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Modulation of gamma and spindle-range power by slow oscillations in scalp sleep EEG of children

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ABSTRACT

Deep sleep is characterized by slow waves of electrical activity in the cerebral cortex. They represent alternating down states and up states of, respectively, hyperpolarization with accompanying neuronal silence and depolarization during which neuronal firing resumes. The up states give rise to faster oscillations, notably spindles and gamma activity which appear to be of major importance to the role of sleep in brain function and cognition. Unfortunately, while spindles are easily detectable, gamma oscillations are of very small amplitude. No previous sleep study has succeeded in demonstrating modulations of gamma power along the time course of slow waves in human scalp EEG. As a consequence, progress in our understanding of the functional role of gamma modulation during sleep has been limited to animal studies and exceptional human studies, notably those of intracranial recordings in epileptic patients.

Because high synaptic density, which peaks some time before puberty depending on the brain region (Huttenlocher and Dabholkar, 1997), generates oscillations of larger amplitude, we considered that the best chance to demonstrate a modulation of gamma power by slow wave phase in regular scalp sleep EEG would be in school-aged children. Sleep EEG was recorded in 30 healthy children (aged 10.7 ± 0.8 years; mean ± s.d.). Time-frequency analysis was applied to evaluate the time course of spectral power along the development of a slow wave. Moreover, we attempted to modify sleep architecture and sleep characteristics through automated acoustic stimulation coupled to the occurrence of slow waves in one subset of the children.

Gamma power increased on the rising slope and positive peak of the slow wave. Gamma and spindle activity is strongly suppressed during the negative peak. There were no differences between the groups who received and did not receive acoustic stimulation in the sleep parameters and slow wave-locked time-frequency analysis. Our findings show, for the first time in scalp EEG in humans, that gamma activity is associated with the up-going slope and peak of the slow wave. We propose that studies in children provide a uniquely feasible opportunity to conduct investigations into the role of gamma during sleep.

1. Introduction

A most characteristic electrophysiological feature of non-rapid eye movement (NREM) sleep is the slow oscillation, visible on scalp electroencephalography (EEG) as a biphasic wave of high amplitude and a fundamental frequency of around 1 Hz (Achermann and Borbély, 1997). This slow oscillation, or slow wave, is the result of the alternation of periods of extended synchronization and desynchronization of the membrane potentials of numerous cerebral cortical neurons (Steriade et al., 1993). During the hyperpolarized phase, often called down state, neurons remain silent for up to a few hundred milliseconds. During the depolarized phase, also called up state, neuronal spike activity takes place, often including burst firing (Steriade et al., 1993).

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Up states are associated with complex and widespread neuronal network activity throughout the brain (Volgushev et al., 2011), including high-frequency oscillations. Especially these oscillations, and their coalescence with slow oscillations, have been implicated in network communication and systems consolidation of memory traces (Diekelmann and Born, 2010; Mölle and Born, 2011; Schwindel and McNaughton, 2011; Van Someren et al., 2011). During the up states of slow oscillations, newly encoded memory representations are thought to be reactivated and redistributed, enabling a shift from temporary storage to long-term storage. Crucial for the dynamical formation of neuronal ensembles and altering of the synaptic connections during the up state is the co-occurring thalamocortical and cortico-cortical neuronal activity in higher frequency bands, notably the 10–15 Hz sleep spindles (Rosanova and Ulrich, 2005) and the >30 Hz gamma oscillations (Steriade et al., 1996; Mena-Segovia et al., 2008; Mena-Segovia and Bolam, 2011).

After the initial demonstration in cats and ferrets that the preferred occurrence of thalamocortical spindles and cortical gamma oscillatory activity is during the depolarizing phase of slow oscillations (Contreras and Steriade, 1995; Steriade, 2006), Mölle and colleagues showed a similar modulating effect of slow oscillations on spindles in the EEG of adult humans (Mölle et al., 2002; Mölle and Born, 2011). Whereas in humans intracortical recordings support a similar modulation of gamma activity by slow oscillations (Csercsa et al., 2010; Le Van Quyen et al., 2010; Valderrama et al., 2012) and magnetoencephalographic (MEG) recordings support concerted modulation of gamma and spindles (Ayoub et al., 2012), gamma modulation along the time course of a slow wave has not yet been demonstrated in human scalp EEG.

The lacking demonstration of such modulation of gamma activity along the time course of a slow wave in human scalp EEG is unfortunate, because it is especially these high frequency oscillations that have been attributed an important role in synchronizing and binding neuronal network activity to support neuronal network processes underlying cognition. Destexhe et al. (2007) proposed them to represent brief fragments of a wake-like state where effective communication between different neuronal systems can take place to support brain function and cognition. Given the proposed functional importance of gamma activity modulation by slow oscillations, it would be highly valuable to be able to measure and quantify them in the human sleep EEG. This was the major aim of the present study. As will be discussed below, we argued that the best chance of achieving this might be in the sleep EEG of children around the age of 11 years.

The expression of slow waves undergoes remarkable changes during development, both with respect to their topographical distribution (Jenni et al., 2005; Kurth et al., 2010; Tarokh et al., 2010), as well as with respect to their amplitude (Feinberg et al., 1990; Feinberg and Campbell, 2010). The amplitude of slow oscillations increases during childhood to peak shortly before puberty (Feinberg et al., 1990). A steep drop occurs during adolescence, decelerating at the age of about 17 years, after which the amplitude declines only slowly (Feinberg and Campbell, 2010). Interestingly, the initial inverted-U shape closely follows the developmental profile of cortical synaptic density (Huttenlocher, 1979; Huttenlocher and de Courten, 1987; Paus et al., 2008). The reason for this parallel is sought in the fact that the amplitude of slow oscillations reflects the degree of synchronization by which cortical neurons switch between up and down states (Ringli and Huber, 2011). Although receiving much less attention, the capacity of a densely connected neuronal network to synchronize its activity may not only be reflected in the amplitude of slow oscillations, but might as well lead to more pronounced oscillations in frequency bands other than the 0.5–4 Hz range. Indeed, power in the theta (4–8 Hz) range declined across puberty and early adolescence (Feinberg et al., 2011). Gaudreau et al. (2001) investigated NREM sleep EEG power in a wider range of frequency bands across the age range of 6 to 60 years. They report a much higher absolute power of theta (4.0–7.75 Hz), alpha (8.0–12.0 Hz) and beta (15.25–31.0 Hz) in the group of children in the range of 6 to10 years, as compared to the groups of adolescents (range 14 to 16 years), young adults (range 19 to 29 years) and middle aged adults (range 36 to 60 years). The largest values for spindle-range power (12.25–15.0 Hz) were found in the adolescent group, suggestive of an inverted-U shape peaking somewhere between the age of about 10 years and late adolescence. Jenni and Carskadon (2004) investigated developmental changes across the 0.6 to 25 Hz NREM-sleep power spectrum and found that children aged 9.6–12.9 years, as compared to children aged 11.8–15.9 years, had significantly higher absolute power not only in the low frequencies up to about 7 Hz, but also in the 12–13 Hz sigma range and 16–17 Hz low beta range. Recently, both Tarokh et al. (Tarokh and Carskadon, 2010; Tarokh et al., 2011) and Baker et al. (2012) applied a within-subject follow-up design rather than the above-mentioned cross-sectional approaches, to confirm that changes in the sleep EEG across adolescence were not restricted to the lower frequency bands, neither to NREM sleep only. Across adolescence, the sleep EEG power decreases over a wide range of frequencies, up to the beta range for at least some derivations. In summary, the above-mentioned developmental studies suggest that a wide range of cortical oscillations measured in the scalp EEG show their maximal signal-to-noise ratio in late childhood, around the age of 11, where the signal of interest is the amplitude of the oscillations and the noise reflects the noise floor of scalp EEG assessment. We argued that the sleep EEG of children around this age may thus provide an optimal opportunity to investigate modulations and associations between different frequency bands in human sleep.

The first aim of the present study was to investigate whether gamma modulation by slow waves can be demonstrated in the sleep EEG of children. We considered it more likely to demonstrate this phenomenon in children of about 11 years of age than in adults, arguing that the capacity of a densely connected neuronal network to synchronize its activity could result in more pronounced oscillations and a better signal to noise ratio in the gamma frequency range. The demonstration of gamma modulation during slow oscillations in scalp EEG recordings would open a door to noninvasive experimental studies on their functional relevance in humans. The integrated second aim of the present study was therefore to evaluate the feasibility and effects of selective mild acoustic perturbation of slow oscillations in children, one of the experimental approaches that we previously showed to affect slow oscillations in adults, using temperature manipulation (Raymann et al., 2008) and acoustic stimulation (Van Der Werf et al., 2009). A further aim of the present study was to evaluate whether the above-mentioned modulation of spindles by slow oscillations in the sleep of adults can be found as well in an earlier developmental stage.

2. Methods

2.1. Participants

Thirty-two children of an elementary school participated in the study. For ethical reasons, i.e. peer group interaction, no child was excluded from the recording session, whereas the data from two participants were discarded due to a diagnosis of pervasive developmental disorder-not otherwise specified. The data presented here thus represent 30 healthy children (19 females, aged 10.7 ± 0.8 years; mean $\pm$ s.d.). The Medical Ethics Committee of the VU University Medical Center, Amsterdam, The Netherlands, approved all procedures and written informed consent was obtained from the parents.

2.2. Procedure

Children were invited to simultaneously undergo a full polysomnography (PSG) during a full night while sleeping in a dedicated sleep lab built on location in a science museum. They were randomly

assigned to one of three conditions: (A) slow wave noise, i.e. slow wave-triggered acoustic stimulation as described before (Van Der Werf et al., 2009), (B) yoked noise, i.e. acoustic stimulation triggered by the occurrence of slow wave sleep of a neighboring child, or (C) no noise, i.e. no acoustic stimulation. Details are described below.

2.3. Sleep recordings

PSG was recorded with 8 electrodes: for electroencephalography (EEG), 2 electrodes were positioned on frontopolar (Fpz) and central (Cz) positions according to the 10–20 system; for electrooculography (EOG) 2 electrodes were positioned diagonally, 1 cm out of the outer canthi of the eyes, one 1 cm below and one 1 cm above for EOG; for electromyography (EMG), 2 electrodes were attached submentally; for ground, one electrode was positioned on the forehead and for reference one on the left mastoid (A1). Signals were recorded with the Embla A10 system (Flaga, Reykjavik, Iceland). The Embla A10 system initially samples the data at 2000 Hz and subsequently down-samples it digitally to 200 Hz after application of an anti-aliasing filter, and furthermore applies high-pass filtering (1 Hz, −3 dB at 0.3 Hz) and a 50 Hz notch filter (1 Hz bandwidth).

2.4. Manipulation: acoustic stimulation triggered by slow waves

All participants were equipped with in-ear headphones (SR 10 Micro HD, Vivaco, Ahrensburg, Germany) and were notified that they might hear beeps during the night. Tape was used to prevent the headphones from dropping out or moving. In group (A), the 'slow wave noise' group, slow wave-triggered acoustic stimulation was accomplished according to previously described methods (Van Der Werf et al., 2009) using a custom analysis plug-in for the Somnologica 2 software (Flaga, Reykjavik, Iceland). The relative contribution of the 0.4–4 Hz band to the frequency spectrum was calculated online continuously. When the contribution of SWA on one of the EEG channels exceeded a threshold level, a beeping noise was played on an in-ear headphone that continued to increase in amplitude in four discrete steps. As measured at 1 cm in front of a driver of the headphone using a 1933 Precision Sound Level Meter & Analyzer (General Audio, Cambridge, MA, USA), this resulted in flat impulse values of 68, 70, 71, and 72 dB and peak impact values of 70, 75, 80, and 85 dB. The sound continued until the level of slow wave activity dropped below the threshold. To avoid erroneous inclusion of slow EOG signals in the 0.4–4 Hz EEG band, the sound was not emitted when the signals from the two EOG leads were negatively correlated, which occurs in case of conjugated eye movements whereas a positive correlation on the other hand reflects leakage of SWA into the EOG leads. In group (B), the 'yoked noise' group, yoked acoustic stimulation was accomplished by playing the same tone that the neighboring participant in group (A) received, thus not corresponding to their own individual slow waves. Finally, participants in group (C), the 'no noise' group, wore in-ear headphones, but no sound was played.

2.5. Qualitative analysis of the sleep macrostructure

EEG recordings were scored visually by an experienced sleep technician (JCV) using the Somnologica software (Flaga, Reykjavik, Iceland). Epochs of 30 s were assigned a sleep/wake stage according to standard sleep scoring criteria (Rechtschaffen and Kales, 1968), based on the EEG at Fpz-A1 and Cz-A1, on the EOG as the difference between the left and right EOG channels, and on the submental EMG. The following measures were derived to quantify sleep macrostructure: time in bed (in minutes), total sleep time (TST, in minutes), sleep efficiency (as percentage of total time in bed), sleep onset latency (in minutes, defined as the start of the first epoch scored as any stage other than wakefulness), latency to 1st REM period (in minutes), wake after sleep onset (in minutes), stage 1 (as percentage of TST), stage 2 (as percentage of TST), slow wave sleep (as percentage of TST), and REM sleep (as percentage of TST).

2.6. Slow wave detection

EEG recordings were visually inspected for segments containing artifacts, which were excluded from all quantitative analyses. Slow waves occurring in sleep stages 3 and 4 were automatically detected using a previously described method (Massimini et al., 2004; Dang-Vu et al., 2008; Piantoni et al., 2013), applied to each channel separately. In brief, custom code written in Matlab 7.13 (MathWorks, Natick, MA) band-pass filtered the EEG signals between 0.16 and 4 Hz, and subsequently defined a slow wave according to the following criteria: (I) a negative zero crossing separated by 0.3–1 s from the subsequent positive zero crossing; (II) a negative peak between these two zero crossings reaching a voltage lower than −75 μV.

2.7. Time-frequency analysis of modulation of spectral content during slow waves

To investigate the changes in the power spectrum associated with the slow wave, we took the negative peak of each slow wave detected throughout the night in stage 3 and 4 as time zero and we analyzed the time windows of 1.5 s preceding and 1.5 s following the negative peak. A time window was discarded when the amplitude range exceeded the absolute threshold of 500 μV or the variance of the original signal in the 3 s time window was above 7500 μV^2. Because of the concern that muscle activity might affect the power in the gamma frequency band (Whitham et al., 2007; Pope et al., 2009), we rejected those epochs whose variance at Fpz and Cz was above 100 μV^2 after the application of a high-pass filter at 40 Hz.

Time-frequency analysis was computed by means of a wavelet transform (Tallon-Baudry and Bertrand, 1999), using FieldTrip (Oostenveld et al., 2011), a Matlab (MathWorks, Natick, MA) toolbox for electrophysiological data. To compute the time–frequency representation of the power changes along the time course of a slow wave, the selected 3 s epochs of EEG signal were convolved with wavelets belonging to a Morlet wavelet family defined by the constant ratio of $f_0/\sigma_f = 7$, where f_0 represents the frequency of interest (ranging from 10 Hz to 90 Hz) and σ_f is the bandwidth of the wavelet in the frequency domain. At 10 Hz, the frequency resolution ($2\sigma_f$) is 2.9 Hz and temporal resolution is 223 ms, while at 90 Hz the frequency resolution ($2\sigma_f$) is 25.7 Hz and temporal resolution is 25 ms. The time-frequency representations around the negative peak of each slow wave were then averaged for each channel in every participant. On each participant's averaged power spectrum, we applied a baseline correction based on the initial period between 1.5 and 1 s prior to the negative peak. The power at each time–frequency point in the −1.5 s to 1.5 s period of interest can be expressed both as the percentage change (%) or significance of change (t-values) relative to the power at that frequency during the baseline period.

2.8. Statistical analysis

One-way ANOVAs were used to evaluate possible group differences with respect to the sleep variables resulting from the qualitative sleep staging using two-sided alpha level of 0.05. Whether slow waves modulated power in the gamma and spindle range in the time window between −1.5 s and 1.5 s around the negative peak was first evaluated in the complete dataset of 30 sleep recordings. For each of the 30 participants, it was first evaluated whether, across all slow waves detected in that participant, percentage change values (power relative to the baseline period) for each of the individual time–frequency points differed systematically from zero according to a t-test for that time-frequency point (usually set at $P \leq 0.05$, the threshold in our analysis was set at $P \leq 0.048$, see below for the justification).

Subsequently, to correct for multiple comparisons, clusters of adjacent significant time–frequency points were evaluated using a cluster-based non-parametric randomization procedure (Maris and Oostenveld, 2007) in the following way. Significant time–frequency points that were adjacent in time and frequency and had the same sign (smaller vs larger power than at baseline) were clustered together. The threshold used for the selection of the significant time–frequency points was lowered slightly from $P \leq 0.05$ to $P \leq 0.048$ to avoid a spurious aggregation of two clearly separate clusters at Fpz, which were connected only by one time–frequency point with $P = 0.049$. By excluding this point, the two clusters were kept separate in the subsequent analysis. Each cluster has a corresponding sum of the *t*-values of its constituent points. To subsequently evaluate how significant the cluster–sum of *t*-values was, a reference probability distribution for cluster-summed *t*-values was created using a Monte Carlo approach, as follows. Repeated 10,000 times, the sign of each *t*-value in the time–frequency was either kept (− remains −, + remains +) or swapped (− to +, + to −), with a probability of 0.5. The largest cluster–sum of *t*-values is obtained from the resulting matrix to be added to the reference distribution. A cluster was considered significant if the sum of its *t*-values was above the 95% threshold of the reference distribution. All the *P*-values reported here refer to the *P*-value of the clusters computed using this procedure and are corrected for multiple comparisons.

Subsequently, we tested whether there were significant differences between the three groups with respect to the modulation in oscillatory activity elicited by the slow wave. The time–frequency representation of the participants belonging to 'slow wave noise' group was compared with that of each of the other two groups, and the 'yoked' group with the 'no noise' group. The time–frequency representations were compared using a two-sample *t*-test and correction for multiple comparisons was achieved using a cluster-based non-parametric procedure, as above. In this case, however, instead of switching the signs of the *t*-values of time points, *t*-values at each time point were either kept (− remains −, + remains +) or swapped (− to +, + to −) between the two compared groups, with a probability of 0.5.

3. Results

3.1. Qualitative analysis of the sleep macrostructure

Table 1 shows the averages and standard errors of the macrostructural sleep variables as calculated both over all participants and separately within each of the three groups. ANOVAs indicated no significant between-group difference for any of the sleep variables (all $P \geq 0.10$).

3.2. Detection of slow waves

Table 1 shows the averages and standard deviations of the density of slow waves detected in slow wave sleep (SWS) at Fpz and Cz as calculated both over all participants and separately within each of the three groups. ANOVAs indicated no significant between-group difference for slow wave density either at Fpz or at Cz (all $P \geq 0.39$). Fig. 1A–B shows the average of all detected slow waves at Fpz and at Cz, respectively, time-locked to the negative peak.

3.3. Time-frequency analysis of modulation of spectral content during slow waves

Fig. 1B represents the changes in the power spectrum in the frequency range 10–90 Hz in the period around the slow waves detected in SWS. Time 0 is the negative peak of the slow wave. Both channels show a very remarkable modulation in spindle and gamma oscillations, with a decrease in activity during the falling slope of the slow wave and an increase during the rising slope and the positive deflection of the slow wave. In particular, at channel Fpz, there were 6 significant clusters, defined by the weighted mean and standard deviation: 1) a positive cluster centered at 15.6 ± 3.1 Hz at −720 ± 120 ms with $P = 0.008$; 2) a positive cluster centered at 48.4 ± 11.1 Hz at −580 ± 80 ms with $P = 0.001$; 3) a negative cluster centered at 15.8 ± 3.3 Hz at −260 ± 130 ms with $P = 0.022$; 4) a negative cluster centered at 50.8 ± 17.1 Hz at −130 ± 40 ms with $P < 0.001$; 5) a positive cluster centered at 16.9 ± 4.1 Hz at 300 ± 230 ms with $P < 0.001$; 6) a positive cluster centered at 55.4 ± 14.8 Hz at 350 ± 90 ms with $P < 0.001$.

At channel Cz, there were 6 significant clusters: 1) a positive cluster centered at 15.4 ± 2.8 Hz at −680 ± 220 ms with $P = 0.004$; 2) a negative cluster centered at 60.4 ± 14.6 Hz at −120 ± 100 ms with $P < 0.001$; 3) a negative cluster centered at 14.8 ± 2.0 Hz at −30 ± 90 ms with $P = 0.045$; 4) a positive cluster centered at 21.2 ± 6.6 Hz at 230 ± 190 ms with $P = 0.001$; 5) a positive cluster centered at 71.9 ± 10.1 Hz at 290 ± 80 ms with $P = 0.002$; 6) a negative cluster centered at 20.4 ± 4.6 Hz at 960 ± 260 ms with $P < 0.001$.

3.4. Comparison between groups

Although the three groups did not differ in the sleep macrostructure nor in the number of slow waves, acoustic stimulation might affect the way gamma and spindle-range power are modulated by slow waves. We compared the time–frequency representation of the group that received acoustic stimulation with the other two groups. There was no significant difference in oscillatory activity time-locked to the negative peak of the slow wave for participants that received acoustic stimulation as compared to the 'yoked noise' group (for both channels, the most significant cluster had $P = 0.126$) and to the 'no noise' group (for both channels, the most significant cluster had $P = 0.065$). There was no significant difference in the comparison between the 'yoked noise' group and the 'no noise' group in any frequency band ($P > 0.196$).

Table 1
Macrostructural sleep characteristics and SWS slow wave density (mean ± standard deviation) aggregated over all 30 children as well as within the subgroups assigned to the slow wave noise, yoked noise and no noise conditions. The rightmost column shows the P-values of the ANOVAs on group differences with respect to each variable.

	All children	Slow wave noise	Yoked noise	No noise	P
Time in bed (min)	465 ± 10	466 ± 9	461 ± 6	467 ± 12	0.38
Total sleep time (min)	432 ± 25	442 ± 25	432 ± 14	425 ± 32	0.33
Sleep efficiency (%)	93 ± 5	95 ± 4	94 ± 3	91 ± 6	0.72
Sleep onset latency (min)	19 ± 13	18 ± 13	22 ± 13	18 ± 13	0.53
Latency to 1st REM period (min)	94 ± 38	97 ± 46	103 ± 36	84 ± 33	0.10
Wake after sleep onset (min)	9.4 ± 11.5	4.9 ± 9.0	7.1 ± 9.7	15.3 ± 13.2	0.14
Stage 1 (%)	3.4 ± 3.4	1.7 ± 1.5	3.3 ± 4.2	4.8 ± 3.5	0.25
Stage 2 (%)	39.0 ± 7.2	42.4 ± 4.6	37.9 ± 7.5	37.2 ± 8.3	0.30
Slow wave sleep (%)	34.0 ± 5.8	31.8 ± 3.0	36.0 ± 7.1	33.9 ± 6.0	0.77
REM sleep (%)	23.7 ± 4.5	24.1 ± 3.2	22.8 ± 4.0	24.2 ± 6.0	0.20
SWS SW density at Fpz (#/min)	21.2 ± 4.9	22.2 ± 4.4	19.6 ± 5.7	22.2 ± 4.5	0.39
SWS SW density at Cz (#/min)	24.7 ± 4.1	25.3 ± 3.7	23.6 ± 5.2	25.5 ± 2.7	0.53

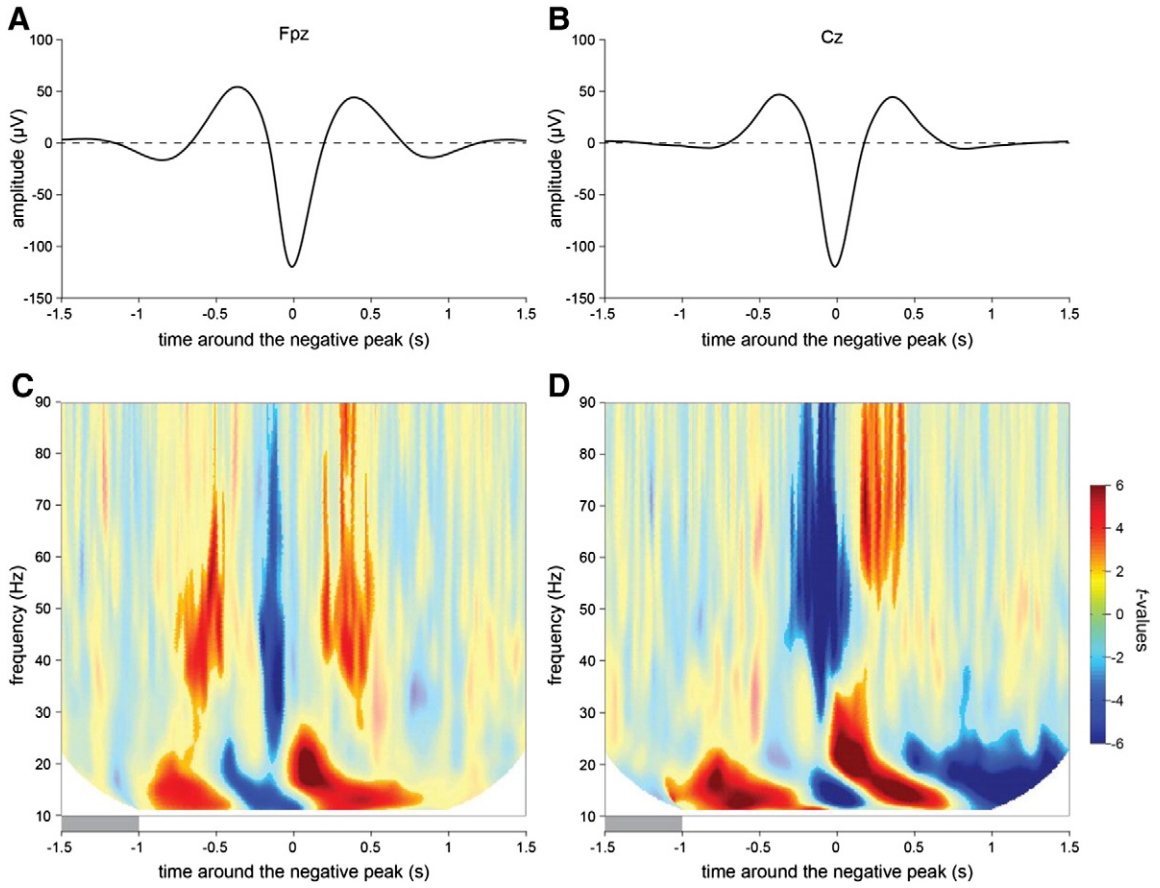

Fig. 1. Modulation of power in the spindle and gamma bands at Fpz and Cz, time-locked to the negative peak of the slow wave. (A–B) Average of all slow waves detected in SWS at Fpz (A) and at Cz (B) time-locked to their negative peak. (C–D) Time–frequency representation at Fpz (C) and at Cz (D). Warm colors represent more significant positive *t*-values, indicating an increase relative to baseline (−1500 to −1000 ms, indicated in gray on the horizontal axis), while cooler colors represent more significant negative *t*-values, indicating a decrease relative to baseline. There were six significant clusters for both Fpz and Cz. Time–frequency points which were not significant after correcting for multiple comparisons are masked in a dimmer color.

4. Discussion

Sleep EEG was recorded from thirty school-aged children of, on average, 11 years of age, with the aim to evaluate the time course of spectral power of higher frequencies along the development of a slow wave. To do so, time-frequency analysis was applied to obtain matrices of spectral power in the frequency ranges of 10–90 Hz for the interval of 1500 ms before to 1500 ms after the negative peak of individually detected slow waves. The results show, for the first time in human scalp EEG, increased gamma power during the rising slope of the slow wave. The results furthermore show, for the first time in children, that a modulation is also present for power in the spindle-range, as has been observed previously in the sleep EEG of adults (Mölle et al., 2002). Of note, the falling slope of the slow wave was associated with a strong silencing of gamma activity, which resumed at the rising slope.

4.1. Slow wave-related gamma activity

Slow waves are defined by a bistable state, characterized by a relatively silent period of neuronal firing (down state) and by a highly synchronized activity during the up state (Steriade et al., 1993; Steriade, 2006; Chauvette et al., 2011). Animal studies have demonstrated that the transition from the down to the up state is accompanied by strong oscillatory activity, in the form of spindles and gamma activity (Steriade et al., 1996; Steriade, 1997). Intracortical recordings in epileptic patients point to similar mechanisms occurring in humans (Csercsa et al., 2010; Le Van Quyen et al., 2010; Valderrama et al., 2012). In one magnetoencephalography (MEG) study in adults, modulations of power in the gamma and spindle-range were found to be associated (Ayoub et al., 2012). No previous human EEG study has revealed a significant modulation associated with slow waves for frequencies above beta (>25 Hz) (Mölle et al., 2002).

Our results show for the first time in human scalp EEG that there is a phasic increase in gamma activity during the rising segment of the slow wave and, moreover, confirm for the first time that the grouping of spindle activity during slow oscillations occurs in children similarly to what has been demonstrated in adult sleep EEG (Mölle et al., 2002). The rising segment of the slow wave corresponds to the onset of the up state, when neurons resume their firing and communication over long distances (Volgushev et al., 2006; Vyazovskiy et al., 2007, 2008). The importance of the up state for brain function and cognition has been frequently discussed in the literature (see Van Someren et al. (2011) for an introduction and overview).

4.2. Cortical maturation

Synaptic density peaks, depending on the cortical area (Huttenlocher and Dabholkar, 1997), some time before puberty (Feinberg et al., 1990; Campbell and Feinberg, 2009). This is also the period in which slow wave amplitude is largest (Ringli and Huber, 2011; Campbell et al., 2012). The larger amplitude of slow waves during childhood has been proposed to be a direct consequence of the higher synaptic density (Feinberg et al., 1990), as higher synaptic density is thought to be

more efficacious in generating post-synaptic potentials. In addition, these post-synaptic events will be more strongly synchronized, due to the interconnectedness of the network. Because local field potentials reflect the synchronized post-synaptic potentials and EEG is the summation of local field potentials measured at the scalp level (Nunez and Srinivasan, 2006), the observed amplitude of the EEG signal directly correlates with the underlying synaptic density.

We suggest that the developmental peak of synaptic density might have facilitated the detection of gamma modulation in the present study. Although the specific neuronal types and network configuration (such as the role of the thalamus) might differ across frequency bands, all oscillatory activity in the brain derives from the well-timed neuronal interaction of, in particular, post-synaptic potentials. Gamma oscillations are generated by a strongly interconnected neuronal network involving excitatory and inhibitory neurons (Bartos et al., 2007). A high synaptic density will likely result in an increase in the signal-to-noise ratio and make it more feasible to find modulations in the scalp EEG.

Another factor that might facilitate the synchronization of neuronal networks and therefore boost the oscillatory activity is the strength of the underlying connections, i.e. the integrity of the white matter (Uhlhaas et al., 2010). In fact, the expression profile of sleep slow waves and spindles is partially determined by the axial diffusivity strength over long-range white matter tracts, as assessed by diffusion-tensor imaging (Piantoni et al., 2013). Higher frequencies including the beta and gamma bands, however, mostly reflect short-distance synchronization, which increases during early development (Thatcher et al., 1987) and might involve short-range white matter axons rather than the long-range white matter tracts studied by Piantoni et al. (2013).

A complementary explanation to the stronger neuronal signal in children would be that their thinner skulls might provide smaller resistance and therefore constitute a weaker filter for the transmission of local field potentials onto the scalp (Akhtari et al., 2002). However, skull thickness does not correlate with the amplitude of slow wave activity (Buchmann et al., 2011), supporting the interpretation of an involvement of synaptic density (Ringli and Huber, 2011).

4.3. Acoustic stimulation

In addition to evaluating whether gamma power was modulated by the slow wave, we investigated whether it would be feasible to experimentally manipulate this modulation through acoustic stimulation. Although in adults acoustic stimulation during slow wave sleep has been shown to decrease delta (0.5–4 Hz) power (Dijk and Beersma, 1989; Aeschbach et al., 2008; Van Der Werf et al., 2009) and suppress the appearance of slow waves (Landsness et al., 2009), we here showed that this is not necessarily the case in children. Neither the sleep macrostructure, nor the number of slow waves or their modulation of higher frequency oscillations differed between children exposed to slow wave-triggered noise, yoked noise and no noise.

The lack of sensitivity is likely due fact that sleeping children have a very high arousal threshold (Busby et al., 1994). There is consistent evidence that waking devices, such as fire alarms, that properly wake adults in case of danger, fail to wake up children (Bruck, 1999, 2001). Therefore, any future study aimed at suppressing slow wave sleep or at presenting sounds during sleep, e.g. to improve memory consolidation (Rudoy et al., 2009), should titrate the sound intensity to a considerably higher level than that used for adults.

4.4. Future studies investigating gamma

As noted, modulation of gamma power along the time course of a slow wave has not been described in human sleep scalp EEG before. Evidence for such modulation was presented in animal studies (Steriade et al., 1996; Steriade and Amzica, 1998; Steriade, 2006) and intracranial recordings in patients with pharmacologically untreatable epilepsy (Csercsa et al., 2010; Le Van Quyen et al., 2010; Valderrama et al., 2012). However, the application of intracortical recordings can be done only sparsely and is limited to studies on neurosurgical patients. Time-locking of gamma and sleep spindles has been reported in the sleep MEG of healthy adults (Ayoub et al., 2012), but it remains to be investigated whether MEG studies can confirm gamma modulation along the time course of a slow oscillation. Even then, the application of MEG during sleep is expensive and limited to a short period of time, making full-night recordings and the investigation of gamma during late sleep unfeasible.

5. Conclusion

We have demonstrated for the first time in human EEG a modulation in gamma power associated with the rising slope of the slow wave. We suggest that sleep EEG in children represents the optimal opportunity to investigate the role of high frequencies during sleep. The widespread availability of EEG, its whole-head coverage, and the possibility to run full-night recordings in children make night EEG recordings in children a feasible and appropriate method to study gamma activity during sleep, its role in memory consolidation, and its contribution to cortical and cognitive development.

Conflict of interest

The authors have no conflict of interest to disclose.

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