Development of the nocturnal sleep electroencephalogram

in human infants

1,2 Oskar G Jenni, 1 Alexander A Borbély, and 1 Peter Achermann

1 Institute of Pharmacology and Toxicology, University of Zurich, 8057 Zurich, Switzerland;
2 University Children’s Hospital, 8032 Zurich, Switzerland

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Address for correspondence (not institution in which the work was done):

Oskar G Jenni, MD

E. P. Bradley Hospital Chronobiology and Sleep Research Laboratory

Department of Psychiatry and Human Behavior, Brown Medical School

Box G-EPB, Providence, RI, 02912

Email Oskar_Jenni@Brown.EDU

Phone (401) 421 94 40 and Fax (401) 453 35 78

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Abstract

The development of nocturnal sleep and the sleep EEG was investigated in a longitudinal study during infancy. All-night polysomnographic recordings were obtained at home at 2 weeks, and at 2, 4, 6 and 9 months after birth (analysis of 7 infants). Total sleep time and the percentage of quiet sleep or non rapid eye movement sleep (QS/NREMS) increased with age, while the percentage of active sleep or rapid eye movement sleep (AS/REMS) decreased. Spectral power of the sleep EEG was higher in QS/NREMS than in AS/REMS over a large part of the 0.75-25 Hz frequency range. In both QS/NREMS and AS/REMS, EEG power increased with age in the frequency range below 10 Hz and above 17 Hz. The largest rise occurred between 2 and 6 months. A salient feature of the QS/NREMS spectrum was the emergence of a peak in the sigma band (12-14 Hz) at 2 months, which corresponded to the appearance of sleep spindles. Between 2 and 9 months, low-frequency delta activity (0.75-1.75 Hz) showed an alternating pattern with a high level occurring in every other QS/NREMS episode. At 6 months, sigma activity showed a similar pattern. In contrast, theta activity (6.5-9 Hz) exhibited a monotonic decline over consecutive QS/NREMS episodes, a trend that at 9 months could be closely approximated by an exponential function. The results suggest that (a) EEG markers of sleep homeostasis appear in the first postnatal months, and (b) sleep homeostasis goes through a period of maturation. Theta activity and not delta activity seems to reflect the dissipation of sleep propensity during infancy.

Keywords: children, sleep homeostasis, EEG spectral analysis
Introduction

Sleep shows dramatic changes across early development. Quiet sleep or non rapid eye movement sleep (QS/NREMS) increases in the course of the first year after birth, while active sleep or rapid eye movement sleep (AS/REMS) decreases (38, 41, 50). Several groups demonstrated that slow wave sleep (SWS, stages 3 and 4 of NREMS) becomes predominant in the first part of the night from age of 2 to 5 months onwards (6, 7, 12, 21). On the basis of these observations, a number of authors postulated developmental, functional and regulatory processes of sleep during ontogeny (7, 41, 50, 51). Roffwarg and colleagues (50) were the first to propose that AS due to its striking abundance in early life may play an essential role in facilitating brain maturation. To understand the function of sleep during early development, the study of sleep regulatory features is a promising approach. In the 1990s, Salzarulo and Fagioli (51) suggested an early appearance of sleep regulatory mechanisms. They based their hypothesis on the temporal structure of infant sleep which is characterized by a higher level of SWS early at night than late at night, a pattern that may reflect the nocturnal dissipation of sleep propensity (7).

The majority of previous studies during ontogeny focused on the quality of sleep, on the percentage and temporal organization of sleep states, and on sleep cycles (6, 7, 12, 31, 35, 38, 41, 50). In studies of adult sleep, quantitative measures of the EEG such as spectral power have been increasingly used to investigate sleep regulatory processes (1, 9). It was shown that specific facets of the EEG can serve as a marker of a sleep process (9). Slow-wave activity (SWA, power in the 0.75-4.5 Hz range) or delta activity was used to delineate a homeostatic process (Process S) in the framework of the two-process model of sleep regulation (8, 13). SWA declines in the course of sleep and its level in the first NREMS episode is determined by the duration of prior wakefulness. Based on these dynamics, SWA was used to delineate the time course of Process S.

Prechtl (47) was among the first to apply spectral analysis of the sleep EEG in human infants. Over the past 30 years, a number of authors have computed power spectra in infants
and have contributed to the knowledge about quantitative characteristics of the sleep EEG during development (29, 30, 42, 52-55, 58). While some reports were based on short daytime recordings (30, 55), others focused on the newborn or preterm age period (29, 30, 42, 54). The development of sleep regulation in early ontogeny, however, was not a major focus of these studies. The aim of the present longitudinal study in human infants was to document state-related and frequency-dependent changes of the sleep EEG. In particular, those facets of the sleep EEG were studied which are markers of the homeostatic process in adults. To obtain a complete picture of sleep regulation, continuous 24-h recordings would have been required. For practical reasons, the experiments had to be limited to the nocturnal period.
Methods

Subjects. 11 healthy full-term infants (5 boys and 6 girls) were recruited from the Department of Gynecology and Obstetrics at the University Hospital of Zurich (Switzerland) and through private sources. Birth weight ranged from 2890 to 4230 g (mean ± S.E.M. was 3724 ± 138 g). The length at birth was between 48 and 56 cm (52.55 ± 0.69 cm) and head circumference between 34 and 37.5 cm (35.25 ± 0.44 cm). Gestational age ranged from 38 to 42 weeks (40.0 ± 0.38 weeks). All infants had a normal birth process with uncomplicated postnatal adaptation. The Apgar scores at 5 min were between 7 and 10 (9.0 ± 0.26). At birth physical examination was normal. All infants were fully breast-fed up to 2 (n=1) or 6 months (n=10) after birth. Written informed consent was obtained from all parents after detailed explanation of the method and aim of the study. The procedures were approved by the ethical committee of the University Children’s Hospital Zurich (Switzerland) and the study was performed according to the Declaration of Helsinki.

Polysomnographic recording procedures. All-night home polysomnography was carried out longitudinally at 2 weeks (17.9 ± 1.3 days), at 2 (69.1 ± 2.2 d), 4 (133.8 ± 2.2 d), 6 (187.9 ± 2.5 d) and 9 months (282.0 ± 2.9 d) after birth. The electroencephalogram (EEG), submental electromyogram (EMG), electro-oculogram (EOG, differential recording), electrocardiogram (ECG) and respiratory movements (using a thoracic respiration belt, Velcro Tab, Newlife Technologies, Midlothian, VA, USA) were recorded with a portable polygraphic amplifier system (PS1, Institute of Pharmacology and Toxicology, University of Zurich, Switzerland). The signals were digitized and transmitted via fiber-optic cable to a portable computer unit with a signal processor board. EEG, EOG and EMG signals were conditioned by two analogue filters: a high-pass filter (~3 dB at 0.16 Hz), and a low-pass filter (~3 dB at 70 Hz, < ~28 dB at 256 Hz). The data were sampled with a frequency of 512 Hz, digitally filtered (EEG
and EOG: low-pass filter at 30 Hz; EMG: band-pass filter between 20 and 50 Hz) and stored on hard disk with a resolution of 128 Hz.

The recordings were performed at home in the habitual sleep environment with usual bedtime routines and were unattended during the night. The parents were contacted by phone prior to each recording session to obtain information on the health status and sleep-wake rhythms during the preceding days. No infant received medication prior and during the recordings. All infants were free of any infectious or respiratory diseases at time of recording and had regular sleep-wake schedules. The experimenter (OGJ) attached the electrodes in the early evening between 18:00 and 21:00 hours preceding the usual bedtime. Occasionally, infants fell asleep during this procedure. EEG electrodes were placed along the antero-posterior axis over both hemispheres (bipolar derivations: F3C3, F4C4, C3P3, C4P4, P3O1, P4O2; referential derivation: C3A2) according to the International 10-20 System. Grass EC-2 electrode paste (Grass Instrument Division, West Warwick, RI, USA) was used for the application of EEG and EOG gold electrodes. Self-adhesive silver silver-chloride electrodes were used for EMG and ECG. The electrodes were secured with a tubular elastic net bandage.

*Recording quality and duration.* At age 2 weeks one recording had technical problems (# 02) and one infant could not be recorded (# 01). However, both were successfully recorded thereafter. In total, 53 nights were judged to be of sufficient quality to provide reliable sleep stage scoring. Total recording time (TRT) ranged from 265 to 777 min (mean 600.6 ± S.E.M. 15.0 min). Two nights were excluded from the analysis because recording duration was less than 5 hours (# 05 at 6 months; # 10 at 9 months). Sleep stage and sleep cycle variables of the infants with complete longitudinal data sets (n=7, Table 1) were calculated for the maximal common TRT of 418.7 min.
Sleep stage scoring. Following generally accepted procedures, scoring of sleep stages was performed according to Anders and co-workers (5) at age 2 weeks and 2 months, while subsequent recordings were scored according to Guilleminault and Soquet (28). The latter scoring rules take into account that spindles and slow waves are present in older infants, and therefore allow to differentiate between stage 1 (S1), stage 2 (S2) and slow wave sleep (SWS). Thus, the scoring criteria resemble those used in adults (48). Sleep was visually scored (referential derivation C3A2) for consecutive 20-s epochs as QS in younger infants (< 4 months) or NREMS in older infants (≥ 4 months) and as AS in younger infants (< 4 months) or REMS in older infants (≥ 4 months). Indeterminate sleep (IS) was scored only at 2 weeks and 2 months. In older infants NREMS was subdivided into S1, S2, and SWS. The criterion for the latter was a peak-to-peak value of 75 nV of slow waves. EEG power in the low delta (0.75-1.75 Hz) and sigma band (12-14 Hz) was displayed on the computer monitor together with the raw data to facilitate the scoring procedure.

Sleep cycle. A sleep cycle was defined as the succession of a QS/NREMS episode lasting at least 15 min and an AS/REMS episode of at least 5 min duration (adapted from 22). For the first AS/REMS episode no minimum criterion was applied. Sleep onset AS/REMS was not considered to be the beginning of a sleep cycle, although it was regularly seen in younger infants. Sleep episodes were terminated when > 10 min of indeterminate sleep or wakefulness occurred. IS occurring at the transition from QS to AS terminated the QS episode and was considered to be part of the subsequent AS episode; IS at the transition from AS to QS was included in the subsequent QS episodes. At least 5 QS/NREMS - AS/REMS cycles (n=7 infants) were completed with the exception of infants at age 2 weeks (who completed at least 3 cycles) and 6 months (only 6 infants completed 5 cycles).

Spectral analysis of the EEG. From seven infants complete longitudinal recordings were obtained at all ages (bipolar EEG derivation C4P4; subjects # 01, 02, 05, 07 were excluded
because of artifacts or detachment of electrodes). The C4P4 derivation was selected on the basis of the maximal amount of artifact free data that was available. Power spectra of consecutive 20-s epochs (FFT routine, Hanning window, averages of five 4-s epochs) were computed resulting in a frequency resolution of 0.25 Hz. The lowest two frequency bins (0.25 and 0.5 Hz) were not used for analysis due to their sensitivity to artifacts (in particular sweating artifacts during AS/REMS). Artifacts were excluded by visual inspection as well as semi-automatically based on low frequency and high frequency EEG power. Only 20-s epochs without artifacts were used for analysis. Spectral data were analyzed up to 25 Hz. Spectra of 20-s epochs were matched with the corresponding sleep stages. Average power spectra (Figure 1 and 2) were based on the first 272 minutes (maximal common sleep time, uncontaminated data devoid of ECG or sweating artifacts), and on the first four cycles (Figure 6). The time course of selected frequency bands (Figure 4 and 5) was based on the available cycles (statistics restricted to number of cycles available in all infants; see legends for details).

Statistics (see also Results). Statistical analysis was performed using the SAS statistical software package (SAS Institute, Inc., NC, USA). Age trends of visually scored sleep and cycle variables were assessed by non-parametric Friedman two-way analyses of variance and within age group effects by Wilcoxon signed rank tests. Absolute EEG power density measures were analyzed by one-way or two-way analyses of variance for repeated measures (rANOVA). Absolute power values were log transformed prior to statistical tests to approximate a normal distribution. Probability values are based on Greenhouse-Geisser corrected degrees of freedom, but the original degrees of freedom are reported. The significance level was set at $p<0.05$. Post-hoc comparisons between and within age groups were performed by paired t-tests if the corresponding main factor or the interaction of the rANOVA was significant.

The mean time course of power in a selected frequency range was calculated by subdividing each QS/NREMS episode into 7 equal intervals and each AS/REMS episode into 3 equal
intervals. For each interval, mean power was calculated for subjects and then averaged across subjects. Statistics were based on mean power per QS/NREMS and AS/REMS episode. A one-way rANOVA with factor ‘episode’ (QS/NREMS episode 1 – 5 or AS/REMS episode 1 – 5, except for age 2 weeks with episodes 1 – 3 only) served to analyze the dynamics in the course of the night. If the rANOVA revealed a significant effect of episode, consecutive episodes were compared by two-tailed paired t-tests. Only complete cycles were analyzed. Incomplete cycles or wakefulness between completed cycles gave rise to gaps in the average curves. Completed cycles were plotted with respect to the average time of occurrence. A gap in the plot was introduced whenever the interval between successive cycles was larger than 18 min (approximately three times the average subdivision of an episode).
Results

Sleep stage and cycle variables derived from visual scoring

Table 1 shows mean values of visually scored sleep variables for each age group. Total recording time varied considerably among subjects and age groups. Therefore, the maximum common length of total recording time (TRT) of 418 min was analyzed. Total sleep time (TST) and sleep efficiency (SE) increased from 2 weeks to 6 months of age, while movement time (MT) and waking after sleep onset (WASO) decreased. However, the decline of the latter did not reach statistical significance. AS/REMS expressed as percentage of TST showed a marked decline during maturation, whereas QS/NREMS increased between 2 weeks and 4 months and stabilized thereafter.

The mean duration of the sleep cycle (53-64 min) showed no age-related variation, while the proportion of its two constituent sleep episodes changed in an opposite direction: QS/NREMS episodes increased and AS/REMS episodes decreased.

EEG power spectra of QS/NREMS and AS/REMS

Spectral power of the sleep EEG (Figure 1, left panels) showed the typical decline with increasing frequency that is well documented for adult sleep (10). A striking developmental change in the QS/NREMS spectrum was the emergence of a peak in the frequency range of sleep spindles (12-14 Hz). The peak appeared at the age of 2 months and was present in each individual spectrum. Visual inspection of the EEG revealed that the spectral peak coincided with emergence of sleep spindles. Analysis of variance with repeated measures (rANOVA) showed significant effects of ‘state’, ‘age’ and their interaction over almost the entire frequency range (Figure 1, panels at bottom right). High F-values were present in the upper delta band for ‘age’, and in the low delta, theta, alpha, sigma, and low beta band for ‘state’.
To visualize sleep state related differences, the spectral values of QS/NREMS were plotted relative to those of AS/REMS (Figure 1, right panels; AS/REMS horizontal line at 1). In newborns power in QS exceeded power in AS in the range of 1 to 15.5 Hz. Also in older infants, power in the low delta band remained higher in QS/NREMS than in AS/REMS. However, some differences between the states were no longer significant in the high delta (2-9 months) and low theta band (2-4 months). Based on these findings, we decided to analyze power specifically in the frequency range of 0.75-1.75 Hz (low delta activity) rather than in the more extended range referred to as slow-wave activity (0.75-4.5 Hz). The largest state-related differences were present in the spindle frequency range, where power in QS/NREMS exceeded power in AS/REMS by a factor of 4-6.

The age-related changes in QS/NREMS and AS/REMS are visualized in Figure 2. Power over the entire frequency range is expressed for each age relative to power at 2 weeks (horizontal line at 1). Although the values increased with age almost over the entire frequency range in both sleep states, the largest rise in power was seen in the delta band. Close to maximal levels of delta power were reached at 6 months for NREMS and already at 4 months for REMS. The emergence of the sigma peak coincident with sleep spindles is evident in the QS/NREMS spectra. Also in the beta range, a conspicuous increase in power was seen between 4 and 9 months.

**Developmental trends in time course of low delta, theta and sigma activity**

Figure 3 illustrates the developmental changes in the dynamics of low delta (0.75-1.75 Hz), theta (6.5-9 Hz) and sigma activity (11.5-13.25 Hz) in an individual. Power in all three frequency bands showed a modulation by the QS/NREMS - AS/REMS cycle with low values in AS/REMS and high values in QS/NREMS. Whereas at 2 weeks and 2 months, low delta and theta activity showed a similar time course, a dissociation became apparent at the age of 4 months (not shown) and thereafter. At 6 and 9 months, theta activity exhibited a declining trend across consecutive sleep episodes. In contrast, low delta activity showed an alternating
pattern with high values in every second NREMS episode. At 2 weeks, sleep spindles were not yet present and accordingly sigma activity was at a very low level. From 2 months onwards, when sleep spindles were clearly recognized visually in QS/NREMS, sigma power exhibited high values in QS/NREMS episodes.

To examine the main features of the age related development and dynamics in specific frequency bands, average data were computed (Figure 4). Sleep cycles were standardized by subdividing QS/NREMS episodes into 7 equal time intervals and AS/REMS episodes into 3 intervals; then the data were averaged across subjects. Instead of showing the typical alternation of high and low delta activity, one subject (# 09) exhibited high power in NREMS episode 1, 4 and 8 at 6 months, and in episode 1, 4, 6 and 8 at 9 months. Waking episodes did not exceed 10 min. In view of this aberrant pattern, this subject was excluded from the analyses at 6 and 9 months shown in Figures 4 and 6 (n=6).

Dynamics of low delta activity (Figure 4, left column). At 2 weeks, a uniform picture with high values in QS and low values in AS was seen. From 2 months onwards, an alternating pattern of high-level and low-level delta activity emerged. There was no conspicuous global decline of low delta activity in the course of the night. At 6 months, the difference in power between consecutive NREMS episodes was largest. At 9 months, the alternating pattern was restricted to the first 4 cycles. The values in AS/REMS episodes were invariably at a low level and no trend was apparent.

At the beginning of a QS episode, low delta activity showed a steep rise at 2 weeks and 2 months. At later ages the intraepisodic build-up became more gradual. Low delta activity showed a steep decline before the onset of AS/REMS (in particular in episodes with high levels of low delta activity). The first data point in all panels of Figure 4 does not correspond to sleep onset since at this age AS/REMS occurred regularly at sleep onset. By definition, cycles started with QS/NREMS, and therefore sleep onset AS/REMS was not included in the first cycle (see Methods). As the occurrence of sleep onset AS/REMS and of interrupted
cycles at the beginning of the sleep episode decreased with age, the first data point was advanced.

*Dynamics of theta activity (Figure 4, middle column).* At 2 weeks, the time course of theta activity resembled that of low delta activity. At 2 months, theta activity was highest in the first QS episode and then was at a uniform lower level in the following QS episodes. At 4 months a gradual decline of theta activity across NREMS episodes emerged, a trend that was most pronounced at 9 months. In contrast to low delta activity, theta activity showed a decline across REMS episodes in the early part of the sleep episode.

The decline of theta activity across NREMS episodes at 9 months is plotted for individual standardized values in Figure 5. An exponential function (for equation see legend Figure 5) was fitted to the data. Its time constant ($t$) was 81.3 min (S.E.M. 13.2 min), the asymptote ($TA_s$) 69.2 % (4.6 %), and the initial value minus the asymptote ($TA_0$) 169.3 % (12.1 %). The asymptotic $r^2$ of 0.81 indicated a good fit.

*Dynamics of sigma activity (Figure 4, right column).* Sigma peak frequency changed with age and sleep cycle. The peak frequency within the sigma band was determined individually for each mean spectrum. The peak frequency increased from 12.6 Hz (S.E.M 0.05) at 2 months to 13.1 Hz (0.14) at 9 months (rANOVA factor ‘age’ p<0.05). Individual 1.25-Hz bands in the sigma range were defined around the individual peak frequency (mid bin; range of 1.25-Hz sigma bands from 11.5 to 13.5 Hz).

From age 2 months onwards, sigma activity was observed in all QS/NREMS episodes. At 6 months when the alternating pattern of low delta activity was most prominent, sigma power showed a parallel, though less pronounced alternating pattern (Figure 4, right column, third panel). A prominent feature of the intraepisodic time course was the rapid increase at QS/NREMS onset and the sharp decline before AS/REMS onset. The intraepisodic U-shape
of sigma activity reported in adults (2, 61) was absent and there was no increase in the course of the night.

**Evolution of EEG spectra in the first 4 episodes**

Figure 6 depicts the evolution of power in the first four episodes of QS/NREMS and AS/REMS. Power in episodes 2 to 4 was expressed relative to the first episode (horizontal line at 1.0).

At age 2 weeks, neither QS nor AS showed significant changes across episodes. Consistent significant variations appeared in QS at 2 months, and in REMS at 4 months.

The pattern in QS/NREMS (age 2 to 9 months) was characterized by a progressive decline of power in the theta and alpha range. This was not the case in the low delta band where the alternating pattern was reflected by lower values in episode 2 than in episode 3. With advancing age, the frequency delimiting the alternating pattern (i.e. the intersection of the curves for episode 2 and 3 indicated by an arrow) shifted from 2.7 Hz at 2 months to 5 Hz at 9 months. At age 6 and 9 months, power in the sigma range exhibited an alternating pattern with lower values in episode 2 than in episode 3. However, statistically significant variations in the sigma range occurred only at 6 months.

In AS/REMS, the significant changes were restricted to a narrower frequency range than in QS/NREMS and encompassed consistently the theta, alpha and beta range. In this frequency range a decrease of power occurred from the first to the second AS/REMS episode, while beyond the second AS/REMS episode the decreasing trend was less evident. The difference between the first and the following REMS episodes was largest at 9 months.
Discussion

In this longitudinal study, the sleep of infants was recorded in the home environment at different stages of development within the first postnatal year. This allowed to specify maturational changes of the sleep EEG and its dynamics across the sleep episode.

Sleep architecture. A conspicuous change in sleep architecture from the age of 2 weeks to 9 months was the decreasing percentage of AS/REMS and the complementary rise in the percentage of QS/NREMS. The altered proportion of the two sleep states came about by the shortening of AS/REMS episodes and the lengthening of QS/NREMS episodes, while the sleep cycle remained stable at approximately 60 min. Similar age-related changes in sleep states have been reported previously (12, 19, 31, 38, 50). With the exception of a single study (23), a stable sleep cycle duration during infancy was observed in earlier studies (19, 21, 38, 59). However, it is still unknown at which age the adult cycle length of 90-110 min is attained. Whereas the cycle duration of 75 min (38) at the age of 2 years was reported to increase to approximately 90 min at 6 years (11), data in the intervening years are not available. On the basis of the presence of SWS in alternate QS/NREMS episodes, Bes and colleagues (7) proposed that the recurrence time of SWS in infants is similar to that in adults indicating that the ultradian component of SWS in infants represents already the adult sleep cycle.

Sleep EEG. A central feature of the present study was the use of quantitative EEG analysis, which complemented the conventional scoring of sleep states. The prominent rise in spectral power within the first 6 months is in agreement with previous findings (58). The largest increase in power was present in the low-frequency range of the spectrum (Figure 2). Since the changes were similar in QS/NREMS and AS/REMS, they may represent a sleep state-independent aspect of EEG development. The EEG reflects changes in post-synaptic potentials of large and distributed neuronal populations (37). The degree of synchronization of these post-synaptic potentials is reflected in the amplitude of the scalp recorded EEG
signal (37). Thus, the marked state-independent increase of EEG power during early infancy may reflect an increase in synaptic connectivity of neuronal assemblies (32), developmental changes in neurotransmitter or neuroreceptor properties (36) and the increasing myelination of the brain’s white matter (44) which are all known to occur during the first year of life.

The EEG spectra of QS/NREMS and AS/REMS differed already at the age of 2 weeks (Figure 1). Between 1 and 16 Hz, power in QS was higher than in AS. State-related differences within the delta band were limited at the age of 2 months and more to the lowest frequencies. Within the middle or higher frequencies of the delta band, power in QS/NREMS and AS/REMS did not differ significantly (Figure 1). This pattern is not present in the adult sleep EEG (10) and may indicate functional differences of distinct parts of the delta band during development. Therefore, instead of relying on the traditional slow-wave activity (0.75-4.5 Hz), the frequency range of 0.75-1.75 Hz was used as a measure of low-frequency EEG activity.

The prominent peak between 12 and 14 Hz emerging in the QS spectrum at 2 months coincided with the appearance of sleep spindles. A close association of sleep spindles and the sigma peak in the power spectrum has been demonstrated for adult sleep (17). Also in previous studies, spindles were not present in newborns and appeared before the age of 2 months (20, 34). The emergence of spindles may be part of the functional transition period from the fetal to the infant state, which occurs during the first weeks of life (46). Spindles constitute a distinct form of synchronous oscillations within the thalamocortical system, depending on intrinsic properties of thalamic reticular and thalamocortical neurons and their synaptic interactions (14, 56, 57). In the postnatal period of experimental animals, thalamocortical neurons are known to undergo profound changes in their morphological and physiological properties (40, 45, 62). Thus, features of the sleep EEG may be useful markers for the development and integrity of the central nervous system in early life (43, 49).

**EEG dynamics and sleep homeostasis.** The present study focused on the dynamics of the infant sleep EEG during the night. For a thorough analysis of sleep homeostasis, it would
have been desirable to record sleep throughout a 24-h period. This was not feasible. Because daytime sleep was found to decrease during infancy from 4.6 hours at age 3 months to 2.8 hours at 9 months (33), sleep propensity in the first part of nocturnal sleep may have changed. It is unlikely, however, that the salient age-related changes in the dynamics of the sleep EEG (such as the alternation of the low delta activity and the monotonic decline of theta activity) can be attributed to developmental changes in daytime sleep.

According to the two-process model of sleep regulation, the homeostatic Process S interacts with a circadian process (Process C) (8, 13). It is important to note that it is difficult to disentangle circadian processes from homeostatic processes without using special experimental protocols (e.g., EEG forced desynchrony paradigms), which are not feasible in human infants.

The typical declining trend of SWS or delta activity in the course of the night was reported for infants at the age of 2 to 5 months (6, 12, 52, 53, 58). If this decline reflects, as in adult sleep, the dissipation of sleep propensity, it would indicate that mechanisms underlying sleep homeostasis emerge early in human life (51). The presence of SWS in alternate QS/NREMS episodes as first described by Bes and colleagues (7), however, is difficult to reconcile with this interpretation. This difficulty is underscored by the present findings that low-frequency delta activity exhibits the alternating pattern at an age as early as 2 months. This striking feature of infant sleep reached its highest prominence at 6 months when the difference between high-level and low-level delta in consecutive NREMS episodes was largest. At this age there was no evidence for a decline of low delta activity in the course of the night. This finding is in agreement with findings from animal experiments (4). Whereas in young rats at age 23 days a declining trend of SWA was not present across the light period (the animals’ predominant sleep period), it was seen within single sleep episodes (4). These ultradian periods were postulated to be miniature versions of the sleep-wake period and indicated that the dynamics of sleep homeostasis may occur on shorter time scale. A tentative interpretation of the present data might be that the alternating pattern of low delta activity may represent a comparable phenomenon arising from a faster dynamics of the homeostatic...
process. Low delta activity and therefore sleep pressure would dissipate within two sleep cycles, and build up again during periods of intermediate wakefulness. However, the present data provide little support for this interpretation because waking periods were not systematically observed after episodes with low levels of delta activity.

In contrast to low-frequency delta activity, theta activity declined in the course of the sleep episode from age 2 months onwards (Figure 4, second column). A gradual decline during the sleep episode prevailed at 9 months and its time course could be approximated by an exponential function. This is an important observation in view of the exponential time course of delta activity during adult sleep, which is a core feature of the two-process model of sleep regulation (8, 10, 13). In adults, the declining trend of EEG power and the increase in its initial level following sleep deprivation are not restricted to the delta band, but extend to the theta and alpha band (2, 10, 18, 24). Moreover, forced desynchrony studies revealed that sleep EEG activity in these bands is essentially independent of the circadian phase (16, 18).

In contrast, theta activity during waking showed homeostatic and circadian components (3, 24). The present findings indicate that theta activity during QS/NREMS may be a marker of sleep homeostasis during development, whereas the generators of delta activity are not yet coupled to the mechanisms involved in sleep homeostasis. The time constant of the exponential decay of theta power at 9 months is inferior to that of delta activity in adults (81.3 min vs. 144.6 min (15)). Based on findings in rat pups (4), it is not unreasonable to assume that the build-up and dissipation of sleep propensity occurs faster during early development. In fact, animal models may give some insights into the ontogeny of sleep regulation (4, 25, 27). It was shown on the basis of sleep deprivation experiments that in rat pups homeostatic sleep regulation is evident as early as the first postnatal weeks (4, 27). While neonatal rats compensated sleep deprivation with increased time spent in QS, the waking-dependent response of delta activity occurred only later in development (27). Whether similar developmental features of homeostatic sleep regulation are present also in human infants remains unknown.
In adults, EEG sigma activity, a correlate of sleep spindles, shows in many respects an inverse relationship to delta activity. It increases over consecutive NREMS episodes and exhibits an U-shaped time course within episodes (2, 17, 61). These adult features of sigma activity were not observed during early development. At 6 months, both low-frequency delta and sigma activity occurred in alternate episodes (Figure 4). The coupling of delta and sigma activity is in accordance with electrophysiological data (56, 57) that demonstrate a close relationship between slow waves and sleep spindles. Visual inspection of the data confirmed a particular morphology of sleep spindles (spiky negative and rounded positive component) and their increasing length in the first 9 months after birth (34, 38, 60).

Taken together, both delta activity and sleep spindles exhibit specific features during development, which may indicate a different functional role in infant and adult sleep. It is conceivable that slow waves and sleep spindles in infants promote the formation of thalamocortical networks by providing endogenous neural signals with repetitive and synchronized activity. Hitherto, AS/REMS was postulated to facilitate brain maturation by internal stimulation when sensory input is still minimal (39, 50). However, there is recent evidence that QS/NREMS in cats may consolidate changes in cortical plasticity during critical periods of brain maturation (26). Thus, both basic substates of sleep, QS/NREMS and AS/REMS, may contribute to brain development at a time period in life when the need for sleep is largest (33).

We conclude that the nocturnal pattern of specific EEG markers such as low delta, theta and sigma activity may reflect their different functional roles in the sleep process during development. Research protocols in which sleep and wakefulness are manipulated are needed to further investigate the development of sleep regulatory processes and to gain insights into the functional role of specific frequency components in the sleep EEG during ontogeny.
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Legends

Table 1.

Sleep stage and cycle variables in human infants across the first 9 months of life. Data derived from visual scoring (based on referential derivation C3A2). The first 418.7 min of total recording time (TRT) were analyzed (n=7, for details see Methods). Mean values (S.E.M. in brackets) are reported. Non-parametric Friedman two-way analyses of variance for age trends and Wilcoxon signed rank tests for between group differences were performed. △ or ▽ indicate significant increase or decrease between successive episodes (p < 0.05), while (▽) or (△) indicate a trend (p < 0.1; ns, non significant). TST: total sleep time; SEF: sleep efficiency (TST as % of TRT); WASO: waking after sleep onset; IS: indeterminate sleep; QS/NREMS: quiet sleep or non rapid eye movement sleep; AS/REMS: active sleep or rapid eye movement sleep; MT: movement time.
Figure 1.

EEG power spectra (bipolar derivation C4P4) of the first 272 min of sleep (maximum common duration of artifact-free sleep). *Left:* absolute spectra in QS/NREMS (thick lines) and AS/REMS (thin lines); *right:* relative spectra (each frequency bin in QS/NREMS expressed relative to the corresponding bin in AS/REMS). Bars at the bottom of each panel indicate frequency bins in which absolute power density in QS/NREMS and AS/REMS differed significantly (p<0.05; two-tailed paired t-test, performed for frequency bins for which a two-way rANOVA factor ‘state’ or the interaction ‘age x state’ was significant; bottom panels at right; only significant F-values (p<0.05) are depicted).

Figure 2.

Evolution of EEG power density spectra as a function of age in AS/REMS and QS/NREMS. Spectra at 2, 4, 6, and 9 months after birth are expressed relative to spectra at 2 weeks (newborn). Black vertical bars above the abscissae represent significant F-values of factor ‘age’ (p<0.05; one-way rANOVA on log-transformed absolute values).

Figure 3.

Hypnogram and time course of low delta (EEG power in the 0.75 – 1.75 Hz range), theta (6.5 – 9 Hz) and sigma activity (11.5 – 13.25 Hz, sigma peak frequency individually determined at each age and 1.25-Hz bands defined with peak frequency as mid bin) of an individual infant (# 03) across the first 9 months of age. Data at the age of 4 months are not presented, but the time course of the variables was similar to those at 6 months of age. EEG power and sleep stages are plotted for 20-s epochs. Bipolar derivation C4P4. M: movement time, W: wakefulness, QS/NREMS: quiet sleep or non rapid eye movement sleep; AS/REMS: active sleep or rapid eye movement sleep, IS: indeterminate sleep, S1: stage 1, S2: stage 2, SWS: slow wave sleep. Gaps in EEG power represent either wakefulness (scored as W) or
disconnection of the headbox (gap in hypnogram). The headbox was disconnected for feeding or soothing the infant. Wakefulness can be assumed for most of this time.

Figure 4.

Average time course of low delta activity (left column of panels, EEG power in the 0.75 – 1.75 Hz range), theta activity (middle column of panels, 6.5 – 9 Hz) and sigma activity (right column of panels) in each age group (2 weeks, 2 and 4 months, n=7; 6 and 9 months, n=6). Since the peak frequency of the sigma range in mean spectra changed as a function of age and varied considerably among subjects and cycles, individual 1.25-Hz bands were defined with the peak frequency as the mid bin (range 11.5 – 13.5 Hz). QS/NREMS episodes were subdivided into 7 and AS/REMS episodes into 3 equal time intervals and averaged across subjects. Vertical lines represent ±1 S.E.M. Black bars and dotted vertical lines delimit AS/REMS episodes. Filled triangles above the abscissae represent QS/NREMS and AS/REMS episodes for which the mean power of the episode differed significantly from the following episode (p<0.05, two-tailed paired t-test). The orientation of triangles specifies the direction of difference between indicated and consecutive episode. Open triangles represent a trend (p<0.1). t-tests were only performed when a one-way rANOVA with factor ‘episode’ revealed a significant effect of episode (p<0.05, indicated in each panel at the top right) over the first 5 episodes (first 3 episodes at 2 weeks). Only completed sleep cycles were included. 2 weeks: cycles 1-3: n=7, cycle 4: n=6; 2 months: cycles 1-6: n=7; 4 months: cycles 1-6: n=7, cycle 7: n=6, 6 months: cycles 1-7: n=6; 9 months: cycles 1-5: n=6, cycles 6-7: n=5. The first data point does not correspond to sleep onset, since in younger age AS/REMS was regularly seen at sleep onset, but was not included in the first cycle (see Methods).
Figure 5.

Time course of theta activity (TA) across the sleep episode at age 9 months. TA was standardized as % of the mean TA in NREMS during the first 272 min of sleep. Mean TA per NREMS episode is plotted at episode midpoints (relative to sleep onset) and pooled for n=7 infants. All NREMS episodes were included. The solid line represent an exponential function that was fitted to the data: $TA(t) = TA_0 \cdot e^{\frac{-t}{W}} + TA_\infty$. $W$: time constant; $TA_\infty$: asymptote; $TA_0$: initial value minus asymptote.

Figure 6.

EEG power density spectra of the first 4 QS/NREMS episodes (left panels) and AS/REMS episodes (right panels). Each curve connects average power density values (2 weeks, 2 and 4 months, n=7; 6 and 9 months, n=6) expressed relative to the corresponding value in the first episode (second episode thick line, third episode medium line, fourth episode thin line) smoothed with a moving median over four 0.25-Hz bins. Arrows represent the upper frequency limit with higher EEG power in episode 3 than in episode 2 (intersection of the curves for episode 2 and 3). Black vertical bars above the abscissae represent significant F-values (p<0.05) of a one-way rANOVA, factor ‘episode’ (1-4). Triangles below the abscissae indicate 0.25-Hz bins which differed significantly from the first episode (p<0.05, two-tailed paired t-test, performed when the one-way rANOVA revealed a significant effect of factor ‘episode’).
Table 1. Sleep stage and cycle variables in human infants across the first 9 months of life.

<table>
<thead>
<tr>
<th></th>
<th>2 weeks</th>
<th>2 months</th>
<th>4 months</th>
<th>6 months</th>
<th>9 months</th>
<th>F age (p) (^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TRT (min)</td>
<td>627.9 (35.1)</td>
<td>609.6 (23.5)</td>
<td>623.9 (20.3)</td>
<td>638.7 (25.9)</td>
<td>632.6 (43.1)</td>
<td>ns</td>
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<tr>
<td>TST of first 418 min TRT</td>
<td>312.6 (9.0)</td>
<td>333.4 (15.2)</td>
<td>365.2 (11.2)</td>
<td>377.1 (9.7)</td>
<td>377.6 (6.3)</td>
<td>3.4 (p &lt; 0.01)</td>
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<tr>
<td>SEF (%)</td>
<td>74.7 (2.2)</td>
<td>79.6 (3.6)</td>
<td>87.2 (2.7)</td>
<td>90.1 (2.3)</td>
<td>90.2 (1.5)</td>
<td>3.4 (p &lt; 0.01)</td>
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<tr>
<td>WASO (%)</td>
<td>21.2 (2.7)</td>
<td>16.2 (4.1)</td>
<td>10.4 (2.9)</td>
<td>8.3 (2.2)</td>
<td>8.0 (1.6)</td>
<td>ns</td>
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<tr>
<td>QS/NREMS (%)</td>
<td>38.7 (3.2)</td>
<td>45.2 (1.7) △</td>
<td>64.1 (1.3)</td>
<td>65.8 (1.1)</td>
<td>70.0 (1.4)</td>
<td>34.4 (p &lt; 0.001)</td>
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<tr>
<td>IS (%)</td>
<td>9.8 (1.0) (▼)</td>
<td>7.0 (0.7)</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
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<tr>
<td>AS/REMS (%)</td>
<td>51.5 (3.6)</td>
<td>47.6 (1.8) ▼</td>
<td>35.9 (1.3)</td>
<td>34.2 (1.1) ▼</td>
<td>30.0 (1.4)</td>
<td>18.6 (p &lt; 0.001)</td>
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<td>MT (min)</td>
<td>17.4 (3.2)</td>
<td>17.6 (2.8)</td>
<td>10.0 (1.8)</td>
<td>7.0 (1.0)</td>
<td>7.6 (0.8)</td>
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Duration (min)

<table>
<thead>
<tr>
<th></th>
<th>2 weeks</th>
<th>2 months</th>
<th>4 months</th>
<th>6 months</th>
<th>9 months</th>
<th>F age (p) (^a)</th>
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<tbody>
<tr>
<td>QS/NREMS episode</td>
<td>26.3 (1.2)</td>
<td>27.8 (0.9) △</td>
<td>32.8 (1.0)</td>
<td>33.1 (1.0) △</td>
<td>37.5 (2.2)</td>
<td>3.7 (p &lt; 0.005)</td>
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<tr>
<td>AS/REMS episode</td>
<td>37.8 (3.5)</td>
<td>29.2 (2.6) ▼</td>
<td>20.2 (0.6)</td>
<td>21.2 (1.4)</td>
<td>20.0 (0.6)</td>
<td>5.6 (p &lt; 0.005)</td>
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<tr>
<td>Sleep cycle</td>
<td>64.1 (3.8)</td>
<td>56.9 (2.7)</td>
<td>53.0 (1.5)</td>
<td>54.3 (1.5)</td>
<td>57.5 (2.4)</td>
<td>ns</td>
</tr>
<tr>
<td>N per subject (range)</td>
<td>3 - 8</td>
<td>7 - 9</td>
<td>8 - 11</td>
<td>7 - 12</td>
<td>7 - 13</td>
<td>-</td>
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</table>
References


Figure 1.
Figure 2.
Figure 3.
Figure 4.
Figure 5.

$r^2 = 0.81$
$t = 81.3\ min$
Figure 6.