EEG Slow (−1 Hz) Waves Are Associated With Nonstationarity of Thalamo-Cortical Sensory Processing in the Sleeping Human

MARCELLO MASSIMINI, MARIO ROSANOVA, AND MAURIZIO MARIOTTI
Department of Clinical Science, Osp. L. Sacco, Faculty of Medicine, University of Milan, 20157 Milan, Italy
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Massimini, Marcello, Mario Rosanova, and Maurizio Mariotti. EEG slow (−1 Hz) waves are associated with nonstationarity of thalamo-cortical sensory processing in the sleeping human. J Neurophysiol 89: 1205–1213, 2003; 10.1152/jn.00373.2002. Intracellular studies reveal that, during slow wave sleep (SWS), the entire cortical network can swing rhythmically between extremely different microstates, ranging from wakefulness-like network activation to functional disconnection in the space of a few hundred milliseconds. This alternation of states also involves the thalamic neurons and is reflected in the EEG by a slow (<1 Hz) oscillation. These rhythmic changes, occurring in the thalamo-cortical circuits during SWS, may have relevant, phasic effects on the transmission and processing of sensory information. However, brain reactivity to sensory stimuli, during SWS, has traditionally been studied by means of sequential averaging, a procedure that necessarily masks any short-term fluctuation of responsiveness. The aim of this study was to provide a dynamic evaluation of brain reactivity to sensory stimuli in naturally sleeping humans. To this aim, single-trial somatosensory evoked potentials (SEPs) were grouped and averaged as a function of the phase of the ongoing sleep slow (<1 Hz) oscillation. This procedure revealed a dynamic profile of responsiveness, which was conditioned by the phase of the spontaneous sleep EEG. Overall, the amplitude of the evoked potential changed systematically, increasing and approaching wakefulness levels along the negative slope of the EEG oscillation and decaying below SWS average levels along the positive drift. These marked and fast changes of stimulus-correlated electrical activity involved both short (N20) and long latency (P60 and P100) components of SEPs. In addition, the observed short-term response variability appeared to be centrally generated and specifically related to the evolution of the spontaneous oscillatory pattern. The present findings demonstrate that thalamo-cortical processing of sensory information is not stationary in the very short period (approximately 500 ms) during natural SWS.

INTRODUCTION

Sequential averaging of many trials is the traditional procedure used to extract reliable responses from the background activity of the brain. This method necessarily assumes that inter-trial variability is due to the linear superimposition of ongoing random activity on a deterministic, reproducible response (Aunon et al. 1981; Dawson 1951). This assumption, although corroborated by experimental observations (Arieli et al. 1996), can be violated if the properties of the circuits that generate the response spontaneously change during the period under analysis (Kisley and Gerstein 1999). In this case, the result of sequential averaging has to be considered a poor or misleading estimate of brain reactivity.

This theoretical problem may become critical when addressing the fundamental issue of brain responsiveness during different states of vigilance, especially as slow wave sleep (SWS) emerges. Indeed, intracellular studies in anesthetized and behaving animals (for review see Steriade 2000, 2001) revealed this latter condition as characterized by the occurrence of spontaneous, fast, and pronounced shifts in the general state of the thalamo-cortical networks. During SWS, the membrane potential of all cortical neurons oscillates, with a periodicity of about 1 s, between depolarized (up state) and hyperpolarized (down state) levels (Steriade et al. 1993). The long-lasting hyperpolarizations are due to recurrent phases of global disfacilitation and rhythmically interrupt the periods of wakefulness-like network activity associated with the up state (Contreras et al. 1996; Steriade et al. 2000; Timofeev et al. 2001). This pattern of alternating states is also associated with the continuous fluctuation of the probability of synaptic release at the cortical level: higher at the end of the down state and reduced by about 40–50% toward the end of the up state (Massimini and Amzica 2001). This slow sleep oscillation is an emergent property of the cerebral cortex (Sanchez-Vives and McCormick 2000; Timofeev and Steriade 1996; Timofeev et al. 2000a) and powerfully entrains thalamic neurons (Contreras and Steriade 1995). In addition, this pattern is prevalent during late stages of sleep and is reflected in the human EEG by a low-frequency (<1 Hz) oscillation synchronized in all leads (Achermann and Borbély 1997; Amzica and Steriade 1997).

According to these data, during SWS, the whole thalamo-cortical system seems to swing rapidly between substantially different configurations, ranging from functional disconnection to network activation. These fast, global changes must be taken into account to obtain a realistic profile of brain reactivity during sleep. However, to our knowledge, the issue of real-time thalamo-cortical responsiveness during natural SWS has never been addressed.

We wondered whether, and in what way, the electrical activity correlated to a sensory stimulation may vary as a function of the fluctuating state of the thalamo-cortical circuits during natural SWS. We chose to address this question directly in the sleeping human brain. In particular, our approach is based on the use of the low-frequency component of the
We demonstrate that sleep slow waves are associated with short-term nonstationarity of sensory processing in the thalamo-cortical networks of humans. Our results are discussed in the light of intracellular and behavioral correlates of SWS.

**METHODS**

All night sessions of stimulation and recordings were carried out on six healthy volunteers (2 females and 4 males). Informed consent was given, and the subjects were requested to undergo only mild sleep deprivation prior to the recording session. No pharmacological substance was used to induce sleep. Median nerve SEPs were evoked by electric stimulation at the right wrist using constant current square-waves pulses (0.1–2 ms), with intensity 10% above the thenar motor threshold. The inter-stimulus interval was set around 180 ms (approximately 6 Hz). Four monopolar leads (C3, C4, P3, and P4) with reference to the contralateral ear were used to monitor the spontaneous EEG, together with electro-oculograms and submental EMG. The afferent peripheral volley was detected at the Erb point, with the other sleep rhythms evolving in the upper range of the delta band. In humans, the slow oscillation prevails during sleep stages 3 and 4 and appears to arise from the rhythmic recurrence of K-complexes (Amzica and Steriade 1997, 1998b). In our protocol, to be considered as representative of the slow oscillation, a single oscillatory cycle had to have a period (negative-to-negative peak) between 0.8 and 1.2 s and had to be synchronized in all leads. We extracted the above criteria from the parameters describing the sporadic K-complexes that we recorded from the same subjects during earlier sleep stages. Indeed, the K-complex represents a clearly identifiable oscillatory pattern, with a regular and slow (approximately 1 Hz) oscillatory pattern. Our aim was to identify the slow oscillation with a minimal confound with the other slow rhythms evolving in the upper range of the delta band. In humans, the slow oscillation prevails during sleep stages 3 and 4 and appears to arise from the rhythmic recurrence of K-complexes (Amzica and Steriade 1997, 1998b). In our protocol, to be considered as representative of the slow oscillation, a single oscillatory cycle had to have a period (negative-to-negative peak) between 0.8 and 1.2 s and had to be synchronized in all leads. We extracted the above criteria from the parameters describing the sporadic K-complexes that we recorded from the same subjects during earlier sleep stages. Indeed, the K-complex represents a clearly identifiable forerunner of the slow oscillation (Amzica and Steriade 1997, 1998b). In Fig. 1A, the time-course of sporadic K-complexes is compared with...
the one of the oscillatory cycles selected for analysis during sleep stage 4 in the same subject. Once the spontaneous pattern was identified, time-stamps were manually inserted, on a cycle-by-cycle basis, in correspondence with the minimum voltage marking the transition from the negative to the positive slope of the selected oscillatory cycles. We chose this point (EEG-Ref) of the spontaneous EEG oscillation as a reference to align the evoked responses, because of its sharpness and because of its intra- and inter-subject reproducibility. Following this procedure, we detected 1,300–2,200 slow oscillatory cycles in each subject during a full night of recording.

Based on the identification of the reference points (EEG-Refs) on the spontaneous signal, we proceeded to select and group the single responses in relation to the phase of the oscillatory cycle. We called the spontaneous signal, we proceeded to select and group the single cycles in each subject during a full night of recording.

The signal-to-noise ratio characterizing Ph-AVGs was lower than that normally achieved by traditional sequential averaging, due to the selection of a relatively small number (615–1,230) of single trials. Even so, the typical components of the somatosensory evoked potential were clearly detectable and easily measurable. To demonstrate that the changes in amplitude characterizing the Ph-AVGs during the different phases of the oscillatory cycle were related to actual changes in the state of the networks rather than due to the superimposition of random residual variability, a statistical test was performed. In each subject, the peak-to-peak amplitudes of the main components of the response (N20, P60, and P100) were calculated on the single trials giving rise to each Ph-AVG. Next, as exemplified in Fig. 2 (where N20 is evaluated), a two-tailed, paired Student’s t-test between the amplitudes of the single responses collected around the negative phase of the EEG oscillation versus the ones falling around the positive phase was performed. A $P < 0.01$ was considered an index of phase-dependent significant changes in responsiveness.

To further evaluate the dependence of cortical responsiveness on the phase of the spontaneous slow EEG, the Ph-AVGs were compared with randomly generated averages (random-AVGs). The latter were produced by shuffling the total pool of single-responses (approximately 10,000), giving rise to the entire sequence of Ph-AVGs. Thus the random-AVGs had the same total mean of the Ph-AVGs but no phase-locking to the oscillatory cycle. To statistically evaluate this trend, a Student’s t-test between populations of single responses falling on opposite phases of the cycle (negative vs. positive EEG peak) was performed. Significant ($P < 0.01$) fluctuations in amplitude were detected for N20 (P15-N20) and
for P60 (N45-P60) in five subjects and for P100 (N70-P100) in three subjects.

To further characterize the share of response variability specifically related to the evolution of the oscillatory cycle, we shuffled the total pool of single-responses to produce random clusters of single trials from which random-AVGs were calculated. In all cases, the dispersion of Ph-AVGs, expressed as the SD from the total mean, was significantly larger than that computed for random-AVGs (Fig. 3B). We called this additional amount of dispersion “phase-dependent variability.” Interestingly, as shown in Fig. 3B, phase-dependent variability was selectively located at latencies corresponding to the typical components (N20, N45, P60, and P100) of cortical somatosensory evoked potentials.

In contrast with the results concerning cortical components, when we performed the same analysis on the afferent peripheral volley, we could not detect any statistically significant difference. Accordingly, in this case, the dispersion of Ph-AVGs around the total mean was small and comparable to that of the corresponding random-AVGs (data not shown).

Time-course of phase-dependent variability

The phase-dependent variability had a reproducible time-course across subjects. In all cases the amplitude of N20 underwent modifications that were temporally correlated with the dynamics of the EEG slow oscillation. The peak-to-peak amplitude of N20 started to increase after the onset of the EEG negative slope and rose progressively until it approached, and in some cases (n = 2) overshot, wakefulness levels around the onset of the positive EEG trend (Fig. 4C). Once past this point, N20 decreased, reaching 60–80% of its maximum around the plateau of the positive EEG wave (Figs. 2B, 4C, and 5C). The phase-dependent fluctuation of N20 was statistically consistent in five of six subjects. By contrast, a clear modulation of the latency of N20 during the oscillatory cycle was found in only one subject. In this case, the latency was increased by 0.7 ms around the negative-to-positive slope transient of the EEG, where the larger responses were found.

A similar dynamics characterized the amplitude-modulation of later positive components (P60 and P100). The amplitude of
P60 fluctuated ($P < 0.01$ in 5 subjects) between a maximum around the onset of the positive EEG deflection and a minimum around the plateau of the positive EEG wave (Figs. 3A, 4A, and 5, A and C). P100, a slower evoked component, was not well represented in all subjects because of the high-pass analog filtering conditioning the evoked signal. However, when clearly detectable, P100 underwent a powerful phase-dependent modulation; it gradually reached its maximal amplitude close to the negative-positive EEG transient and virtually faded before the beginning of the negative drift (Figs. 3A, 4A, and 5, A and C).

Overall, the global amplitude of the evoked potential oscillated significantly and coherently with the spontaneous EEG slow fluctuation, increasing and approaching wakefulness levels along the negative slope of the EEG and decaying below SWS levels during the positive slope. In particular, in correspondence with the positive phase of the EEG oscillation, very little stimulus-correlated activity was still detectable (at least, in the explored frequency band: 30–3,000 Hz) at latencies longer than 60 ms. As shown in Figs. 4B and 5B, in terms of amplitude, the response could vary more in the short temporal window (approximately 540 ms) in between Ph-AVG-3 and Ph-AVG0, than in the transition from SWS to wakefulness. Therefore the phase-dependent, short-term, response variability appeared to be of the same order, if not larger, when compared with the state-dependent one (see also Figs. 4C and 5C).

**DISCUSSION**

The major finding of our study is that thalamo-cortical sensory processing, during deep SWS, is not stationary in the very short period (approximately 500 ms), being significantly conditioned by the evolution of a spontaneous cortical oscillation (Figs. 2A, 3A, and 4A). Indeed, depending on its actual timing with respect to the EEG cycle, the amplitude of the evoked potential fluctuated significantly around the mean sleep value calculated by standard sequential averaging.

To extract phase-dependent variability, we adopted a model based on the hypothesis that the slow ($<1$ Hz) oscillation of the sleep EEG may reflect relevant changes in the functional (biophysical/computational) state of the thalamo-cortical networks. This prediction is based on a wealth of intracellular data systematically collected over the last 10 years, both in anesthetized and behaving cats (reviewed in Steriade 2001). The final output of our procedure is the Ph-AVG, a signal that contains 180 ms of stimulus-correlated electrical activity produced around a given phase of the spontaneous EEG oscillation.

During the evolution of the cycle, the response varied significantly depending on the phase of the oscillation as confirmed by the statistical evaluation performed on the different pools of single responses (Fig. 2). This phase-dependent variability was preferentially located at given latencies (around 20, 40, 60, and 100 ms) as indicated by the profile of dispersion of the Ph-AVGs around the total mean and was lost when random-AVGs were calculated (Fig. 3B). Moreover, the modulation of responsiveness associated with the sleep EEG oscillation appeared to be introduced at the central level, since the Ph-AVGs computed on the signal recorded at the brachial plexus overlapped precisely.

In all subjects, we observed a progressive increase of the evoked potential amplitude during the negative trend of the EEG slow fluctuation. The largest responses were observed around the transient from the negative to the positive slope of...
the EEG. Past this point, the amplitude of stimulus-correlated activity started to decrease and, around the end of the positive slope, showed a marked depression, particularly of later components (P60 and P100). The interpretation of these results in the light of the precise cellular and network events underlying the slow oscillation cannot be straightforward. Several studies, both using EEG recordings (Achermann and Borbely 1997; Amzica and Steriade 1997) and magnetoencephalography (Simon et al. 2000), have confirmed the presence of a slow oscillation in the human sleep EEG. Indeed, also in the sleeping human, the slow oscillation coherently reflects synchronous fluctuations of the membrane potential of cortical neurons. However, the exact phase relation between the scalp-recorded human sleep EEG and the underlying intracellular dynamics are not known and can only be extrapolated from the results of animal experiments. According to field potential recordings performed with high-impedance electrodes in the ketamine-xylo-amine anesthetized cat (Amzica and Steriade 1998), the long-lasting hyperpolarization of cortical neurons is associated with a surface-negative deflection, while the beginning of the depolarizing phase is marked by the onset of a surface-positive one. In the discussion that follows we will assume that a similar rule also applies to human EEG scalp recordings.

When calculated by means of standard sequential averaging, N20, a scalp-recorded reflection of the first evoked depolarizing event in the primary somatosensory cortex (Allison et al. 1991), increased in latency (0.5–1.3 ms) and slightly decreased in amplitude (by 5–25%) during SWS compared with wakefulness. This result is in accordance with previous studies about state-dependent changes of SEPs in humans (Addy et al. 1989; Emerson et al. 1988; Goff et al. 1966; Noguchi et al. 1995). However, a new and different picture emerged when N20 was computed with relation to the phase of the EEG cycle. Here the amplitude of N20 fluctuated, approaching wakefulness levels in correspondence with the onset of the positive EEG trend and decreasing below SWS mean values, around the positive plateau. This rapid fluctuation of the amplitude of a primary somatosensory response might be related to rhythmic spontaneous changes occurring both at the thalamic and the cortical level.

Indeed, a first possible role could be played by a fluctuation in the level of thalamic gating. This possibility is suggested by
intracellular data (Timofeev et al. 1996) recorded in the ventro-lateral thalamus of anesthetized cats in which the brachium conjunctivum was stimulated. This study clearly demonstrated the role played both by recurrent thalamic hyperpolarizations and the cyclical shunting of the membrane of relay neurons in periodically preventing prethalamic inputs from being transferred to the cortex during the slow oscillation. While we have detected a clear modulation of the response by the slow oscillation, we have never observed, at any point of the cycle, a complete failure of thalamo-cortical transmission. This discrepancy with the intracellular study could be explained by the fact that 1) our responses were collected during the natural slow oscillation, a pattern more variable and less marked than the one obtained during ketamine-xylazine anesthesia; and 2) we stimulated a peripheral nerve, thus recruiting different fibers with various time-constants, possibly allowing for temporal summation of excitatory postsynaptic potentials (EPSPs) up to firing threshold in yet hyperpolarized thalamic relay neurons.

On the other hand, the major source of phase-dependent variability could be at the cortical level. Indeed, important changes in postsynaptic cortical responsiveness are expected to occur during the oscillatory cycle; taking into account the time-course of the fluctuations involving both the input resistance of cortical neurons (Contreras at al. 1996) and the probability of synaptic release (Massimini and Amzica 2001), a progressive increase of the cortical postsynaptic response is expected to occur toward the transition between the down state and the up state, thus presumably, around the negative-to-positive EEG slope transition. We actually measured the largest responses around this point. In addition to the abovementioned mechanisms, a possible role of glial cells in the cyclical modulation of cortical excitability during SWS has been recently suggested (Amzica and Massimini 2002; Amzica et al. 2002) and cannot be ruled out.

Later components of the response, which reflect further cortical processing (Allison et al. 1989; Desmedt et al. 1983), were also strongly conditioned by the phase of the spontaneous EEG oscillation. P60 displayed a variation in amplitude of about 50% and rose and decayed in phase with the EEG negative-positive fluctuation. The next positive component of the evoked potential, P100, had a similar time-course but a more dramatic evolution, virtually disappearing in correspondence with the positive EEG plateau. As indicated by intracellular studies in the cerebral cortex of cats, during the evolution of the depolarizing phase, the probability of transmitter release...
progressively decreases (Massimini and Amzica 2001) and synaptic interactions among cortical neurons gradually run down, leaving leak currents to prevail (Contreras et al. 1996; Timofeev et al. 2000b). This spontaneous drift, bringing the cortical network toward a state of disfacilitation and functional disconnection may explain the marked obliteration of long-latency, stimulus-correlated potentials observed around the end of the positive EEG slope.

In summary, we tested the reactivity of the human thalamo-cortical system by means of simple, sensory stimulation during SWS. In line with previous results, during this state, the average response was delayed and the amplitude slightly reduced with respect to wakefulness. Surprisingly, when responsiveness was evaluated in relation to the actual microstates run through by the slowly oscillating thalamo-cortical networks, large-amplitude, wakefulness-like evoked potentials were found to rapidly alternate with low-amplitude ones. This fluctuation in thalamo-cortical reactivity may reflect the main feature of the slow sleep oscillation: the spontaneous and rhythmic recurrence of phases of global disfacilitation within periods of increased network excitability. Of course, the large amplitude responses, observed around the negative-to-positive slope change of the EEG, do not necessarily reflect a wakefulness-like elaboration of sensory information. Nevertheless, the presence of short temporal windows, during which the thalamo-cortical system seems to be more open to external stimuli, is consistent with the well-known notion that even the deeply sleeping brain can detect and process meaningful events (Langford et al. 1974; Oswald et al. 1960; Portas et al. 2000).

On the other hand, our results show that the slow oscillation, an emergent feature of the slow sleep oscillation: the spontaneous and rhythmic recurrence of phases of global disfacilitation within periods of increased network excitability. Of course, the large amplitude responses, observed around the negative-to-positive slope change of the EEG, do not necessarily reflect a wakefulness-like elaboration of sensory information. Nevertheless, the presence of short temporal windows, during which the thalamo-cortical system seems to be more open to external stimuli, is consistent with the well-known notion that even the deeply sleeping brain can detect and process meaningful events (Langford et al. 1974; Oswald et al. 1960; Portas et al. 2000).

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