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EEG FUNCTIONAL CONNECTIVITY PRIOR TO SLEEPWALKING: EVIDENCE OF INTERPLAY BETWEEN SLEEP AND WAKEFULNESS

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Abstract

Study objectives: Although sleepwalking (somnambulism) affects up to 4% of adults, its pathophysiology remains poorly understood. Sleepwalking can be preceded by fluctuations in slow-wave sleep EEG signals, but the significance of these pre-episode changes remains unknown and methods based on EEG functional connectivity have yet to be used to better comprehend the disorder.

Methods: We investigated the sleep EEG of 27 adult sleepwalkers (mean age: 29±7.6 years) who experienced a somnambulistic episode during slow-wave sleep. The 20-sec segment of sleep EEG immediately preceding each patient's episode was compared with the 20-sec segment occurring two minutes prior to episode onset.

Results: Results from spectral analyses revealed increased delta and theta spectral power in the 20 sec preceding the episodes' onset as compared to the 20 sec occurring 2 minutes before the episodes. The imaginary part of the coherence immediately prior to episode onset revealed (i) decreased delta EEG functional connectivity in parietal and occipital regions, (ii) increased alpha connectivity over a fronto-parietal network, and (iii) increased beta connectivity involving symmetric inter-hemispheric networks implicating frontotemporal, parietal and occipital areas.

C**onclusions:** Taken together, these modifications in EEG functional connectivity suggest that somnambulistic episodes are preceded by brain processes characterized by the co-existence of arousal and deep sleep.

Keywords: Electrophysiology; Parasomnias; Pathophysiology; Slow-wave sleep; Somnambulism; Functional connectivity

Statement of Significance: Although sleepwalking has long been conceptualized as a state comprised of both sleep and wakefulness, the disorder's pathophysiology remains poorly understood. The present study, the first to use EEG functional connectivity metrics to study sleepwalkers, reveals changes in brain connectivity prior to the emergence of somnambulistic episodes. Specifically, observed changes in pre-episode connectivity were found to reflect the simultaneous presence of deep sleep and arousal, a finding that provides new insights into sleepwalking's pathophysiology while bolstering its conceptualization as a disorder of arousal. Given that EEG functional connectivity can yield highly valuable and complementary information to that obtained via brain imaging and polysomnography, its use is encouraged in the investigation of both normal and pathological sleep.

Introduction

Somnambulism (sleepwalking) is a non-rapid eye movement (NREM) sleep parasomnia involving behaviours of varying complexity, usually initiated during slow-wave sleep (SWS).[1, 2] Most behavioural episodes are characterized by misperception and relative unresponsiveness to the environment, mental confusion, perceived threat or agitation, and variable retrograde amnesia.[2]

Sleepwalking is far more common in adults than commonly acknowledged, affecting up to 4% of adults,[3] and represents a leading cause of sleep-related violence and self-injury.[4, 5] Despite its high prevalence and its well-described clinical characterization, the pathophysiology of the disorder remains poorly understood. Somnambulism has long been conceptualized as a "disorder of arousal"[6] because of the autonomic and motor arousal which propels the patient towards incomplete wakefulness. Consistent with this view, studies of electroencephalographic (EEG) activity during adult sleepwalkers' arousals from SWS[7] as well as during somnambulistic episodes[8] indicate that approximately 50% of sleepwalkers' post-arousal EEG contain clear evidence of delta activity. This suggests that sleepwalkers are caught between NREM sleep and full EEG arousal.

Studies have also shown that somnambulism is characterized by an inability to sustain stable, consolidated SWS. While sleepwalkers' overall sleep architecture and cycling among sleep stages is essentially the same as that of controls,[1, 9, 10] sleepwalkers show an unusually elevated number of spontaneous awakenings and EEG arousals out of SWS, even on nights without episodes.[9, 11, 12] Sleepwalkers' SWS also shows anomalies in sleep intensity as measured quantitatively by slow wave activity (SWA; spectral power between 0.5 and 4.5 Hz)[9, 13] as well as atypical patterns in the cyclic alternating pattern, an endogenous rhythm considered to be a physiologic marker of NREM sleep instability.[14-16]

Spectral analysis of sleep EEG signals prior to behavioural episodes in individuals with sleepwalking and/or sleep terrors indicate that the onset of the parasomnia is likely to be preceded by an increase in SWA[12] or in low delta power (0.25-2.0Hz),[13] with the later peaking about 10 to 12 sec prior to an episode. Similarly, one investigation[17] reported an abrupt increase in high amplitude slow oscillations (< 1 Hz) in the 20 sec immediately preceding the episodes. These changes in sleep EEG represent deeper sleep and may reflect the brain's attempt to maintain sleep despite the occurrence of internal or external processes or stimuli that may give rise to behavioural episodes.[17] Relatedly, one recent study[18] found that SWA and slow oscillation density were significantly greater prior to sleepwalkers' episodes as compared to their non-behavioural awakenings recorded from matched sleep cycles and sleep stage, suggesting that the observed effect is specific to patients' episodes.

There is also evidence to suggest that pre-episode fluctuations in patients' sleep EEG may also reflect an interplay between wakefulness and NREM sleep. Using stereo electroencephalography, Terzaghi and colleagues[19] reported a single case study in which beta activity (indicative of wakefulness) was recorded in the motor and central cingulate cortices 5 sec before a sleepwalking episode along with increased delta bursts (indicative of sleep) in the frontal and parietal dorsolateral associative cortices. Similarly, using electromagnetic tomography (eLORETA) in 15 adult sleepwalkers, Januszko and colleagues[20] reported a significant activation in the 24-30 Hz beta frequency range 4 sec prior to the onset of somnambulistic episodes. Taken together, these findings indicate that changes in sleep EEG are observed prior to the onset of behavioural episodes and suggest

that sleepwalking is characterized by a state reflecting the interplay between the states of deep sleep and wakefulness.

One way to better understand the pre-episode dynamics of sleep and wakefulness is through the study of functional connectivity. Studies of connectivity using functional magnetic resonance imaging (fMRI) have shown that the transition from wakefulness to light sleep is associated with reduced thalamocortical connectivity and increased corticocortical connectivity.[21] Functional connectivity then breaks down during SWS, as reflected by reduced corticocortical connectivity.[21] Testing the hypothesis that impaired consciousness during NREM sleep is associated with increased modularity in brain activity, fMRI studies have shown that NREM sleep is characterized by a decrease of large-scale networks and increase of smaller independent modules as reflected by high clustering ratios and low inter-modular connectivity.[22-25] Using transcranial magnetic stimulation (TMS) and high density (HD) EEG to assess how a TMS pulse delivered to the premotor cortex propagates in the brain during both sleep and wakefulness, Massimini and colleagues[27] found that when compared to a wakefulness propagated response, the response during NREM sleep (as recorded with HD-EEG) was stronger but extinguished more rapidly and did not propagate beyond the stimulation site. Thus, the fading of consciousness observed during deeper sleep stages may be related to a breakdown in long-range cortical connectivity. In support of this hypothesis, total brain connectivity is negatively correlated to SWA during NREM sleep[24] and a direct link has been described between increased EEG delta power and a breakdown in inter-modular connectivity.[25]

Given this collection of findings, the investigation of functional connectivity in relation to somnambulism appears both promising and warranted. In fact, the results of one recent study[26] suggest that when compared to NREM sleep of healthy controls, the NREM sleep of adults presenting with a disorder of arousal (e.g., sleep terrors or sleepwalking) is characterised by the presence of local sleep differences in EEG SWA power, even in the absence of clinical episodes. These results highlight the importance of investigating local sleep activity and interaction of brain regions in relation to parasomnias such as sleepwalking. One metric that provides information about brain activity synchronization and interaction is EEG functional connectivity, defined as the temporal correlation (in terms of statistically significant dependence) in EEG activity recorded from different brain regions. This method offers very good temporal resolution, which is optimal when investigating short time windows. We thus used EEG functional connectivity analysis to clarify the nature of sleep changes preceding the onset of somnambulistic episodes. Spectral power analyses were also performed on our data set to replicate previous findings and thus confirm that our episodes were representative of those reported in previous studies.

Given that somnambulistic episodes are conceptualized as interplay between SWS and wakefulness, a dynamic known to affect brain connectivity, it was hypothesized that significant modifications in EEG functional connectivity would be found prior to the episodes' onset. Since sleepwalking is preceded by spectral power changes indicative of deeper SWS, we predicted that EEG functional connectivity prior to the episodes would also reflect consolidated SWS. Specifically, and in line with findings suggesting an association between SWS and a decrease in large-scale networks and an increase in local

connectivity, we predicted that episodes would be preceded by reduced long-range connectivity and/or by increased local connectivity.

Materials and methods

Data acquisition and segmentation

Subjects were 27 adult sleepwalkers (13 men, 14 women, mean age: 29 ± 7.6 years) referred to the Sleep Disorders Clinic of the Hôpital du Sacré-Coeur de Montréal by a physician for suspected somnambulism. They were selected on the basis of having experienced a spontaneous sleepwalking episode during an overnight polysomnographic (PSG) assessment at the sleep laboratory. In addition, patients included in the study had to report a clinical history (including over the past 6 months) of somnambulism or somnambulism and sleep terrors that was not of a traumatic, neurological or medication-induced origin and received a final diagnosis of sleepwalking according to the International Classification of Sleep Disorders.[2] Exclusion criteria were the following: (1) presence of neurological or psychiatric condition, (2) sleep disorder other than sleepwalking, (3) history of head injury, (4) history of epilepsy, and (5) use of medications altering vigilance or sleep (antidepressants, benzodiazepines, psychostimulants, hypnotics). PSG recordings were conducted on a 32-channel Grass polygraph (sensitivity, 7 μV/cm; bandpass, 0.3–100 Hz). Signals were digitized at a sampling rate of 256 Hz using commercial software (Harmonie, Stellate Systems, Montreal, QC, Canada). PSG recordings included 19 electrodes EEG on the scalp (Fp1, Fp2, F3, F4, F7, F8, Fz, C3, C4, Cz, T3, T4, T5, T6, P3, P4, Pz, O1 and O2) referenced on mastoids (10-20 international system), an electrooculogram, an electromyogram at the chin and the legs, nasal and oral cannulas, a transcutaneous oximetry apposed on a finger

and thoracic and abdominal straps. All patients were continuously videotaped during sleep assessment and sleep stages were identified according to the standardized criteria.[28] The study was approved by the hospital's ethics and scientific committee and signed consent was obtained from each participant.

Behavioural manifestations arising out of patients' SWS were evaluated using EEG activity and time-synchronized video recordings according to AASM criteria[2]: stereotyped or repetitive movements, confusion, agitation or disorientation during the event. One SWS episode per subject was included in the study. Two time windows were investigated: 20 sec immediately preceding episode onset (-20 sec to 0 sec prior to episodes' onset), and 20 sec occurring 2 minutes before episode onset (-140 sec to -120 sec prior to episode; See figure 1). EEG segments were visually inspected to insure they contained no sleep stage transition or evidence of arousal.

Spectral analysis

EEG power spectra was calculated for 4-s epochs using Fast Fourier Transform and the Welch estimator at a resolution of 0.25 Hz with Hamming window taper and a 75% window overlap ratio. Six frequency bands were analyzed: delta (0.5–4.00 Hz), theta (4.00–8.00 Hz), alpha (8.00–12.00 Hz), sigma (12.00–14.00 Hz), beta 1 (14.00–22.00 Hz) and beta 2 (22.00-32.00 Hz). In order to evaluate a possible localization effect, analyses were performed on Fz, Cz and Pz. As no electrode effect was found, only results on Fz are presented.

EEG functional connectivity

Magnitude-squared coherence (Msc) and the Imaginary part of the Coherence (ICoh) were used as two complementary measures of EEG functional connectivity. Msc

is a well-established measure of linear relationship between two time series at specific frequencies.[29-31] Although this metric is well grounded in theoretical and empirical literature,[32] it is known to be sensitive to volume conduction and common reference channel, resulting in spurious zero-lag interactions not attributed to neural sources.[33] It should be noted that the problem posed by common reference channels is not an issue in the current study as it is controlled for by the within-subject design. However, results from Msc can be difficult to interpret as it does not discriminate "true" coherence and volume conduction. Icoh has been introduced to address this concern.[33] By capturing the part of synchronization that occurs with a non-zero time lag, it is only sensitive to signals that are time-lagged to each other and therefore isolates the part of coherency which necessarily reflects true interaction.[33-35] While making this key discrimination possible, Icoh is also likely to miss some brain interaction due to its severity.[33] Authors suggest the importance of considering different connectivity metrics in order to obtain more complete estimate of the EEG signals' functional connectivity.[36-38] Values for Msc and Icoh vary between 0 and 1. A value of 0 indicates a complete absence of synchrony between electrical signals of two EEG derivations while a value of 1 indicates perfect synchronization.[30, 33]

Functional connectivity metrics were computed and analyzed using Matlab (The MathWorks Inc., Natick, MA) on EEG signals segmented with Brainstorm.[39] Statistical analyses were performed on each EEG frequency between 0 and 40 Hz (39 segments of 2 Hz bandwidth with a 50% overlap). This method offers the advantage of increased precision as it does not average data in predefined frequency bands.[40] Measures of Msc and Icoh were obtained by applying the following steps to EEG segments (20 sec) : (1)

For each time window, the EEG activity of each electrode was transformed in each frequency band using Fast Fourier Transform; (2) a measure of the coherence was computed for every possible electrode pair for each 4-sec window in each frequency segment; and (3) measures of Msc and Icoh were obtained from the averaged coherence over the total length of the selected EEG recordings for each subject. Therefore, a measure of Msc and Icoh was obtained for each subject for all possible electrode pairs (19 x 9; all channels to itself comparisons were excluded), for each of the 39 frequency segments (See Figure 1). A Yates replacement was applied to replace connectivity data related to epochs of specific EEG channels showing muscular activity artifacts in 1 or 2 derivations in a total of 5 subjects. This represents less than 2% of the data, well below the 10% cut-off for reliable results.[41]

Statistical analysis

Statistical differences between the two conditions (20 sec immediately preceding the episode; 20 sec occurring 2 minutes before the episode) for spectral analyses were computed using IBM SPSS Statistics (IBM SPSS Statistics for Windows, Version 17.0). A logarithmic transformation (log10) was applied to normalize spectral data. Time windows were compared using paired t-tests for each frequency band.

Statistical analyses to compare EEG functional connectivity data between the two conditions were assessed with a Monte Carlo p-value in a nonparametric design using Matlab (The MathWorks Inc., Natick, MA). Coherence studies generally face the problem of multiple comparisons as they include a large number of frequency bins while comparing a large number of electrode pairs. Moreover, parametric statistical tests of coherence differences rely on the asymptotic normality of the Fourier mode's

assumption, which is not always met under such circumstances.[42] The nonparametric design proposed by Maris & al. is adapted for coherence studies as it does not require data to be normally distributed and allows testing of multiple electrode pairs and frequency bins.

For each condition, p-values were compared to a critical alpha-level calculated by randomly partitioning the trials. To do so, the trials of the two conditions were first pooled into a single set and randomly sampled within our two conditions. Then, the test statistic of this random partition was computed and this resampling was repeated 1500 times to construct the histogram of the null hypothesis test statistic.[42] To bypass the multiple comparisons problem at the level of the electrode pairs, statistics were averaged over spatial clusters as proposed by Maris and colleagues.[42] For each frequency bin separately, the test statistic that was actually observed for the pairs is compared with the Monte Carlo p-value threshold to obtain significant links (See Maris and colleagues[42] for more details).

Results

Results from spectral power analyses are summarized in Table 1. There was a significant increase in the spectral power of delta and theta frequency bands in the 20 sec immediately preceding the episodes' onset when compared to the 20 sec segment occurring 2 minutes prior to each episode. These results are in line with previous PSG studies reporting increased SWA immediately preceding somnambulistic episodes.

EEG functional connectivity with Msc did not show any significant differences between the two conditions (time segments). Results with Icoh revealed significant differences in EEG functional connectivity networks between the two conditions for three

frequency bands: 0.1-2 Hz (low delta), 9-11 Hz (alpha) and 22-24 Hz and 26-29 Hz (beta). EEG functional connectivity results are presented in Figure 2.

Results in the low-delta band showed lower functional connectivity for networks that involve parietal and occipital regions ($p<0.05$) in the 20 sec immediately preceding the episodes' onset as compared to the 20 sec segment occurring 2 minutes prior to each episode. Results in the alpha band showed higher connectivity between frontal and parietal regions ($p<0.05$). Results in the beta band show higher connectivity for symmetric inter-hemispheric networks ($p<0.05$) involving frontotemporal, parietal and occipital areas.

Discussion

This study aimed to characterize changes in EEG functional brain connectivity prior to the onset of sleepwalking episodes. We found that somnambulistic episodes were preceded by decreased connectivity in the delta frequency band over posterior areas and an increased functional connectivity in alpha and beta frequency bands over a wide anteroposterior network. Increased spectral power in delta and theta frequencies was also observed. These functional changes in EEG connectivity suggest the concomitant presence of arousal and deep sleep processes prior to the onset of sleepwalking episodes.

EEG functional connectivity as a marker of imminent shift towards somnambulistic episodes

Pre-arousal increases in delta spectral power, as shown in the present study, have been viewed as cortical attempts to maintain sleep in the occurrence of internal or external processes that may give rise to behavioural episodes.[1, 17] In parallel, we observed a reduction in delta connectivity over centro-parietal regions. Posterior regions were

previously identified in adults presenting with disorders of arousal (sleepwalking or sleep terrors) as exhibiting local decreases in SWA in comparison to controls, especially during NREM sleep.[26] The present finding of lowered connectivity in the delta frequency band thus highlights the importance of investigating this frequency band, including with respect to localized changes in EEG activity, to better document mechanisms believed to underlie sleepwalking's pathophysiology. Since our data reveal that this lower connectivity is found locally rather than over anterior and posterior electrodes, it may be interpreted as a decrease in local connectivity. Because SWS is characterized by high local connectivity,[22-25] our findings prior to episode onset likely reflect a transition towards wakefulness or, at the very least, a lighter sleep stage. While results in the delta band show decreased connectivity in posterior areas, a completely different portrait was observed in higher frequency bands, where posterior regions show increased connectivity over a wide antero-posterior bilateral network. Since SWS is not characterized by activation of long-range connectivity networks,[22, 24, 25, 27] our pre-episode inter-modular connectivity networks may also be interpreted as a manifestation as a shift towards wakefulness.

As suggested by several lines of inquiry, functional connectivity fluctuates during the transition between sleep and wakefulness and these fluctuations have been linked to differences in consciousness and information processing characterizing these states.[21, 27] Since the transition from deep sleep to wakefulness is accompanied by increased long-range connectivity and decreased local connectivity,[22-25] our findings of a concomitant decrease of local connectivity in posterior regions and increased long-range antero-posterior connectivity suggest the brains of sleepwalkers undergo a shift from deep sleep

to a partial arousal before an episode even begins. Thus, increased delta power and lower connectivity in the delta frequency band as well as increased long-range connectivity in higher frequency bands observed prior to sleepwalking could represent an early manifestation of the brain state changes underlying episode occurrence. These findings of concomitant lower delta connectivity associated with increased delta spectral power refines our understanding of the pathophysiology of somnambulism by highlighting the fact that the presence of delta activity prior to episode onset might not simply reflect a mechanism of deepening sleep. Instead, it suggests that the onset of somnambulism is preceded by a relatively gradual and complex arousal process that occurs preferentially over posterior regions of the brain.

SWA and inter-modular connectivity have been shown to be negatively correlated during NREM sleep.[24, 25] Therefore, SWA decreases and inter-modular connectivity increases in a highly coordinated fashion during awakenings from deep sleep.[25] By contrast, the transition from deep sleep to episodes of somnambulism shows a coexistence between SWA power and sleep-wake transition connectivity patterns (as reflected by an increase in long-range/inter-modular connectivity). This suggests that the coexistence of sleep and wakefulness is not only present during the episodes themselves, but also prior to their behavioural onset. This line of reasoning is also consistent with recent findings suggesting the coexistence of wake-related activity and deep sleep in adults with disorders of arousal, both immediately preceding the onset of behavioral episodes[20] as well as during whole-night sleep periods devoid of clinical events.[26]

Since up to 90% of arousals occurring out of NREM sleep have been shown to be preceded by elevated delta activity,[43] many NREM sleep activation phenomena have

been conceptualized as variants of the same process: an initial high-voltage slow-wave EEG activation reflecting the readiness of the cerebral cortex to shift towards stronger activating effects of rapid EEG rhythms.[44-48] According to this line of reasoning, sleepwalking may not occur abruptly out of stable NREM sleep, but rather represent the end product of a more prolonged cortical process.

It is also relevant to note that our spectral power analysis revealed a non-significant (p=0.06) decrease in sigma activity, a frequency band associated with sleep spindles. This observation supports the interpretation of our results as reflecting a gradual process of state transition towards behavioural episodes. SWA and sleep spindles hold a complex relationship during normal SWS, as both increase at the start of a NREM sleep cycle while showing opposing relationships during transitions from NREM sleep to REM sleep.[49] It has been proposed that the temporary disappearance of spindles (thought to reflect thalamic stimuli filtering) before an arousal provides a time window for improved sensorial transmission through the thalamic relay.[50] Thus, the inverse relationship observed between delta and sigma spectral power prior to episode onset could reflect the imminent transition towards a brain state facilitating the occurrence of behavioural manifestations and information processing.

Arousal mechanisms in NREM parasomnias

Beta activity has been documented prior to the onset of NREM parasomnias and linked to behavioral manifestations during sleep.[19, 20] The pre-episode increase in beta EEG connectivity found in our subjects involved symmetrical frontal-occipital networks which may be related to motor manifestations underlying somnambulistic episodes. Specifically, these fast activity connectivity networks were found to coexist with delta

activity in associative brain areas, a result in line with findings in parasomniac patients suggesting the coexistence of slow activity in frontal areas and fast activity in motor areas both before[19] and during sleepwalking episodes.[19, 51] Increased EEG functional connectivity in these frequency bands may thus be understood as activation markers that mobilize the brain to upcoming EEG changes associated with eventual behavioural episodes.

Neuroanatomical basis of sleepwalking

Although little is known about how sleepwalking may be related to neuroanatomic dysfunctions or how episodes manifest themselves at the cerebral level, our results suggest that somnambulism is preceded by a sudden increase in beta functional connectivity networks linking frontotemporal regions and central, parietal and occipital regions. These symmetrical interhemispheric networks may be related to brain regions previously described as showing increased activity in association with NREM parasomnias. These areas include the motor cortex,[19, 52] cingulate,[19, 20, 51, 52] insular, amygdalar and temporopolar cortices,[52] as well as the anterior cerebellum.[51] Taken together, these results suggest beta functional connectivity networks may be related to brain activity in motor, cingulate and temporopolar areas and may constitute an early manifestation of somnambulistic episodes.

Limitations and implication for future studies

While measures of EEG functional connectivity have been used to clarify key processes underlying various neurological disorders and neurodegenerative diseases,[53-55] this is the first time that these methods have been applied to the study of a parasomnia. Our results indicate that the study of EEG functional connectivity represents a useful and

promising venue to better understand pathophysiological mechanisms underlying disorders of arousal.

The literature on EEG functional connectivity, however, is characterized by significant variations in the methodologies and metrics employed, thus hindering an unequivocal interpretation of our results. It is also important to note that while Icoh provided significant results in three distinct frequency bands, Msc did not show any significant differences between conditions. This may have been due to volume conduction which is known to affect Msc but not Icoh. Furthermore, since the EEG segments being compared were close to each other in time, it is possible that they were equally affected by volume conduction thereby obscuring underlying differences. That said, it would be of great interest to investigate NREM sleep parasomnias with other connectivity tools such as fMRI graph theoretical methods.[21]

Recent findings based on scalp and source power topography suggest that the NREM sleep of adults with sleepwalking is characterized by local decreases in SWA in posterior regions such as cingulate and motor cortices.[26] These results are hypothesized to be related to localized differences in arousal thresholds that may predispose affected individuals to somnambulistic episodes. Consistent with this view, the results from the present study suggest that posterior regions exhibit localized differences in functional connectivity that may gave rise to a favourable environment for episode occurrence in predisposed patients. It would therefore be especially worthwhile for studies to investigate scalp as well as source coherence topography in subjects with sleep arousal disorders to better elucidate how these observations may be empirically related and to

provide a broader conceptual understanding of the pathophysiological mechanisms believed to underlie these disorders.

It should be noted that arousal processes are believed to play a role in the pathophysiology of other sleep disorders, including obstructive sleep apnea and periodic leg movements during sleep. For instance, sleep-disordered breathing is known to be associated with changes in the EEG seconds before respiratory recovery.[56] Similarly, it is well documented that periodic leg movements during sleep are associated with EEG arousals[46, 57-59] and phase A manifestations of the cyclic alternating pattern, a measure of NREM sleep instability.[60] This suggests that leg movements are associated with a complex arousal process, progressing from sympathetic activation to increased EEG delta activity to higher-frequency rhythms.[46, 57, 61] It would therefore be relevant to explore the extent to which arousal-related processes implicated in sleepwalking show similarities to analogous processes believed to underlie other sleep disorders.

That said, it should be noted that electrophysiological markers of arousal, including of apparent coexistence of sleep and wake-like processes at sleep onset as well as during NREM sleep, are not exclusively observed in subjects presenting with arousal disorders, but have been well documented in healthy subjects.[62-66] It would thus be erroneous to consider these sleep processes as necessarily pathological. In this sense, the localized coexistence of sleep and wake-like EEG activity observed in sleepwalkers may reflect a deregulation of an intrinsic property of the brain that can culminate in clinical episodes of somnambulism in predisposed individuals.[67] Relatedly, one study of pre-arousal EEG activity in adult sleepwalkers showed that increased SWA and slow oscillation density was specific to clinical episodes rather than generalized to all sleep-

wake transitions.[18] It would thus be of interest to investigate the extent to which the patterns of EEG functional connectivity observed in the present study are specific to somnambulistic episodes.

Conclusion

Sleepwalking can be conceptualized as a brain state reflecting the co-existence of sleep and wakefulness as demonstrated by the persistence of sleep in frontal areas and wake-related activity in motor areas.[51, 52]

Our results suggest that somnambulistic episodes are preceded by changes in brain processes that are relatively gradual in nature and that the interplay between sleep and wakefulness can be observed through EEG functional connectivity networks before the onset of such clinical events. These results are, to our knowledge, the first to provide such evidence.

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Abbreviations

ICoh: Imaginary part of the coherence

Msc: Magnitude-squared coherence

NREM: Non-rapid eye movement sleep

PSG: polysomnographic

SWA: Slow wave activity

SWS: Slow-wave sleep

Caption for Figures

Figure 1: Illustration of the method for one pair of electrodes (F3-F4) for 0.1-2 Hz frequency bin.

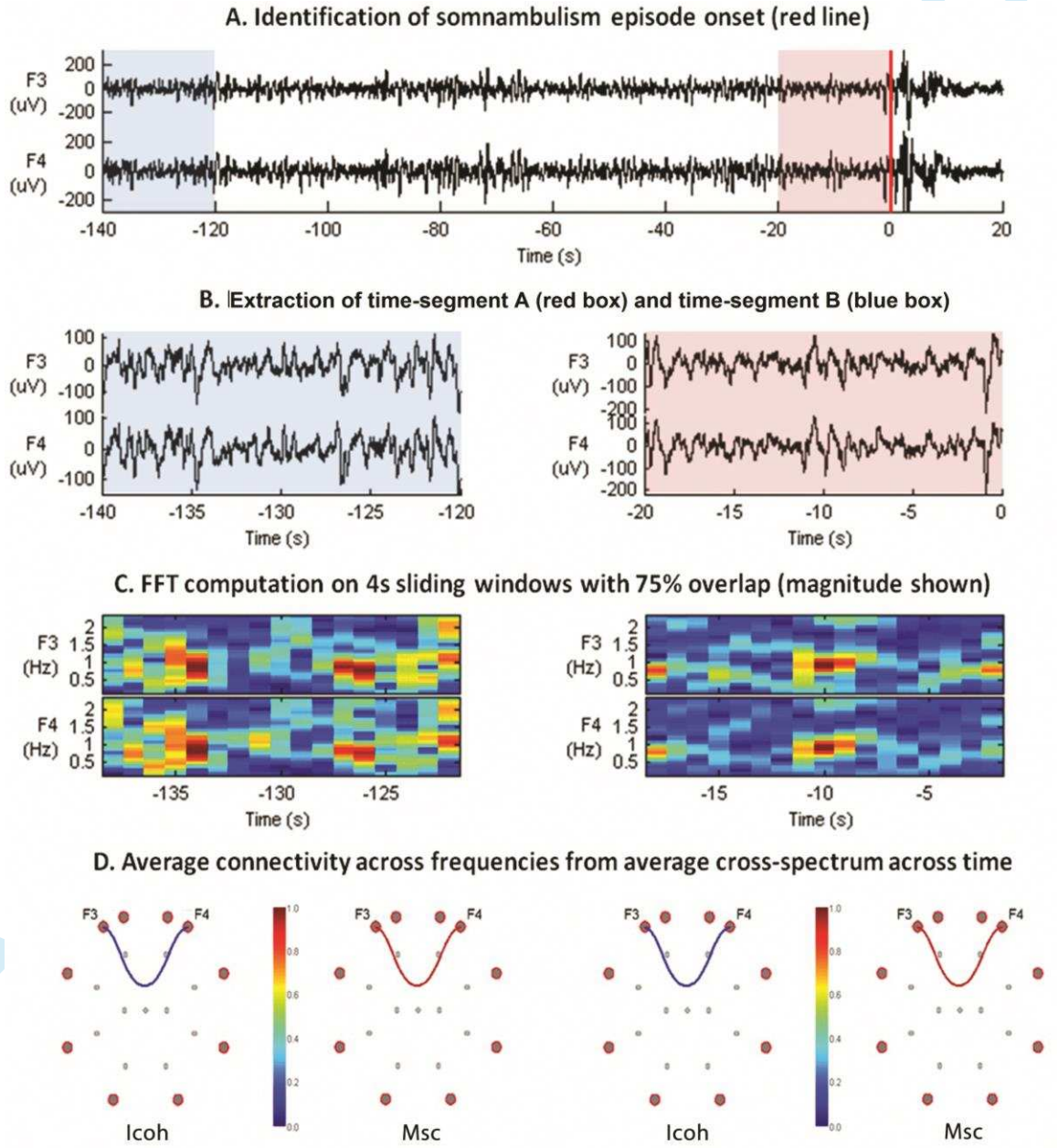

Figure 2: Graphical representations of EEG functional connectivity.

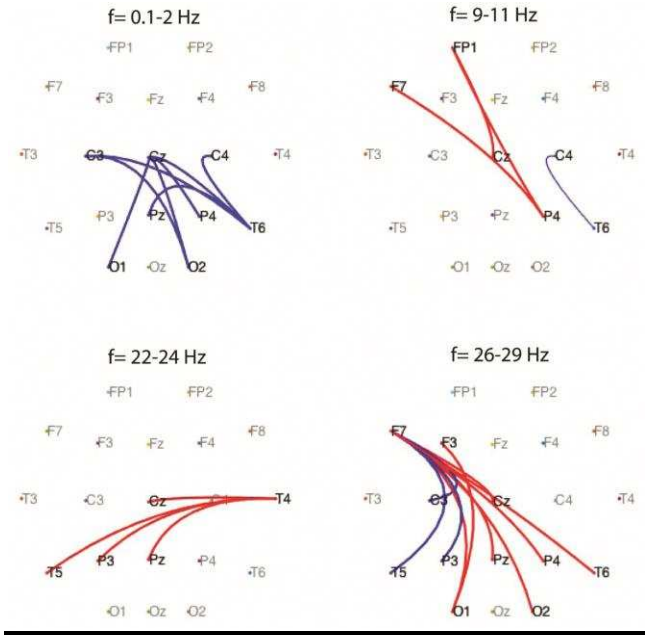

Figure Legends

Figure 1: (**A**) The selected time segments are identified. (**B**) Segments are extracted for further analysis. (**C**) Segments are subjected to Fast Fourier transformation. (**D**) A measure of the coherence is computed for each window of 4 sec in the segment; and then averaged coherence determined over the total length of the selected segment for each subject.

Figure 2: Graphical representations of electrode pairs showing significant differences between the 20 sec immediately preceding the episodes' onset and the 20 sec segment occurring 2 minutes prior to each episode time segments using ICoh ($p<0.05$); red links

represent increases of connectivity immediately preceding episode onset, blue links represent decreases of connectivity.

Tables

Table 1: Statistical differences between the two time segments for spectral power data band using paired T-tests (Log10 transformed)

Frequency band (N = 27 subjects)	**Paired difference Mean (SD)**	**df**	**t-value**	**p-value**
Delta (0.5-4 Hz)	0.11 (0.13)	26	4.35	0.000
Theta (4-8 Hz)	0.09 (0.12)	26	3.81	0.001
Alpha (8-12 Hz)	0.14 (0.21)	26	0.34	0.737
Sigma (12-14 Hz)	-0.07 (0.19)	26	-1.96	0.061
Beta 1 (14-22 Hz)	0.001 (0.15)	26	0.06	0.956
Beta 2 (22-32 Hz)	0.01 (0.08)	26	0.77	0.450

***N* = sample size; *SD* = standard deviation; *df* = degrees of freedom**