



Cerebral correlates of delta waves during non-REM sleep revisited

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We aimed at characterizing the neural correlates of delta activity during Non Rapid Eye Movement (NREM) sleep in non-sleep-deprived normal young adults, based on the statistical analysis of a positron emission tomography (PET) sleep data set. One hundred fifteen PET scans were obtained using H₂¹⁵O under continuous polygraphic monitoring during stages 2–4 of NREM sleep. Correlations between regional cerebral blood flow (rCBF) and delta power (1.5–4 Hz) spectral density were analyzed using statistical parametric mapping (SPM2). Delta power values obtained at central scalp locations negatively correlated during NREM sleep with rCBF in the ventromedial prefrontal cortex, the basal forebrain, the striatum, the anterior insula, and the precuneus. These regions embrace the set of brain areas in which rCBF decreases during slow wave sleep (SWS) as compared to Rapid Eye Movement (REM) sleep and wakefulness (Maquet, P., Degueldre, C., Delfiore, G., Aerts, J., Peters, J.M., Luxen, A., Franck, G., 1997. Functional neuroanatomy of human slow wave sleep. *J. Neurosci.* 17, 2807–2812), supporting the notion that delta activity is a valuable prominent feature of NREM sleep. A strong association was observed between rCBF in the ventromedial prefrontal regions and delta power, in agreement with electrophysiological studies. In contrast to the results of a previous PET study investigating the brain correlates of delta activity (Hofle, N., Paus, T., Reutens, D., Fiset, P., Gotman, J., Evans, A.C., Jones, B.E., 1997. Regional cerebral blood flow changes as a function of delta and spindle activity during slow wave sleep in humans. *J. Neurosci.* 17, 4800–4808), in which waking scans were mixed with NREM sleep scans, no correlation was found with thalamus activity. This latter result stresses the importance of an extra-thalamic delta rhythm among the synchronous NREM sleep oscillations. Consequently, this rCBF distribution might preferentially reflect a particular modulation of the cellular processes

involved in the generation of cortical delta waves during NREM sleep.

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Introduction

Non Rapid Eye Movement (NREM) sleep is characterized by specific oscillations on electroencephalographic recordings (EEG): spindles, delta and slow rhythms. Spindles, a prominent feature of light NREM sleep (i.e., sleep stage 2), are defined in humans as waxing-and-waning oscillations within the 12–15 Hz (sigma band) frequency range, lasting at least 0.5 s (Rechtschaffen and Kales, 1968). During deep NREM sleep (sleep stages 3 and 4), the EEG is mainly characterized by a slower oscillation in the delta range (1.5–4 Hz). A slow rhythm (<1 Hz) occurs both during light and deep NREM sleep and manifests itself, respectively, as the regular recurrence of spindles every 3–10 s, K-complexes, or as slow waves below 1 Hz (Achermann and Borbély, 1997; Steriade and Amzica, 1998).

The mechanisms that generate spindles are well documented. Following a reduction in activating input from brainstem reticular formation, repetitive spike-bursts arise from GABAergic thalamic reticular neurons (RE), that generate inhibitory post-synaptic potentials (IPSPs) in glutamatergic thalamocortical (TC) neurons. The latter are entrained to oscillate within the sigma frequency range (Dijk et al., 1993; Steriade, 1999; De Gennaro and Ferrara, 2003). Although neurophysiological studies in animals suggest comparable mechanisms for the generation of delta waves, the process of delta wave generation is less clear; delta oscillations have been studied in vitro or in vivo under anesthesia and two types of delta activity have been identified. First, when TC neurons reach a sufficient level of hyperpolarization, a clock-like delta rhythm is generated in these neurons by the interplay between two

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hyperpolarization-activated currents (McCormick and Bal, 1997). Second, another delta oscillation is generated within the cortex because it can be recorded even after extensive thalamectomy (Frost et al., 1966; Ball et al., 1977; Steriade et al., 1993a; Steriade, 2003). The mechanisms of this cortical delta oscillation remain poorly understood.

In humans, the cerebral correlates of delta activity have been characterized previously in terms of changes in regional cerebral blood flow (rCBF), using positron emission tomography (PET) with oxygen-15-labeled water ($H_2^{15}O$) (Hofle et al., 1997). Delta activity recorded at the scalp was shown to correlate negatively with rCBF in the thalamus, brainstem reticular formation, cerebellum, anterior cingulate, and orbitofrontal cortex. According to the authors, these negative correlations reflected the active inhibition of thalamocortical relay neurons in association with delta waves, and the neural substrates underlying the progressive attenuation of sensory awareness, motor responsiveness, and arousal that occur during slow wave sleep (SWS). However, several peculiarities of the experimental design adopted by Hofle et al. may have biased their results. First and most importantly, the analysis looking for delta-related variations of regional brain activity included scans obtained during wakefulness. Since delta oscillations are much more abundant during NREM sleep than during wakefulness, this procedure is confounding the genuine effect of delta generation with a condition effect (wakefulness versus NREM sleep). Furthermore, subjects were partially sleep-deprived on the night preceding the PET acquisition, which might have modified the observed pattern of rCBF. It is known that sleep deprivation modifies Slow Wave Activity (SWA; 0.25–4 Hz) during ensuing sleep (Borbely et al., 1981; Knoblauch et al., 2002), and that extended wakefulness leads to rCBF decreases in frontal areas (Muzur et al., 2002). Finally, this study was based on a reduced data sample (32 scans), which considerably weakens the statistical power of the experiment.

In the present paper, our aim was to more specifically characterize the cerebral correlates of delta activity during human NREM sleep. To do so, we conducted a meta-analysis on a data set of 115 PET scans acquired during NREM sleep, in a non-sleep deprived population of normal subjects. In a preliminary analysis, we additionally included 46 scans acquired during wakefulness to provide a replication of the Hofle et al. study.

Methods

Subjects and experimental protocol

Data were obtained from previous sleep studies conducted in our center using the $H_2^{15}O$ technique (Maquet et al., 2000; Peigneux et al., 2003). These studies were approved by the Ethics Committee of the Faculty of Medicine of the University of Liège. All subjects were young, healthy, and right-handed male volunteers ($n = 23$; mean age = 22.9 years, range = 20.5–27, standard deviation = 3.5) who gave their written informed consent. Subjects were recruited after a detailed interview which assessed the regularity of their life habits, schedules, and quality of sleep for the 3-month period before the experiment. They were asked to keep a regular sleep–wake schedule and to fill in a sleep diary for the 2 weeks before the first night in the scanner. They were also asked to abstain from alcohol and restrict caffeine for the week before the experiment. Subjects were accustomed to the

sleeping conditions of the scanner during the two nights before the experimental night. Polysomnographic recordings were used to screen for sleep abnormalities, including excessive sleep fragmentation and insufficient total sleep time. Only subjects whose sleep duration and quality was sufficient, and who could maintain 20 min of continuous stage 2, stages 3–4 of NREM sleep and Rapid Eye Movement (REM) sleep on both acclimatization nights were selected for the third, experimental, night. During this night, PET scans were performed when the electrophysiological recording showed steady characteristic patterns following standard polysomnographic criteria (Rechtschaffen and Kales, 1968), for the sleep stage in which the PET scan was intended. Waking (W) scans were obtained at rest just before and after the sleep episode, with eyes closed in complete darkness. At least two W, two stage 2, and two stages 3–4 scans were obtained in each subject, with a total of 161 scans for all subjects (W = 46, stage 2 = 50, stages 3–4 = 65).

EEG acquisition

Polysomnography was recorded using a Synamp (Neuroscan, NeuroSoft, Sterling, Virginia) system, at 500 or 1000 Hz sampling rate, with a bandwidth 0.15 to 100 Hz. EEG on (at least) C3–A2 and C4–A1 channels, electro-oculogram, and chin electromyogram were recorded on bipolar montages. Polysomnographic recordings were scored according to standard international criteria (Rechtschaffen and Kales, 1968).

PET acquisition

PET scans were acquired on a Siemens CTI 951 R 16/31 scanner in three-dimensional mode, reconstructed using a Hanning filter (cutoff frequency 0.5 cycles/pixel) and corrected for attenuation and background activity. A transmission scan was performed before the first emission scan of the night to allow a measured attenuation correction. The subject's head was stabilized by a thermoplastic facemask secured to the head holder (Truscan Imaging, Annapolis, Maryland), and a venous catheter was inserted in a left antebraclial vein. When polysomnography showed steady characteristic patterns, cerebral blood flow was estimated, with a maximum of 12 scans per subject. Each scan consisted of two frames: a 30-s background frame and a 90-s active frame. Six millicuries (mCi) equivalent to 222 megabecquerels (MBq) were injected for each scan, in 5 cubic centimeters (cc) saline, over a period of 20 s, starting 10 s before the onset of the active frame. The infusion was totally automated in order not to awake the subject during the scanning period.

PET and EEG analysis

Raw polygraphic data were considered for the time intervals corresponding to the duration of each PET scan acquisition (90 s). Each time series was visually checked for ocular and muscular artifacts. Most 90-s EEG epochs were artifact-free; whole epochs and corresponding CBF data were eliminated in cases in which artifacts lasted more than 5 s. Delta power spectral density (1.5–4 Hz) was computed on C3–A2 and C4–A1 electrodes, using the Welch's averaged, modified periodogram method, with 4-s Hamming symmetric windows overlapping by 1 s (Werth et al., 1997). Averaged values of delta power over each active frame were used as covariates of interest in the analysis of rCBF modifications.

PET data were analyzed using statistical parametric mapping (SPM2; Wellcome Department of Cognitive Neurology, Institute of Neurology, London, UK) implemented in MATLAB (Mathworks, Sherborn, Massachusetts). For each subject, all scans were realigned together, then normalized to a standard PET template and smoothed (16 mm full width at half maximum). Two analyses were separately conducted. The first one reproduced the analysis published by Hofle et al. (1997). It included observations obtained during wakefulness, light and deep NREM sleep. The analysis looked for the brain areas in which rCBF correlated with delta power density values across these three different states. The second analysis aimed at specifying the cerebral correlates of delta rhythm specifically and exclusively during NREM sleep. It included only data obtained during light and deep NREM sleep and excluded waking scans. The analysis looked for the brain areas in which rCBF correlated with delta power density values across light and deep NREM sleep only. The resulting set of voxel values for each analysis constituted a map of the t statistic [SPM(t)], thresholded at $P \leq 0.001$ ($Z \geq 3.09$). Statistical inferences were then obtained at the voxel level corrected for multiple comparisons in the whole brain volume ($P^{\text{corr}} < 0.05$).

Results

The results presented in this section are based on correlations with values of delta power computed from C3–A2 derivation. Values from C4–A1 derivations are not reported here since they

were highly correlated to C3–A2 values (Spearman correlation $r > 0.95$, $P_s < 0.001$) and yielded nearly identical results.

In the first analysis, negative correlations of delta power across NREM sleep and wakefulness were found with rCBF in a set of brain areas including the medial frontal cortex, the orbitofrontal cortex, the basal forebrain and the anterior hypothalamus, the striatum (putamen), the thalamus, the anterior part of the insula, the anterior cingulate gyrus, the posterior cingulate gyrus, and the precuneus (Fig. 1, left panel). Positive correlations were found in parieto-occipital white matter, primary and secondary visual areas ($P_s^{\text{corr}} < 0.05$) (data not shown).

In the second analysis, after the withdrawal of waking scans and corresponding delta power values, negative correlations of delta power during NREM sleep only were found with rCBF in the medial frontal cortex, the orbitofrontal cortex, the anterior cingulate gyrus, the basal forebrain and the anterior hypothalamus, the striatum (putamen), the anterior part of the insula, and the precuneus (Table 1; Fig. 1 middle/right panel, and Fig. 2 left panel). A positive correlation was found in the white matter of the left parietal region ($P_s^{\text{corr}} < 0.05$) (data not shown). At variance with the results of the first analysis, no correlation was detected between NREM sleep delta power and rCBF in the thalamus, even at a very low statistical threshold ($P < 0.05$ uncorrected).

Discussion

NREM sleep rhythms entrain large neuronal populations in synchronous oscillations throughout the entire cerebrum. Accord-

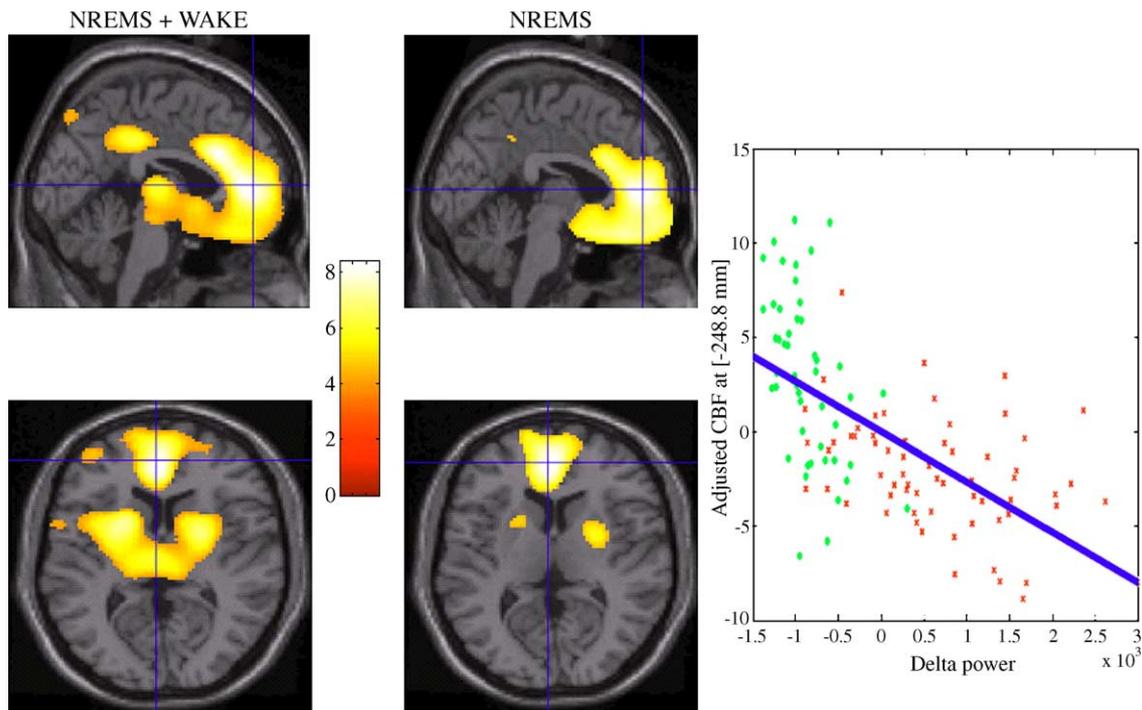


Fig. 1. (Left panel) rCBF decreases as a function of delta power during wakefulness and NREM sleep (stages 2–4). Images sections are centered on the ventromedial prefrontal cortex at the following coordinates: $x = -2$ mm, $y = 48$ mm, $z = 8$ mm ($P^{\text{corr}} < 0.05$) (Talairach and Tournoux, 1988). (Middle panel) rCBF decreases as a function of delta power during NREM sleep after exclusion of waking scans and corresponding delta values. Images sections are centered at the same coordinates ($P^{\text{corr}} < 0.05$). The color scale between left and middle sections indicates the range of Z values for the activated voxels in both panels. (Right panel) Plot of the adjusted rCBF responses (arbitrary units) in the ventromedial prefrontal cortex in relation to the adjusted delta power values (μV^2) during NREM sleep (corresponding to middle panel pictures): rCBF activity decreases when delta power increases. Each circle/cross represents one scan: green circles are stage 2 scans, red crosses are stages 3–4 scans. The blue line is the linear regression.

Table 1

Negative correlations between rCBF and scalp EEG delta activity during NREM sleep

	Side	x	y	z	Z
Medial frontal gyrus (BA 9/10)	Left	-2	48	8	7.36
Medial frontal gyrus (BA 9/10)	Right	2	48	8	7.26
Orbital gyrus (BA 11)	Right	6	36	-22	7.31
Orbital gyrus (BA 11)	Left	-2	60	-10	6.42
Striatum (putamen)	Right	32	0	-4	6.70
Striatum (putamen)	Left	-16	8	-6	5.89
Insula (anterior part)	Right	38	6	-8	6.41
Insula (anterior part)	Left	-34	10	-4	5.23
Anterior cingulate gyrus (BA 24)	Left	-2	26	24	6.35
Precuneus (BA 31)	Left	-4	-44	42	4.49
Basal forebrain (caudal orbital)	Right	14	18	-16	5.63
Basal forebrain (anterior hypothalamus)	Right	6	4	-10	5.10

x , y , and z coordinates (Talairach and Tournoux, 1988) correspond to the local maxima of significant negative correlation between rCBF and activity in the delta frequency band (1.5–4.0 Hz) during stages 2–4 of NREM sleep. x , distance (in millimeters) to right (+) or left (-) of the midsagittal line; y , distance anterior (+) or posterior (-) to the anterior commissure; z , distance above (+) or below (-) the intercommissural line. Z = statistical Z score. For cortical locations, Brodmann's cytoarchitectonic areas are given (BA). All reported values are significant at $P^{\text{corr}} < 0.05$ after correction for multiple comparisons in the whole brain volume. Positive correlations are not shown.

ingly, a global decrease in the cerebral blood flow is reported in (deep) NREM sleep (Braun et al., 1997; Kajimura et al., 1999). Here, our aim was not to confirm this global decrease in CBF with delta power density but to specify the brain areas where the regional blood flow is most decreased in relation with values of delta power, calculated from scalp EEG data at central electrodes.

First, we replicated the previously described analysis (Hofle et al., 1997), in which scans and delta power values acquired during stages 2–4 of NREM sleep but also during wakefulness were included. The results of this analysis yielding negative correlations between delta power values and rCBF in the thalamus, the

orbitofrontal cortex, and the anterior cingulate cortex (Fig. 1, left panel) are in agreement with that preceding work.

However, since delta oscillations are more profuse during NREM sleep than during wakefulness in normal human subjects and as this study was aimed at exploring the cerebral correlates of rhythms that characterize NREM sleep, the presence of waking values of delta power is likely to obscure the interpretation of the results. Therefore, in our second analysis, data obtained during wakefulness were discarded from the statistical analysis. As shown in Fig. 1, this analysis run on data exclusively recorded during NREM sleep yielded markedly different results, and notably failed to detect any significant correlation of delta activity with rCBF in the thalamus. The discrepancy suggests that wakefulness data might indeed have played an important confounding effect in the identification of the cerebral correlates of delta waves.

The present analysis shows, in non-sleep-deprived normal young adults, that rCBF in a set of brain areas is negatively correlated during NREM sleep with delta power values measured at the central scalp. These regions include cortical areas (medial frontal and orbitofrontal cortex, anterior cingulate gyrus, anterior part of insula, precuneus), the basal ganglia, and the basal forebrain (Fig. 2, left panel). This distribution is actually closely similar to the previously published map of brain areas in which rCBF significantly decreased during NREM sleep as compared to REM sleep and wakefulness, except for the absence of the thalamus (Maquet et al., 1997) (Fig. 2, right panel). These similarities underline the notion of delta power as a prominent feature of NREM sleep.

Thalamic versus cortical delta oscillations

As mentioned above, our analysis did not show any significant relationship between delta activity and rCBF in the thalamus in NREM sleep, even at low statistical thresholds. In the comparison between wakefulness and NREM sleep, thalamic deactivation is the most reproducible pattern observed by neuroimaging techniques in humans (Maquet, 2000): it was demonstrated in glucose

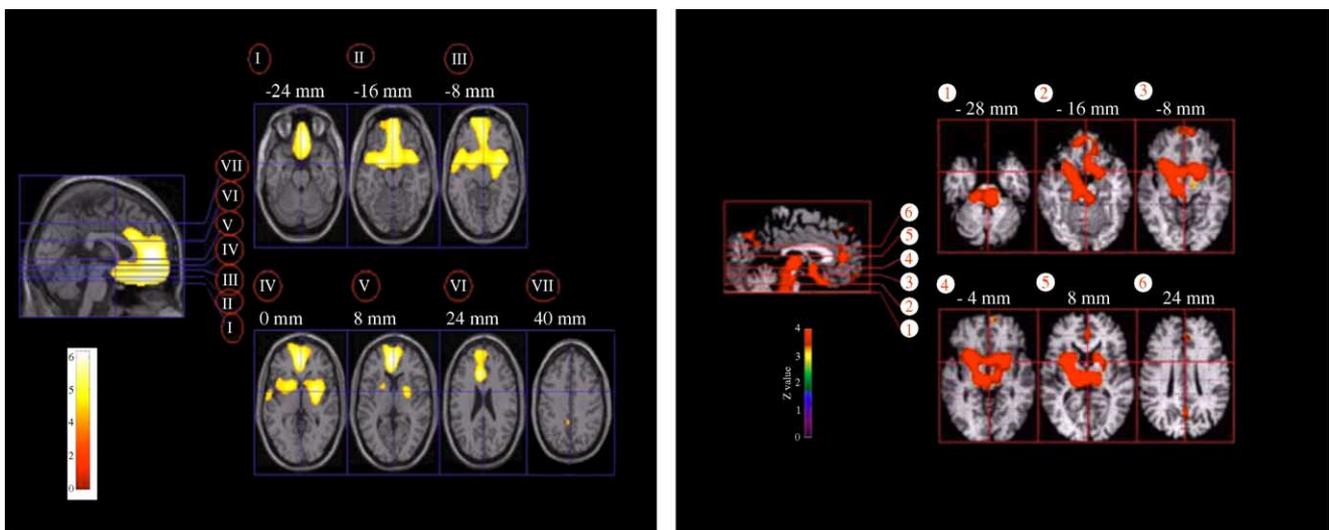


Fig. 2. (Left panel) rCBF decreases as a function of delta power during NREM sleep (stages 2–4). Image sections are displayed on different levels of the z axis as indicated on the top of each picture (Talairach and Tournoux, 1988). The color scale indicates the range of Z values for the activated voxels. Displayed voxels are significant at $P < 0.05$ after correction for multiple comparisons. (Right panel) Statistical map showing the brain areas in which rCBF decreases during NREM sleep as compared to wakefulness and REM sleep (Maquet et al., 1997). Note the striking similarity of the regional blood flow distribution between left and right panels. Copyright 1997 by the Society for Neuroscience.

metabolism studies (Buchsbau et al., 1989; Maquet et al., 1990) as well as in more recent $H_2^{15}O$ studies (Braun et al., 1997; Maquet et al., 1997; Andersson et al., 1998). Regional CBF decrease in the thalamus during NREM sleep is explained by the bursting mode of neuronal firing underlying spindle and delta oscillation generation, summarized in the Introduction (Steriade and Amzica, 1998; Steriade, 1999). Due to the temporal resolution of PET scans, the net effect of the hyperpolarization periods on rCBF exceeds that of the bursts resulting in a decrease in rCBF in thalamic nuclei (Maquet, 2000). However, our analysis is methodologically different from these previous PET studies. Here, the functional relationship between rCBF and delta power density was assessed exclusively in NREM sleep. The absence of significant correlation between the thalamic rCBF and delta power density suggests that the bursting firing pattern adopted by the thalamic neurons has a similar impact on local cerebral blood flow whatever the rhythm generated (spindle or delta), and irrespective of the degree of hyperpolarization or cellular processes involved in the generation of these sleep oscillations in TC neurons. In contrast, in some cortical areas, in the basal forebrain and in the basal ganglia, rCBF does correlate with delta power density. This result shows a different modulation of rCBF in these areas in response to delta power and suggests that this peculiar rCBF distribution is related to the regional modulation of delta oscillation within the cortex. Electrophysiological data in animals have shown the existence of a cortical delta rhythm, distinct from the clock-like delta waves, which survive extensive thalamectomy (Frost et al., 1966; Ball et al., 1977; Steriade et al., 1993a). More recent animal studies have shown that selective destruction of thalamic RE neurons leads to reduced delta power values after 1 day. However, there was no complete abolition in most animals and a recovery of delta activity was observed after 2 weeks (Marini et al., 2000). The specific characteristics, intracortical topography, and cellular mechanisms of this cortical delta rhythm are still unknown.

Local field potentials and multi-unit recordings in the cat cerebral cortex have shown a remarkable large-scale synchronization of delta waves during natural SWS (Destexhe et al., 1999). A computational model proposed a mechanism for such large-scale synchrony based on thalamocortical loops (Destexhe et al., 1998). Our results do not allow to discard the involvement of thalamus in this synchronization mechanism and therefore do not challenge this hypothesis. Nevertheless, animal data suggest that delta waves are synchronized by a slow oscillation (usually 0.6 to 1.0 Hz), generated intracortically as it survives extensive thalamic lesions (Steriade et al., 1993a), disappearing in the thalamus of decorticated animals (Timofeev and Steriade, 1996), and whose synchronization is disrupted by interruption of intracortical synaptic circuits (Amzica and Steriade, 1995). This oscillation would be able to group the other sleep rhythms, including spindles (Steriade, 1999). It is known that during the depolarizing phase of the slow oscillation, corticothalamic synaptic volleys succeed in synchronizing pools of thalamic cells by activating GABAergic thalamic RE neurons that project to thalamic relay cells and hyperpolarize them (Steriade et al., 1991). This process results in the coherence of the thalamic component of delta waves. On the other hand, the intracortical component of delta waves has not been systematically studied at the intracellular level. Although its hypothetical synchronization is less documented, it has been shown that typical delta waves, at a frequency of 2–4 Hz, generated by both regular-spiking and intrinsically bursting cortical neurons, are grouped within sequences recurring with the slow rhythm (Steriade et al.,

1993a). Likewise in humans, sleep EEG data have described the periodic recurrence of sequential mean amplitudes of delta waves with the rhythm of slow oscillation (Steriade et al., 1993b). Therefore, intracortical synchronization of cortical delta waves appears to be a plausible hypothesis. Our results speak for a regional modulation of these synchronization processes within the cortex, irrespective of their thalamocortical or genuinely cortical origin. Further studies are needed to clarify these issues.

Neocortical correlates of delta waves: a role for ventromedial prefrontal areas?

Our results show that delta activity is not homogeneously correlated with cortical rCBF but correlates predominantly with rCBF in the medial frontal, anterior cingulate, and orbitofrontal regions (Figs. 1 and 2). These areas were commonly referred to as ventromedial prefrontal cortex (VMPFC) (Damasio, 1994).

A frontal predominance of delta activity during NREM sleep has been outlined in several electrophysiological studies (Buchsbau et al., 1982; Zeitlhofer et al., 1993; Werth et al., 1997). This EEG frontal (Finelli et al., 2001) or medio-frontal (Happe et al., 2002) dominance might thus be linked to structures inside the VMPFC. However, at present, the implication of the VMPFC in the generation of delta waves remains speculative. Detailed investigations in animals should particularly focus on the generation of delta rhythm in cortical areas homologous to VMPFC.

One interpretation for the frontal predominance of rCBF changes in relation to EEG delta power is that frontal association areas display higher sleep intensities because of an intensive daytime use. Arguments for this theory came from the demonstration that the rebound in SWA after extended wakefulness yields a clear frontal predominance (Cajochen et al., 1999; Finelli et al., 2000; Knoblauch et al., 2002). These regional changes in EEG have been corroborated to different levels of activation by neuroimaging PET studies. Frontal association areas including VMPFC are among the most markedly deactivated brain regions during SWS, potentially reflecting the frontal dominance of delta activity, but also among the most activated areas during wakefulness (Braun et al., 1997; Andersson et al., 1998; Kajimura et al., 1999; Maquet, 2000).

Also, VMPFC is one of the most active brain areas during the awake resting state (Gusnard and Raichle, 2001). While the reasons for such a high waking activity are not clearly identified, it appears that VMPFC is involved in several important cognitive processes. For instance, VMPFC has been related to action monitoring (Luu et al., 2000), planning of tasks executed in expected sequences (Koechlin et al., 2000), explicit processing of sequential material (Destrebecqz et al., 2003), self-referential mental activity (Gusnard et al., 2001), guessing (Elliott et al., 1999), holding in mind goals while processing secondary goals (Koechlin et al., 1999), and decision making (Bechara et al., 1998). On the other hand, VMPFC is closely related to brain arousal systems because of its widespread connections with many structures of a distributed ascending activating system (Morecraft et al., 1992). Besides, increasing activity in VMPFC was found in situations of heightened arousal levels associated with some psychiatric conditions such as obsessive–compulsive disorder (Baxter et al., 1987), post-traumatic stress disorder (Rauch and Shin, 1997), and depression (Nofzinger et al., 2000). These data consistently support the interpretation that VMPFC is a site of

arousal regulation and important higher-order cognitive processes during wakefulness, which undergoes a deep deactivation during NREM sleep reflected by the frontal predominance of EEG delta activity.

Other negative correlations of rCBF with delta

The spatial resolution of PET does not allow to differentiate the anterior hypothalamus from the basal forebrain itself (Maquet, 2000). *Basal forebrain/anterior hypothalamus (BF/AH)* forms a functionally and structurally heterogeneous structure (Szymusiak, 1995) implicated in both arousal and sleep generation processes. GABAergic BF/AH neurons promote delta activity during SWS (Jones, 2004). They would represent a minority (16%) of BF/AH neurons. A majority (74%) of BF/AH neurons (including cholinergic neurons) are involved in cortical activation during wakefulness and REM sleep (Lee et al., 2004). Thus, the negative correlation of rCBF in BF/AH with delta power would be compatible with a lower activity of these arousal-promoting neurons during NREM sleep.

Arguments for a role of *striatum* in sleep regulation have emerged from pathologic conditions involving a dysfunction of basal ganglia. In particular, changes in the sleep–wake cycle, including an irregular delta activity and a decrease in SWS, have been reported in patients suffering from Huntington's disease (Sishta et al., 1974; Wiegand et al., 1991b), a pathology characterized by severe damage in the striatum (Sanberg and Coyle, 1984). Moreover, a direct association has been outlined between striatal atrophy and reduced SWS in Huntington's disease (Wiegand et al., 1991a). However, it is unclear whether these sleep disturbances are caused by an impaired function of striatum in sleep regulation or are simply non-specific consequences of the disease. Several elements support the former hypothesis. It is known that choreic movements largely cease during sleep in patients with Huntington's disease (Fish et al., 1991). Experimental models have shown that the sleep disturbances induced by striatal excitotoxic lesions in rats occurred on the 30th day post-lesion, at a time when motor abnormalities were no longer observed (Mena-Segovia et al., 2002). These data suggest the possible participation of the striatum in the regulation of the sleep–waking cycle, independently of locomotor activity. Two main alternative hypotheses may be put forward to explain the mechanisms by which the striatum would be implicated in sleep–wake regulation and could then be in agreement with the negative correlation of rCBF in this area with delta activity. The first one, which integrates the striatum into a cortical–basal ganglia–thalamic–cortical loop, relies on the observation that frontal cortex and thalamus are both among the most deactivated brain areas during SWS and major afferents to basal ganglia (Macchi et al., 1984; Selemon and Goldman-Rakic, 1985; Sadikot et al., 1990). Activity in these regions could entrain the basal ganglia neuronal population in highly synchronized oscillations (Maquet, 2000) with long phases of hyperpolarization alternating with bursts of discharges (Wilson, 1993). On the other hand, recent experiments suggest a striatal role in arousal processes by its connections with the pediculopontine tegmental nucleus (PPT), where afferents arising from the striatum would entrain PPT disinhibition and result in cortical activation (Mena-Segovia and Giordano, 2003). Decreasing activity in the striatum during NREM sleep, as seen in other PET studies (Braun et al., 1997; Maquet et al., 1997; Kajimura et al., 1999), could thus be also related to a lower propensity to arousal.

We found a negative correlation of delta activity with rCBF measured in the anterior part of the *insula*. However, there is no evidence at present for a clear relationship between this structure and sleep-dependent processes. The anterior insula has been found deactivated in a study assessing rCBF during SWS (Braun et al., 1997). Braun et al. suggested that the anterior insula, as belonging to the paralimbic structures, would serve as an interface between the external and internal milieus, allowing the limbic core structures (hippocampus, amygdala) to be functionally disconnected during SWS from the brain regions that directly mediate their interactions with the external environment. This disengagement of limbic structures, entrained by the paralimbic deactivation, would be a precondition for their homeostatic restoration (Braun et al., 1997).

Finally, the *precuneus* is known to be deactivated during SWS (Braun et al., 1997; Maquet et al., 1997; Andersson et al., 1998) but also, intriguingly, during mental states of decreased consciousness like pharmacological sedation (Fiset et al., 1999), hypnotic (Maquet et al., 1999) and vegetative states (Laureys et al., 1999). The precuneus is also a region particularly active during wakefulness, which would suggest that its activity reflects more brain operations taking place in conscious wakefulness than in sleep (Maquet, 2000). The negative correlation of delta power with rCBF in precuneus probably represents a decrease during NREM sleep of waking-dependent processes taking place in the precuneus, along with mechanisms potentially mediating levels of consciousness and deserving further investigations.

Conclusions

This analysis of a set of PET scans acquired in non-sleep-deprived subjects characterizes the cerebral correlates of delta waves during NREM sleep with rCBF in a set of brain areas. These regions include areas in which rCBF decreases during SWS compared to REM sleep and wakefulness (Maquet et al., 1997), underlining the notion of delta activity as a valuable feature of NREM sleep. A strong association was observed between rCBF in the ventromedial prefrontal regions and delta power, in agreement with electrophysiological studies. Noticeably, the absence of thalamic correlation stresses the importance of an extra-thalamic delta rhythm among the synchronous NREM sleep oscillations. Consequently, this rCBF distribution might preferentially reflect a particular modulation of the cellular processes involved in the generation of cortical delta waves during NREM sleep. A genuine localization of the brain sites of generation for these slow brain rhythms exceeds the scope of this meta-analysis. A combination of advanced neuroimaging and electrophysiological topographical techniques in future, dedicated, studies should enlighten this crucial issue.

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References

- Achermann, P., Borbely, A.A., 1997. Low-frequency (<1 Hz) oscillations in the human sleep electroencephalogram. *Neuroscience* 81, 213–222.
- Amzica, F., Steriade, M., 1995. Disconnection of intracortical synaptic linkages disrupts synchronization of a slow oscillation. *J. Neurosci.* 15, 4658–4677.
- Andersson, J.L., Onoe, H., Hetta, J., Lidstrom, K., Valind, S., Lilja, A., Sundin, A., Fasth, K.J., Westerberg, G., Broman, J.E., Watanabe, Y., Langstrom, B., 1998. Brain networks affected by synchronized sleep visualized by positron emission tomography. *J. Cereb. Blood Flow Metab.* 18, 701–715.
- Ball, G.J., Gloor, P., Schaul, N., 1977. The cortical electromicrophysiology of pathological delta waves in the electroencephalogram of cats. *Electroencephalogr. Clin. Neurophysiol.* 43, 346–361.
- Baxter Jr., L.R., Phelps, M.E., Mazziotta, J.C., Guze, B.H., Schwartz, J.M., Selin, C.E., 1987. Local cerebral glucose metabolic rates in obsessive–compulsive disorder. A comparison with rates in unipolar depression and in normal controls. *Arch. Gen. Psychiatry* 44, 211–218.
- Bechara, A., Damasio, H., Tranel, D., Anderson, S.W., 1998. Dissociation of working memory from decision making within the human prefrontal cortex. *J. Neurosci.* 18, 428–437.
- Borbely, A.A., Baumann, F., Brandeis, D., Strauch, I., Lehmann, D., 1981. Sleep deprivation: effect on sleep stages and EEG power density in man. *Electroencephalogr. Clin. Neurophysiol.* 51, 483–495.
- Braun, A.R., Balkin, T.J., Wesenten, N.J., Carson, R.E., Varga, M., Baldwin, P., Selbie, S., Belenky, G., Herscovitch, P., 1997. Regional cerebral blood flow throughout the sleep–wake cycle. An H2(15)O PET study. *Brain* 120 (Pt 7), 1173–1197.
- Buchsbaum, M.S., Mendelson, W.B., Duncan, W.C., Coppola, R., Kelsoe, J., Gillin, J.C., 1982. Topographic cortical mapping of EEG sleep stages during daytime naps in normal subjects. *Sleep* 5, 248–255.
- Buchsbaum, M.S., Gillin, J.C., Wu, J., Hazlett, E., Sicotte, N., Dupont, R.M., Bunney Jr., W.E., 1989. Regional cerebral glucose metabolic rate in human sleep assessed by positron emission tomography. *Life Sci.* 45, 1349–1356.
- Cajochen, C., Foy, R., Dijk, D.J., 1999. Frontal predominance of a relative increase in sleep delta and theta EEG activity after sleep loss in humans. *Sleep Res. Online* 2, 65–69.
- Damasio, A., 1994. *Descartes's Error. Emotion, Reason, and the Human Brain.* Grosset/Putnam, New York, NY.
- De Gennaro, L., Ferrara, M., 2003. Sleep spindles: an overview. *Sleep Med. Rev.* 7, 423–440.
- Destexhe, A., Contreras, D., Steriade, M., 1998. Mechanisms underlying the synchronizing action of corticothalamic feedback through inhibition of thalamic relay cells. *J. Neurophysiol.* 79, 999–1016.
- Destexhe, A., Contreras, D., Steriade, M., 1999. Spatiotemporal analysis of local field potentials and unit discharges in cat cerebral cortex during natural wake and sleep states. *J. Neurosci.* 19, 4595–4608.
- Destrebecqz, A., Peigneux, P., Laureys, S., Degueldre, C., Del Fiore, G., Aerts, J., Luxen, A., van der Linden, M., Cleeremans, A., Maquet, P., 2003. Cerebral correlates of explicit sequence learning. *Brain Res. Cogn. Brain Res.* 16, 391–398.
- Dijk, D.J., Hayes, B., Czeisler, C.A., 1993. Dynamics of electroencephalographic sleep spindles and slow wave activity in men: effect of sleep deprivation. *Brain Res.* 626, 190–199.
- Elliott, R., Rees, G., Dolan, R.J., 1999. Ventromedial prefrontal cortex mediates guessing. *Neuropsychologia* 37, 403–411.
- Finelli, L.A., Baumann, H., Borbely, A.A., Achermann, P., 2000. Dual electroencephalogram markers of human sleep homeostasis: correlation between theta activity in waking and slow-wave activity in sleep. *Neuroscience* 101, 523–529.
- Finelli, L.A., Borbely, A.A., Achermann, P., 2001. Functional topography of the human nonREM sleep electroencephalogram. *Eur. J. Neurosci.* 13, 2282–2290.
- Fiset, P., Paus, T., Daloz, T., Plourde, G., Meuret, P., Bonhomme, V., Hajj-Ali, N., Backman, S.B., Evans, A.C., 1999. Brain mechanisms of propofol-induced loss of consciousness in humans: a positron emission tomographic study. *J. Neurosci.* 19, 5506–5513.
- Fish, D.R., Sawyers, D., Allen, P.J., Blackie, J.D., Lees, A.J., Marsden, C.D., 1991. The effect of sleep on the dyskinetic movements of Parkinson's disease, Gilles de la Tourette syndrome, Huntington's disease, and torsion dystonia. *Arch. Neurol.* 48, 210–214.
- Frost Jr., J.D., Kellaway, P., Gol, A., 1966. Single-unit discharges in isolated cerebral cortex. *Exp. Neurol.* 14, 305–316.
- Gusnard, D.A., Raichle, M.E., 2001. Searching for a baseline: functional imaging and the resting human brain. *Nat. Rev. Neurosci.* 2, 685–694.
- Gusnard, D.A., Akbudak, E., Shulman, G.L., Raichle, M.E., 2001. Medial prefrontal cortex and self-referential mental activity: relation to a default mode of brain function. *Proc. Natl. Acad. Sci. U. S. A.* 98, 4259–4264.
- Happe, S., Anderer, P., Gruber, G., Klosch, G., Saletu, B., Zeitlhofer, J., 2002. Scalp topography of the spontaneous K-complex and of delta-waves in human sleep. *Brain Topogr.* 15, 43–49.
- Hofle, N., Paus, T., Reutens, D., Fiset, P., Gotman, J., Evans, A.C., Jones, B.E., 1997. Regional cerebral blood flow changes as a function of delta and spindle activity during slow wave sleep in humans. *J. Neurosci.* 17, 4800–4808.
- Jones, B.E., 2004. Activity, modulation and role of basal forebrain cholinergic neurons innervating the cerebral cortex. *Prog. Brain Res.* 145, 157–169.
- Kajimura, N., Uchiyama, M., Takayama, Y., Uchida, S., Uema, T., Kato, M., Sekimoto, M., Watanabe, T., Nakajima, T., Horikoshi, S., Ogawa, K., Nishikawa, M., Hiroki, M., Kudo, Y., Matsuda, H., Okawa, M., Takahashi, K., 1999. Activity of midbrain reticular formation and neocortex during the progression of human non-rapid eye movement sleep. *J. Neurosci.* 19, 10065–10073.
- Knoblauch, V., Krauchi, K., Renz, C., Wirz-Justice, A., Cajochen, C., 2002. Homeostatic control of slow-wave and spindle frequency activity during human sleep: effect of differential sleep pressure and brain topography. *Cereb. Cortex* 12, 1092–1100.
- Koechlin, E., Basso, G., Pietrini, P., Panzer, S., Grafman, J., 1999. The role of the anterior prefrontal cortex in human cognition. *Nature* 399, 148–151.
- Koechlin, E., Corrado, G., Pietrini, P., Grafman, J., 2000. Dissociating the role of the medial and lateral anterior prefrontal cortex in human planning. *Proc. Natl. Acad. Sci. U. S. A.* 97, 7651–7656.
- Laureys, S., Goldman, S., Phillips, C., Van Bogaert, P., Aerts, J., Luxen, A., Franck, G., Maquet, P., 1999. Impaired effective cortical connectivity in vegetative state: preliminary investigation using PET. *NeuroImage* 9, 377–382.
- Lee, M.G., Manns, I.D., Alonso, A., Jones, B.E., 2004. Sleep-wake related discharge properties of basal forebrain neurons recorded with micropipettes in head-fixed rats. *J. Neurophysiol.* 92 (2), 1182–1198.
- Luu, P., Fleisch, T., Tucker, D.M., 2000. Medial frontal cortex in action monitoring. *J. Neurosci.* 20, 464–469.
- Macchi, G., Bentivoglio, M., Molinari, M., Minciacci, D., 1984. The thalamo-caudate versus thalamo-cortical projections as studied in the cat with fluorescent retrograde double labeling. *Exp. Brain Res.* 54, 225–239.
- Maquet, P., 2000. Functional neuroimaging of normal human sleep by positron emission tomography. *J. Sleep Res.* 9, 207–231.
- Maquet, P., Dive, D., Salmon, E., Sadzot, B., Franco, G., Poirrier, R., von Frenckell, R., Franck, G., 1990. Cerebral glucose utilization during sleep–wake cycle in man determined by positron emission tomography and [18F]2-fluoro-2-deoxy-D-glucose method. *Brain Res.* 513, 136–143.
- Maquet, P., Degueldre, C., Delfiore, G., Aerts, J., Peters, J.M., Luxen, A., Franck, G., 1997. Functional neuroanatomy of human slow wave sleep. *J. Neurosci.* 17, 2807–2812.
- Maquet, P., Faymonville, M.E., Degueldre, C., Delfiore, G., Franck, G., Luxen, A., Lamy, M., 1999. Functional neuroanatomy of hypnotic state. *Biol. Psychiatry* 45, 327–333.
- Maquet, P., Laureys, S., Peigneux, P., Fuchs, S., Petiau, C., Phillips, C., Aerts, J., Del Fiore, G., Degueldre, C., Meulemans, T., Luxen, A.,

- Franck, G., Van Der Linden, M., Smith, C., Cleeremans, A., 2000. Experience-dependent changes in cerebral activation during human REM sleep. *Nat. Neurosci.* 3, 831–836.
- Marini, G., Ceccarelli, P., Mancina, M., 2000. Effects of bilateral microinjections of ibotenic acid in the thalamic reticular nucleus on delta oscillations and sleep in freely-moving rats. *J. Sleep Res.* 9, 359–366.
- McCormick, D.A., Bal, T., 1997. Sleep and arousal: thalamocortical mechanisms. *Annu. Rev. Neurosci.* 20, 185–215.
- Mena-Segovia, J., Giordano, M., 2003. Striatal dopaminergic stimulation produces c-Fos expression in the PPT and an increase in wakefulness. *Brain Res.* 986, 30–38.
- Mena-Segovia, J., Cintra, L., Prospero-Garcia, O., Giordano, M., 2002. Changes in sleep–waking cycle after striatal excitotoxic lesions. *Behav. Brain Res.* 136, 475–481.
- Morecraft, R.J., Geula, C., Mesulam, M.M., 1992. Cytoarchitecture and neural afferents of orbitofrontal cortex in the brain of the monkey. *J. Comp. Neurol.* 323, 341–358.
- Muzur, A., Pace-Schott, E.F., Hobson, J.A., 2002. The prefrontal cortex in sleep. *Trends Cogn. Sci.* 6, 475–481.
- Nofzinger, E.A., Price, J.C., Meltzer, C.C., Buysse, D.J., Villemagne, V.L., Miewald, J.M., Sembrat, R.C., Stepe, D.A., Kupfer, D.J., 2000. Towards a neurobiology of dysfunctional arousal in depression: the relationship between beta EEG power and regional cerebral glucose metabolism during NREM sleep. *Psychiatry Res.* 98, 71–91.
- Peigneux, P., Laureys, S., Fuchs, S., Destrebecqz, A., Collette, F., Delbeuck, X., Phillips, C., Aerts, J., Del Fiore, G., Degueldre, C., Luxen, A., Cleeremans, A., Maquet, P., 2003. Learned material content and acquisition level modulate cerebral reactivation during posttraining rapid-eye-movements sleep. *NeuroImage* 20, 125–134.
- Rauch, S.L., Shin, L.M., 1997. Functional neuroimaging studies in posttraumatic stress disorder. *Ann. N. Y. Acad. Sci.* 821, 83–98.
- Rechtschaffen, A., Kales, A., 1968. A Manual of Standardized Terminology, Techniques and Scoring System for Sleep Stages of Human Subjects. Brain Information Service/Brain Research Institute, University of California, Los Angeles.
- Sadikot, A.F., Parent, A., Francois, C., 1990. The centre median and parafascicular thalamic nuclei project respectively to the sensorimotor and associative-limbic striatal territories in the squirrel monkey. *Brain Res.* 510, 161–165.
- Sanberg, P.R., Coyle, J.T., 1984. Scientific approaches to Huntington's disease. *CRC Crit. Rev. Clin. Neurobiol.* 1, 1–44.
- Selemon, L.D., Goldman-Rakic, P.S., 1985. Longitudinal topography and interdigitation of corticostriatal projections in the rhesus monkey. *J. Neurosci.* 5, 776–794.
- Sishta, S.K., Troupe, A., Marszalek, K.S., Kremer, L.M., 1974. Huntington's chorea: an electroencephalographic and psychometric study. *Electroencephalogr. Clin. Neurophysiol.* 36, 387–393.
- Steriade, M., 1999. Coherent oscillations and short-term plasticity in corticothalamic networks. *Trends Neurosci.* 22, 337–345.
- Steriade, M., 2003. The corticothalamic system in sleep. *Front Biosci.* 8, d878–d899.
- Steriade, M., Amzica, F., 1998. Coalescence of sleep rhythms and their chronology in corticothalamic networks. *Sleep Res. Online* 1, 1–10.
- Steriade, M., Dossi, R.C., Nunez, A., 1991. Network modulation of a slow intrinsic oscillation of cat thalamocortical neurons implicated in sleep delta waves: cortically induced synchronization and brainstem cholinergic suppression. *J. Neurosci.* 11, 3200–3217.
- Steriade, M., Nunez, A., Amzica, F., 1993a. Intracellular analysis of relations between the slow (<1 Hz) neocortical oscillation and other sleep rhythms of the electroencephalogram. *J. Neurosci.* 13, 3266–3283.
- Steriade, M., Nunez, A., Amzica, F., 1993b. A novel slow (<1 Hz) oscillation of neocortical neurons in vivo: depolarizing and hyperpolarizing components. *J. Neurosci.* 13, 3252–3265.
- Szymusiak, R., 1995. Magnocellular nuclei of the basal forebrain: substrates of sleep and arousal regulation. *Sleep* 18, 478–500.
- Talairach, J., Tournoux, P., 1988. Co-Planar Stereotaxic Atlas of the Human Brain. George Thieme, Stuttgart.
- Timofeev, I., Steriade, M., 1996. Low-frequency rhythms in the thalamus of intact-cortex and decorticated cats. *J. Neurophysiol.* 76, 4152–4168.
- Werth, E., Achermann, P., Borbely, A.A., 1997. Fronto-occipital EEG power gradients in human sleep. *J. Sleep Res.* 6, 102–112.
- Wiegand, M., Moller, A.A., Schreiber, W., Lauer, C., Krieg, J.C., 1991a. Brain morphology and sleep EEG in patients with Huntington's disease. *Eur. Arch. Psychiatry Clin. Neurosci.* 240, 148–152.
- Wiegand, M., Moller, A.A., Lauer, C.J., Stolz, S., Schreiber, W., Dose, M., Krieg, J.C., 1991b. Nocturnal sleep in Huntington's disease. *J. Neurol.* 238, 203–208.
- Wilson, C.J., 1993. The generation of natural firing patterns in neostriatal neurons. *Prog. Brain Res.* 99, 277–297.
- Zeitlhofer, J., Anderer, P., Obergottberger, S., Schimicek, P., Lurger, S., Marschnigg, E., Saletu, B., Deecke, L., 1993. Topographic mapping of EEG during sleep. *Brain Topogr.* 6, 123–129.