (Schönwald et al., 2011) in order to include a subroutine whereby a two-step ridge pursuit procedure as proposed by Gribonval (2011) is implemented using a Gabor chirplet set of functions. This procedure represents an advantage for EEG signal representation, as it makes use of a set of atoms that are "less rigid" in comparison to a purely orthonormal chirplet basis (where there are parameter dependencies between HW, frequency and chirp factor beta). In addition, it is also more conservative with respect to the beta factor. Chirplet functions (S. et al., 1998) account for frequency modulation as opposed to wavelet functions used by the original MP procedure, that lack this parameter ($\beta = 0$) (Mann and Haykin, 1995). Hence, in the ridge pursuit procedure (Gribonval, 2011), the best Gabor atom (without chirp, $\beta = 0$) is initially found in the iteration as in the original MP approach; then it is explored whether an atom with a chirp parameter ($\beta \neq 0$) can better fit the signal (Fig. 1), which may result in a better signal description if local frequency variability is significant. It should be noted that linear chirp, as opposed to exponential chirp, is measured through this procedure. Therefore, the expressions "acceleration" and "deceleration" have been employed in this study in order to convey, to a clinically-oriented reader, the general concept of time interval changes between individual oscillations. It is also noteworthy that an individual MP atom fulfilling detection criteria is not conceptually equivalent to a sleep spindle, and the procedure is primarily robust and reliable at the statistical level. Moreover, as abovementioned, the ridge pursuit procedure is particularly conservative regarding the β factor. However, considering a highly non-stationary signal such as the EEG, transient elements fulfilling criteria for spindles could be theoretically described with $\beta \neq 0$ by chance alone. This would result in a chirp value distribution best represented as a steep Gaussian curve centered at zero and quasi-zero values, with no chirp preference (negative or positive). This is, however, different from what has been previously described for visually detected sleep spindles, where chirp values (β) are mainly negative (Schönwald et al., 2011). Therefore, a putative sleep spindle chirp tendency has to be interpreted within the context of a very non-stationary signal with a background of random chirp production.

Performance (sensitivity and specificity) of the modified (MP-chirp) code has been tested for automatic spindle detection against that of an original, freeware, "chirpless" MP algorithm (Durka et al., 2001), using an approach identical to what was described in Schönwald et al. (2006), and found to be similar to the original algorithm performance (Schönwald et al., 2011). The modified code is available from authors upon request.

After subsampling to 128 Hz in order to reduce computational requirements, each whole-night EEG series was segmented into juxtaposed bins of 2048 digital points and subjected to MP decomposition with a dictionary size of 10^6 atoms, stopping at 96 iterations. As the MP uses Gabor atoms (sine-modulated Gaussian functions), chosen for EEG microstructure description because they provide optimal joint time–frequency localization with a small number of parameters to be determined (Durka and Blinowska, 1996; Durka et al., 2001), each atom obtained has a central point in time and frequency and limits established by a half-width (HW) corresponding to ±standard deviation of a Gaussian curve. Atoms (hereafter called spindles) had duration between 0.5 and 2 s, central frequency between 11 and 16 Hz (typical parameters for sleep spindles) and chirp rate from −2 to 2 Hz/s. Chirp rate threshold was arbitrated in ±2 Hz/s for two reasons. Firstly, frequency difference between slow and fast scalp spindles is in average at least 2 Hz (Jankel and Niedermeyer, 1985; Werth et al., 1997; Anderer et al., 2001; De Gennaro and Ferrara, 2003; Huupponen et al., 2008); therefore avoiding, as much as possible, data contamination from superimposition of slow and fast spindles. Secondly, short elements with chirp rate beyond this range were rare in the dataset (<1%) and associated with signal inhomogeneity.

2.4. Amplitude threshold determination

Amplitude threshold was individualized throughout the analysis in order to account for inter-subject spindle amplitude variability (Huupponen et al., 2000; Bódizs et al., 2009; Ray et al., 2010). It has been shown that using high amplitude atoms ensures optimal specificity for MP sleep spindle description (Zygierewicz et al., 1999; Schönwald et al., 2006). It has also been described that higher voltage spindles are more likely to chirp negatively (Schönwald et al., 2011). It is important not to confuse atom amplitude with spindle voltage directly measured over the time series. The atom is a representation of a signal segment and higher amplitude atoms tend to represent larger spindles; however, the relationship is not linear. Therefore, to determine the most appropriate amplitude threshold (AT parameter in MP) to study chirp we assessed the percentage of spindles with chirp parameter ≠ 0 (chirping spindles) according to different amplitude thresholds (from top 5% to top 30% amplitude atoms) for the scalp locations under study. There was little variability in the amount of spindles best described with a chirp ≠ 0 within this threshold range, suggesting that threshold arbitration for high amplitude atoms is not detrimental in chirp analysis. We thus selected the top 15% amplitude atoms from each subject and EEG channel due to high specificity conferred by greater amplitude threshold for sleep spindle detection, while maintaining adequate sample size and homogenous percentage of chirping spindles across scalp topography.

2.5. Additional data modeling

Spindles were further divided into slow (<13 Hz) and fast (⩾13 Hz) types according to central frequency. Chirping (chirp ≠ 0) spindles were further categorized into positively (chirp > 0) and negatively (chirp < 0) chirping types.

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2.6. Statistical analysis

Continuous variables were compared between groups with Kruskal–Wallis analysis of variance followed by Dunn's post hoc pairwise comparisons due to limited sample size and/or non-normal distributions. Unless otherwise specified, these results were represented as medians (interquartile range). Categorical variables were analyzed by means of Chi-Square tests. For chirping variable comparisons across and within groups, analyses were stratified by topography and/or sleep spindle type (slow and fast), as appropriate. Correlation between percentage of positive and negative chirping spindles and AHI in different scalp regions was verified by the Spearman's rank correlation coefficient. Binomial test was used to verify whether the percentage of chirping types (positive and negative) are statistically different from that expected randomly ($p = 0.5$) for every group and for different sleep spindle types in all studied locations. Statistical significance was assumed for two-tailed p-values <0.05. Analyses were performed with PASW Statistics 18 (SPSS Inc, Chicago, IL, USA) statistical package.

3. Results

3.1. Overall chirp percentage and distribution among groups

The majority of spindles collected in our sample presented chirp, which tended to be mainly negative. Mean chirping spindle percentage was 89.5 ± 2.8% (ranging from 83.9% to 94.2%). Within chirping spindles, mean negative chirp percentage was 62 ± 6% (ranging from 50.4% to 74.8%). The overall median chirp rate was −0.11 (0.72) Hz/s. Total chirping spindle percentage was not different among OSA severity groups. There were no differences in total chirping spindle percentage among scalp regions.

As expected, the contribution of slow spindles was larger in frontal regions (73.9%), followed by central (47%) and parietal regions (30.7%), where fast spindles were more prevalent (53% and 69.3%, respectively). Considering the sample as a whole, there was no statistically significant difference between fast and slow spindles in the expression of chirping types (positive and negative) across the studied regions, although slow spindles are consistently more associated with negative-chirp spindle types overall.

When the distribution of negative- and positive-chirp spindle types – as expressed in every OSA severity group for slow and fast spindle types, and for every studied topography – was compared to a random binomial distribution, with a probability of 50% for both chirp types, all analyzed distributions had a very low probability of being generated by chance ($p < 0.01$), except for the distribution of negative- and positive-chirp types within slow spindles in parietal regions of moderate OSA subjects ($p = 0.176$).

3.2. Distribution of negative-chirp spindles according to topography and OSA severity

Fig. 2 shows negatively chirping spindle percentage across scalp topography for Non-OSA, Mild and Moderate OSA groups. When slow spindles were considered, there was a distinct gradient on negative-chirp percentage across OSA severity groups. Negative-chirp percentages decreased from Non-OSA to Mild and Moderate OSA in all studied locations. This difference was statistically significant for the comparison between Moderate vs. Mild OSA and Non-OSA patients for frontal (62.2% vs. 67.9%, $p = 0.026$; 62.2% vs. 68.4%, $p = 0.027$, respectively) and parietal (53.5% vs. 61.9%, $p = 0.023$; 53.5% vs. 65.7%, $p = 0.011$, respectively) regions. A trend was also noticed for a difference between Moderate OSA and Non-OSA subjects regarding negative-chirp percentage on central regions (57.3% vs. 60.9%, $p = 0.066$). There was no statistically significant

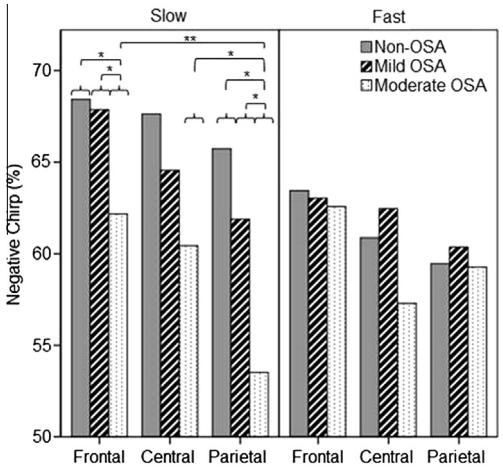

Fig. 2. Bars represent the percentage of negative chirp spindles out of all chirping spindles according to spindle type (slow and fast), topography and OSA severity. For slow spindles (<13 Hz), a decreasing gradient is seen across OSA severity groups (from Non-OSA to Moderate OSA) and from anterior to posterior regions for Moderate OSA patients. No significant differences are seen for fast (⩾13 Hz) spindles. OSA, Obstructive Sleep Apnea. Statistical significance defined as: * for $p < 0.05$ and ** for $p < 0.01$, Chi-Square Test.

difference between Non-OSA and Mild OSA patients in any region under study. Still considering slow spindles only, there was an anterior-posterior gradient on negative-chirp percentage that reached statistical significance for Moderate OSA patients, when frontal (62.2%, $p = 0.005$) and central (60.4%, $p = 0.031$) regions were compared to parietal locations (53.5%). Within the same subgroup, median chirp value was also different in the parietal region (0 (0.67) Hz/s), when compared to frontal (−0.10 (0.85) Hz/s) and central (−0.08 (0.8) Hz/s) regions, with post hoc p-values <0.01.

When only fast spindles were considered, no statistically significant differences in the distribution of negative-chirp spindles were seen among OSA severity groups and/or across scalp topography.

3.3. Slow spindle chirp rate distribution across topography and OSA severity groups

In order to investigate in better detail the decrease in negative chirp percentage seen in slow spindles for Moderate OSA, slow spindle chirp rate distribution (i.e., across the −2 to +2 Hz/s range) was examined across OSA severity groups in different scalp locations. On the frontal region (Fig. 3), in comparison to Mild OSA and Non-OSA groups, Moderate OSA patients had a loss of negative chirp contribution which was more pronounced in the −0.72–0 Hz/s range. For central and parietal locations (results not shown), the negative chirp loss was more diffuse, resulting from a more homogeneous decrease in negative chirp spindle contribution along the −2–0 Hz/s chirp rate distribution.

Moderate OSA median chirp rate (0 (0.67) Hz/s) was statistically different from that of Mild OSA (−0.11 (0.7) Hz/s, $p < 0.01$) and Non-OSA (−0.08 (0.6) Hz/s, $p < 0.05$) subjects on the parietal region. Chirp rate was not different among groups for frontal and central regions.

3.4. Correlation between AHI and percentage of negatively-chirping slow spindles

Percentage of negative-chirp slow spindles correlated negatively with AHI in the parietal region ($r_s = -0.519$, $p = 0.005$) (Fig. 4), but not in central ($r_s = -0.259$, $p = 0.183$) or frontal

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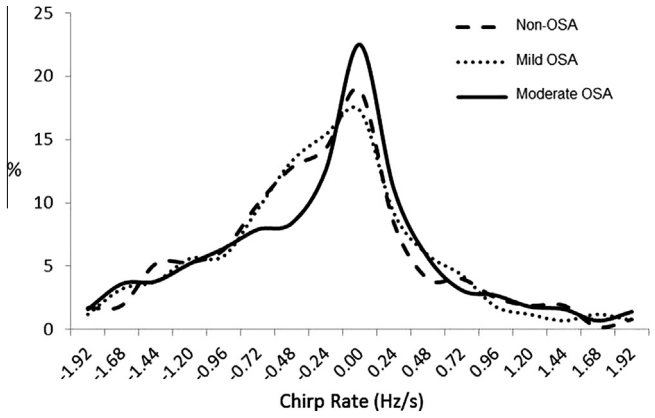

Fig. 3. Curves represent the relative distribution of chirp rate of slow spindles for every group (Non-OSA, Mild OSA and Moderate OSA) in the frontal region. In Moderate OSA patients, there was a loss of negative chirp contribution limited to the −0.72–0 Hz/s range, when compared to Non-OSA and Mild OSA groups. Chirp values ≠ 0 grouped to 0.24 Hz/s juxtaposed class intervals. OSA, Obstructive Sleep Apnea.

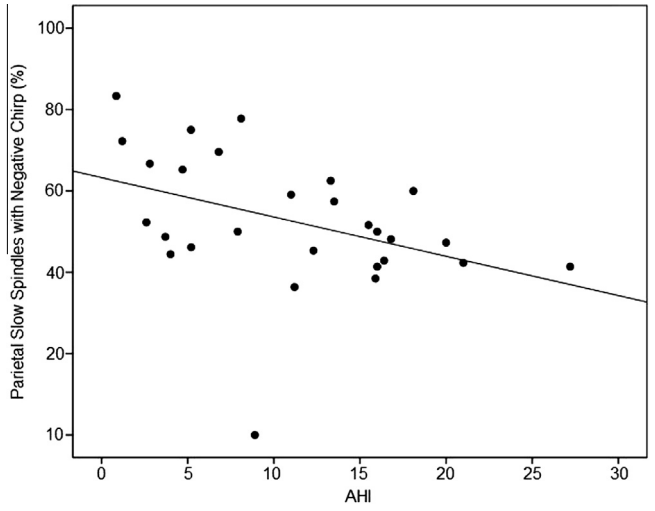

Fig. 4. Scatter plot shows a negative correlation between AHI and negative-chirp slow spindle percentage in the parietal region ($r_s = -0.519$, $p = 0.005$). AHI, apnea-hypopnea index.

($r_s = -0.251$, $p = 0.198$) locations (results not shown). It is noteworthy that the percentage of slow spindles positively correlates with the AHI especially in the parietal regions ($r_s = 0.509$, $p = 0.006$), but also in central ($r_s = 0.499$, $p = 0.007$) and frontal ($r_s = 0.460$, $p = 0.014$) locations, which replicates previous findings (Schönwald et al., 2012). As parietal EEG channels are relatively close to occipital regions, we performed an a posteriori analysis to assess whether the correlation between parietal negative-chirp slow spindle percentage and AHI was affected by negative-chirp slow spindles occurring on occipital regions. Although sleep spindles are not primarily expected to be detected on occipital channels, short alpha bursts may be expected to increase in prevalence with increasing AHI due to arousals associated with respiratory events. Such short-time events may be confounded with slow spindles (Schönwald et al., 2012). As internal frequency variability has not (to our knowledge) been studied in alpha bursts, no firm theoretical assumptions can be made on this matter; however, an increase in occipital alpha bursts could cause an increase in non-chirping slow frequency elements (potentially detected as spindles in our procedure), or alternatively, similar (random) proportions of negative and positive chirps, in either way reducing the relative percentage of negative chirping spindles in association with OSA. Although the percentage of slow-frequency elements did increase with AHI in the occipital region ($r_s = 0.444$, $p = 0.018$) (possibly representing alpha-burst activity), it was not associated with decreased negative-chirp slow spindle percentage in the parietal region ($r_s = -0.136$, $p = 0.489$).

4. Discussion

In this study, slow and fast sleep spindle chirping rate and negative chirp percentage were studied in different brain regions, in patients with and without OSA. Moderate OSA subjects showed a decrease in negative chirp percentage, or loss of sleep spindle deceleration, which was detected for slow spindles only, in frontal and parietal regions. In other words, they showed a loss of the expected physiological negative bias in chirp distribution which had been previously reported for healthy subjects, when central scalp regions were studied without discrimination of fast/slow spindle types (Schönwald et al., 2011). As the sleep EEG signal is very non-stationary, random chirp modulation is highly expected as the result of signal decomposition for short transients, so that a loss of negative chirp modulation alone is theoretically expected to generate similar distributions of positive and negative chirp types that could be generated by chance, such as the distribution of negative and positive chirp types seen here for parietal slow spindles of Moderate OSA patients, which was not different from one generated randomly (Binomial test). For all other analyzed distributions, there was a bias towards spindle deceleration. The previous findings showing a higher contribution of spindle frequency deceleration were therefore reproduced and extended to other scalp regions.

The amount of sleep spindles with chirping modulation in the present sample is slightly higher than in the previous study (89.5% vs. the 82.9%), where visually detected spindles had been analyzed. Considering the differences in methodology between both studies, including the important facts that the present study relies solely on automatic spindle detection and that an individualized, top 15% amplitude threshold was applied, chirping spindle percentage may be considered similar between studies. Limiting the threshold to higher amplitude elements could have filtered out false-positives, as alpha-burst activity, which is less expected to have frequency modulation, and could have biased the sample towards negative chirp spindles, as negative chirp elements had been shown to have higher amplitude when compared to zero chirp and positive chirp elements on the previous study. Nevertheless, when considering only chirping spindles, percentage of negative chirp elements was surprisingly similar between studies (61% vs. reportedly 60.2%), corroborating to the consistency and robustness of Matching pursuit procedure with Gabor chirplet functions for spindle frequency analysis. This study thus provides further evidence for the existence of a physiological mechanism of internal sleep spindle frequency modulation.

A distinct gradient on negative chirp percentage across OSA severity groups was seen for slow spindles in all studied locations (a trend in central regions, and statistically significant in frontal and parietal sites). There was also an anterior-posterior gradient that was more pronounced and reached significance in Moderate OSA, with the lowest negative chirp slow spindle percentage occurring in parietal regions. This is particularly interesting when one considers that for OSA patients, (1) spindle number is diminished (Ondze et al., 2003; Himanen et al., 2003; Schönwald et al., 2012) and (2) parietal regions show increased proportions of slow spindles (Schönwald et al., 2012). Indeed, in the present study, there was a significant positive correlation between parietal slow

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spindle percentage (regardless of chirping type) and AHI, while percentage of negative-chirp slow spindles correlated negatively with AHI in the same location. Also, in a previous study comparing Moderate OSA patients and non-OSA subjects (Schönwald et al., 2012), parietal increase in slow spindle percentage was far more pronounced (50.9% vs. 17.9%) than that occurring in frontal regions (78.8% vs. 66.4%), when the night was considered as a whole. It has been suggested that the increase in slow sleep spindles in OSA could be associated to a global increase of low-frequency bands (delta and theta) in NREM sleep (Morisson et al., 1998; Ondze et al., 2003; Xiromeritis et al., 2011).

OSA represents a unique experiment in nature, whereby sleep fragmentation and intermittent hypoxia combine to create a complex scenario of brain dysfunction, including chronic sleep deprivation with increased homeostatic pressure (Gozal, 2013; Rosenzweig et al., 2013). This context may share some similar characteristics with acute sleep deprivation, whereby sleep spindles decrease in density, become slower and lose intra-spindle frequency variability, supposedly due to higher levels of synchronization of thalamic and cortical oscillations under high sleep pressure (Dijk et al., 1993; Knoblauch et al., 2003). It is possible that slow sleep spindles generated through this process are abnormal and lack some modulatory influence, reducing the proportion of negative chirping. Therefore, spindle production in the context of OSA may be compromised in several ways. There may be a decrease in spindle production, with an increase in the relative proportion of slow spindles, which display loss of physiological internal frequency deceleration. This hypothesis is in agreement with recent findings in cats suggesting the existence of a thalamo-cortical feedback loop whereby cortical regions modulate initiation and termination of spindle oscillations (Bonjean et al., 2011) and may represent a disruption of the physiological spatio-spectral-temporal evolution of spindles (Dehghani et al., 2011).

Consideration has been given to the hypothesis that increased slow spindle proportions with decreased frequency deceleration in parietal regions of Moderate OSA patients might be a simple and direct consequence of an over-representation of short-time alpha activity bursts related with brief arousals from sleep. Indeed, this may still be the case, but our data seems to indicate otherwise. Although percentage of slow-frequency spindles did increase with AHI in the occipital region, possibly representing alpha burst activity, it was not associated with decreased negative-chirp slow spindle percentage in the parietal region. Internal frequency variability (chirp) should also be assessed in alpha bursts in further studies.

In the frontal region, the loss of negative chirp contribution seen for Moderate OSA patients in comparison to Mild OSA and Non-OSA groups was limited to the −0.72–0 Hz/s range, while for central and parietal locations, negative chirp loss was more diffuse. This finding may be consequent to intrinsic functional and/or structural differences between frontal and parietal spindles. It also highlights the concept that slow spindles expressed in anterior regions are not necessarily similar to slow spindles expressed in more posterior sites. Variability in the effect of OSA over chirp modulation in different brain regions may be an epiphenomenon of different modulatory mechanisms affecting sleep spindles across the brain (Nir et al., 2011; Andrillon et al., 2011).

Our study was unable to detect statistically significant changes in spindle chirp characteristics in central derivations across OSA groups. This could be a result of a less important role of central locations in the generation of spindle oscillations as opposed to frontal and parietal regions, which have been associated with slow and fast spindle generation, respectively. However, this hypothesis has to be considered carefully. The absence of significant differences may result from a lack of statistical power in our analysis (as a trend was obtained even with non-parametric data, which are prone to type II errors), and changes may be more diffuse. Further studies with larger sample sizes should clarify this issue.

No significant differences in the distribution of negative-chirp spindles were seen among OSA severity groups and/or across scalp topography when only fast spindles were considered. Several explanations are possible. Primarily, spindle abnormalities in OSA could be tightly related to spindle frequency slowing, so that the expression of fast spindles could be the result of remaining 'healthy' fast spindle generators. Alternatively, slow spindle generator circuits could be more sensitive to repetitive hypoxia than those responsible for fast spindles, which would then be able to maintain their original characteristics in face of sustained obstructive respiratory disturbance. However, it should also be born in mind that fast spindles tended to have lower negative chirp proportions when compared to slow spindles. Regardless of the explanation, this finding is intriguing, as the occurrence of fast spindles immediately prior to slow cortical oscillations associated with slow spindles has been proposed as a key mechanism in sleep-dependent memory processing (Mölle et al., 2011). As sleep spindles are being increasingly studied in the context of verbal and non-verbal learning and memory consolidation (Gais et al., 2002; Schabus et al., 2004; Clemens et al., 2005; Fogel and Smith, 2006; Tamaki et al., 2009), future studies on spindle characteristics in the context of OSA-associated cognitive impairment may be useful.

To the best of our knowledge, this study is the first to quantify sleep spindle frequency modulation or chirping rate in the context of OSA. The meaning and significance of this particular sleep spindle characteristic is yet to be determined; however, our findings suggest that chirping analysis may be useful for an improved description and interpretation of sleep spindle dynamics in the context of neurophysiology and pathophysiology. Although it is not clear how spindle chirp is modulated, the anterior-posterior gradient found in Moderate OSA patients may indicate the existence of a differential cortical response to apnea and subsequent effects in the expression and modulation of spindle oscillations throughout thalamo-cortical feedback loops. As spindle negative chirp modulation seems to be physiologically mediated, its loss may either represent sleep spindle dysfunction or a compensatory mechanism whereby sleep homeostasis and brain processes such as memory consolidation and learning may be affected.

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