## Week-by-week changes in sleep EEG in healthy full-term newborns

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ABSTRACT

Spectral analysis of neonatal sleep is useful for studying brain maturation; however, most studies have analyzed conventional broad bands described for awake adults, so a distinct approach for EEG analysis may disclose new findings.

OBJECTIVES. To extract independent EEG broad bands using principal component analysis (PCA) and describe week-by-week EEG changes in quiet (QS) and active sleep (AS) during the first 5 weeks of postnatal life in healthy, full-term newborns.

METHODS. Polysomnography of spontaneous sleep was recorded in 60 newborns in 5 groups at 41, 42, 43, 44 and 45 weeks (n = 12 each) postconceptional age (POST-C). QS and AS stages were identified. Absolute power (AP) for 1 Hz bins between 1-30 Hz was subjected to PCA to extract independent broad bands.

RESULTS. PCA rendered three independent broad bands distinct from conventional bands. They explained 82.8% of variance: 2-10 Hz, 10-16 Hz and 17-30 Hz. ANOVAs (group x age x derivations) showed significant higher power at 2-10 Hz with greater age, higher power in QS than AS in all three bands, and significantly higher AP in the left central region, and in the right occipital and temporal areas, in both sleep stages.

CONCLUSION. A different method of analyzing sleep EEG generated new information on brain maturation. The Sigma frequencies identified suggest that sleep spindle maturation begins by at least 41 weeks of POST-C age. Inter-hemispheric asymmetries during sleep suggest earlier development of the central left region and the right occipital and temporal areas.

KEYWORDS: EEG spectral analysis; development; mechanisms of REM sleep generation; REM/NREM cycles; REM sleep; sleep spindles; newborns; preterm; active sleep; quiet sleep; slow wave sleep
Analyses of sleep EEGs in neonates generate important information on brain maturation, but current studies use the same broad bands defined for awake adult humans. Applying principal component analysis to absolute power in clearly-defined, week-by-week, post-conceptional ages and polysomnography-defined sleep stages revealed an independent band of 10-16 Hz that corresponds to sleep spindle frequencies and suggests the presence of oscillatory activity in the thalamo-cortical network as early as the first week of life, though this is not yet visually-identifiable. This approach improves our understanding of EEG development during the first postnatal month and, with additional studies, may serve as a diagnostic tool for brain maturation.
INTRODUCTION

Neonatal electroencephalographic activity (EEG) during sleep has been considered a good biological marker of early brain development by permitting the objective characterization of brain function.\textsuperscript{1,2} Important changes in brain electrogenesis occur neonatally and alterations in these ontogenetically-reproducible sleep EEG patterns can be identified.\textsuperscript{3,4} Early detection of abnormalities in brain development in this period is essential for determining the commencement of early neuro-habilitation therapies.\textsuperscript{5} While some excellent analyses of waking EEG activity in neonates exist,\textsuperscript{6} EEG analysis during sleep is preferred because it is easier to obtain reliable, high-quality EEG recordings, since babies spend 16-18 h per day sleeping, their waking periods are brief, and they often move or cry. Also, wakefulness may be hard to differentiate reliably from active sleep.\textsuperscript{3,7} Although visual analyses of neonatal EEGs accompanied by physiological variables are still the gold standard for identifying sleep stages,\textsuperscript{3,4,8-13} power spectra analyses of EEGs allow quantification of the frequency components of signals\textsuperscript{1,14} and can extract useful information on neonatal EEGs, thus permitting the neurobiological and electrophysiological integration that is required to better understand EEG maturation.\textsuperscript{15} Spectral evolution of sleep EEGs has been amply described in large samples of newborns\textsuperscript{2,6,10,11,13,16,17} and has generated important results on the development of sleep EEGs, but most studies have been performed with the conventional EEG broad bands: Delta (0.5-3.5 Hz), Theta (4-7.5 Hz), Alpha (8-12 Hz), and Beta (13-30 Hz), defined through visual
inspection of awake adult humans\textsuperscript{18}. Broad band limits are important for understanding EEG activity during sleep but using a different method to obtain frequency broad bands based on the intrinsic values of the power spectra themselves, instead of \textit{a priori} definitions, could reveal new information on neonatal EEG maturation. Using principal component analysis (PCA) of the power spectra, we found that while conventional broad bands are clearly-identified as independent during wakefulness, they differ during NREM and REM sleep\textsuperscript{19}. Thus, the broad bands might also differ from the conventional EEG bands of waking adults in the sleep patterns of newborns and yield new information on EEG maturation.

Quiet (QS) and Active sleep (AS) EEG evolution from pre- to full-term newborns has been described extensively\textsuperscript{6-13} but approaches vary. While most studies have used correlation between age and EEG, not all reports distinguish sleep stages, some examined mixed term and pre-term newborns, and others failed to clearly divide the neonates into different age groups, or only focused on one age, such as one week, one month, three months or 40 days\textsuperscript{2,8-13} Also, analyses have often been limited to only two EEG derivations with scant information on how maturation develops in the remaining regions, or have used bipolar leads\textsuperscript{5,16,17} Because the first 28 days of the newborn period are marked by important maturational changes in sleep stages and brain maturation, weekly changes could be expected\textsuperscript{20} However, the week-by-week postnatal evolution of EEG activity in groups of newborns at specific post-conceptional (POST-C) weeks of age in polysomnographically-identified sleep stages during the first 5 weeks post-delivery has not been described in detail.
The QS and AS sleep stages can be identified clearly in full-term neonate EEGs, but their EEGs are not yet fully state-dependent as in adults. Several signs of immaturity that gradually disappear with neonatal maturation persist in the first postnatal weeks. Complete concordance of physiological criteria to identify sleep stages has not been attained and the signs of QS and AS can be mixed, as occurs in transitional sleep, which is identified when electrophysiological features for these two sleep stages are discordant.

During the neonatal period, the EEG activity of QS, an early precursor of NREM sleep, alternates with a discontinuous pattern (trace alternant) that consists in bursts of high-amplitude slow activity alternating with inter-burst intervals (IBIs) with minimal amplitudes. This pattern is frequent during QS from 1-3 weeks, but gradually disappears between weeks 3 and 6. Delta brushes, consisting of slow waves with fast superimposed frequencies, are frequent in QS up to 43 weeks of age. In AS, EEGs are continuous and low voltage irregular activity predominates, though the immature pattern of AS onset persists for about three months.

Brain growth and developmental changes are massive in neonates and include myelination, the multiplication of synapses in cortico-cortical neural circuits, inhibitory and feedback-controlling mechanisms in maturing intracortical and thalamic inhibitory circuits accompanied by the shift from depolarizing to hyperpolarizing action of GABA, and the establishment of new cortico-cortical and mature thalamo-cortical connections. These are all necessary conditions for the joint activation of cortical areas and allow a more concordant establishment of state-dependent electrical activity.
Given that broad band limits are important for analyzing EEG spectral characteristics, one objective of this study was to explore maturational changes in the sleep EEGs of newborns using a different method, such as principal component analysis (PCA), to extract information from spectral analysis. This method decomposes the original data –absolute power (AP)– into orthogonal frequency bands derived from the intrinsic structure of the data themselves, instead of using broad bands defined a priori. Considering that weekly changes in the EEGs of QS and IBIs of trace alternant of QS and AS are expected during the newborn period, our second aim was to describe sleep EEGs in 5 groups of healthy, full-term newborns at 41, 42, 43, 44 and 45 weeks of POST-C age in polysomnographically-defined sleep stages. We hypothesized that: i) the broad bands defined by PCA will differ from conventional ones and may provide new information on developing sleep EEGs; and ii) important changes will occur in EEG frequencies during sleep stages that will increase our knowledge of EEG evolution during this important developmental stage.

MATERIALS AND METHODS

Participants

Sixty healthy, full-term newborns were evaluated in a prospective, cross-sectional study divided by POST-C age at the time of study in 5 groups: 41, 42, 43, 44 and 45 weeks of age (n = 12, each). Table 1 shows the anthropometric, neonatal data and average and age ranges of the study groups.

All babies were born at the Children’s and Women’s Specialized Hospital (Querétaro, Mexico) between September 2013 and November 2017. Newborns were considered eligible
upon applying the following inclusion criteria: 1) singleton newborns with gestational age
at birth of 38-41.5 weeks, defined by a first-trimester prenatal Doppler ultrasound study; 2)
birth weight between the 10th and 90th percentiles according to local standards; 3) normal
results in prenatal Doppler ultrasound studies; and 4) the absence of prenatal, perinatal or
neonatal morbidity

Table 1 about here

The Hospital’s Ethics Committee approved the experimental protocol, which follows the
principles of the World Medical Association’s Helsinki Declaration (2013). All parents gave
their written consent to participate in the study.

Procedures

Sleep recording

Polysomnography of spontaneous sleep was recorded after feeding between 10 a.m. and
12 p.m., or 1:00 and 3:00 p.m., taking advantage of the polyphasic pattern of neonatal sleep.
Gold cup electrodes were placed at Fp3, Fp4, C3, C4, O1, O2, T3 and T4, and EOGs were
recorded on 2 channels through an electrode located 1 cm below the external angle of the
right eye, and another placed 1 cm above the external angle of the left eye, each one
referenced to linked mastoids. Sub-mental electromyogram (EMG), thoracic respiratory
effort and electrocardiograms were also recorded following standard guidelines.\textsuperscript{3,4} The
ground electrode was placed at Fpz. Electrodes were fixed in place with a size-4 tubular
elastic mesh bandage that fit the head circumference of the neonate. Electrode impedances
were below 10 kΩ. Filters were set between 1-30 Hz for EEG and EOG, and between 10-100
Hz for EMG. All polygraphic signals were digitalized at a sampling rate of 200 Hz using a Medicid-3E EEG system (Neuronic Mexicana S.A., Mexico City).

Slow waves

Wakefulness, AS, QS and transitional sleep were identified visually off-line by a clinical neurophysiologist with over 20 years’ experience in newborn sleep scoring (LCR) in 30-second segments using standard criteria.\textsuperscript{3,4} QS was characterized by behavioral quiescence, closed eyes and regular breathing. Those EEGs were dominated by slow (1-4 Hz), continuous, symmetric, synchronous large waves with amplitudes of 50-150 μV and frontal or occipital predominance, alternating with three or more trace alternant episodes marked by alternations between large-amplitude, slow-wave bursts lasting 5-6 seconds, and interburst intervals (IBIs) or periods of smaller amplitude (25-50 μV).

AS was identified by irregular, low-voltage activity at frequencies between 5-8 Hz and amplitudes of 20-35 μV. Single or bursts of rapid eye movements were present with frequent facial movements and body activity interspersed with periods of quiescence. Respiration was typically irregular and very low-voltage EMG was present. Wakefulness, in turn, was identified when artifacts with diffuse low-voltage, strong motor activity, open eyes, irregular breathing and high-amplitude tonic and phasic EMG predominated. Transitional sleep occurred in epochs in which 3 QS criteria co-occurred with 2 for AS. Phasic events like arousals and apneas were also identified.

The percentages of sleep stages were calculated over total sleep time. Interburst interval duration (IBIs) and the maximum discontinuity duration of trace alternant were also
calculated. The percentage of wakefulness was calculated over total recording time. Apneas and arousals were quantified.

Quantitative EEG analysis

At least 15 artifact-free (median=16), 2.8-second EEG segments were selected visually for QS, AS and IBIs of *trace alternant* by a clinical neurophysiologist experienced in visualizing neonatal EEGs (LCR). A second neurophysiologist (JRG) subsequently confirmed that the epochs selected for analysis corresponded to QS, IBIs of *trace alternant* and AS. The power spectra of 40 sec of EEG activity (continuous or divided into 2 sec segments) do not differ significantly from the power spectra of 1 or 2 minutes and meet the stationary and stability criteria and clean signals required for quantitative analysis.27

Segments with sudden variations of the EEG signal, such as delta brushes, abrupt changes from baseline, transients with paroxysmal aspect, or contamination by rapid eye movements or electromyograms were excluded from the quantitative EEG analysis. Waking periods were not analyzed. The IBIs of *trace alternant* of QS were analyzed separately from continuous QS activity because these two patterns of QS EEG activity have opposed maturational trends, given that *trace alternant* disappears but continuous activity tends to increase with gestational age.8,9,11 Therefore, a separate analysis could yield additional information on sleep EEG maturation. Delta brushes were excluded from the quantitative analysis because they also disappear as age increases.

The artifact-free EEG segments were subjected to Fast Fourier Transform using the TrackWalker system (Neuronic Mexicana S.A.). The absolute power (AP) for 1 Hz narrow
bands from 1-30 Hz was obtained for each newborn, derivation and sleep stage. The digital
files from the Fp3 derivation were damaged in two patients in groups 41, 43, 44 and 45
weeks, so this derivation was excluded from the quantitative analysis.

AP spectra were subjected to principal component analysis to reduce variables by obtaining
frequency bands with common variance. PCA is a multivariate statistical technique that
decomposes data into constituent, uncorrelated orthogonal components derived from the
intrinsic values—in this case, the AP of every 1 Hz bin—of the power spectra themselves,
instead of broad bands defined \textit{a priori}. Each derived component successively explains a
maximum of data variance, but they can be totaled to recover the original observed AP
spectra. After component extraction, the loading pattern may be rotated with VARIMAX
rotation to maintain the orthogonal relationships between components, maximize high
component loading and minimize low loading values.\textsuperscript{28,29} The original data consisted of 30
variables (AP of 1 Hz bins) for each newborn and experimental condition; that is, age, sleep
stage and derivations that reflect a source of variance. Varimax rotation was applied. To
determine the frequency bands with common variance, Eigenvalues above 1 for
eigenvectors and factor-loading above 0.60 were used to include or exclude a frequency
in/from a factor or eigenvector. This limit was chosen based on the following criteria: 1)
Those frequencies contiguous with the highest eigenvalues must be grouped together; 2)
To avoid overlapping frequencies in two or more components, and 3) To avoid the exclusion
of any frequency. In our case the clear cut limit was 0.6.

Two PCA were run, one for high voltage slow activity of QS, and AS together, a second for
the IBIs of \textit{trace alternant}, because maturation trends of high voltage QS activity and \textit{trace}
alternant follow opposed curves; that is, QS increases while trace alternant decreases with
POST-C age.\textsuperscript{3,4}

The component scores derived for each experimental condition and subject can be treated with conventional statistical analysis; in this case, mixed three-way ANOVAs: age × sleep stage × derivations, or mixed two-way ANOVAs: age × derivations, in the case of the IBIs of trace alternant. AP of the frequencies included in each new band was then averaged for each newborn, derivation and sleep stage to illustrate results. Since PCA analysis grouped the 2-10 Hz frequencies in one independent band that spanned the traditional Delta, Theta bands and part of the Alpha band, we subjected the AP of these frequencies to PCA analysis (with the AP of 2-10 Hz as variables) to determine whether the variance within this frequency range could be separated into independent components.

Statistical analyses

The normality distribution of all variables was assessed by a Shapiro Wilk’s test. The AP of each broad band was subjected separately to 3-way mixed ANOVAs with POST-C age groups as the independent variable (41, 42, 43, 44, 45 weeks) and sleep stage (QS, AS) and derivations as within-subject variables. The AP of the broad bands from the IBIs were compared by 2-way mixed ANOVAs with age (41, 42, 43, 44 weeks) as the independent variable and derivations as the within-subject variable. Spectral energy values were log-transformed before statistical analysis. The confidence level was 99% (α≤0.01). Tukey’s studentized t-tests were used for post-hoc, pair-wise comparisons after family-wise error correction for multiple comparisons between age groups, derivations and interactions.
When non-normality was found, a Kruskall-Wallis test was applied for comparisons. Finally, Pearson coefficient correlations between POST-C age and the average AP of each broad band and derivation of the 60 newborns were calculated for the sleep stages (QS, AS) and IBIs. For statistical analyses, the R program, 3.2.5 version was used.

RESULTS

The mean duration of the polysomnographies was 66.8 minutes (range: 50-87 min) with 95% lasting 60 min or more. At least one sleep cycle with AS and QS was observed in all studies. Table 2 shows the means, standard deviations and statistical results from sleep for the variables in the 5 study groups. The percentage of trace alternant and the number and maximum duration of the IBIs decreased, while the percentage of QS increased with greater POST-C age, as was expected. AS showed significant differences among groups, but no clear pattern associated with POST-C age. There were no differences in the percentages of wakefulness, transitional sleep or arousals, or in the amount and duration of apneas.

Table 2 about here

EEG quantitative analysis

Results of PCA applied to the AP values of the EEG frequencies in QS and AS rendered three independent broad bands that explained 82.8% of the variance found. Table 3 and Figure 1 show the frequency spectra and 3 broad bands for QS and AS identified: a first component for frequencies of 17-30 Hz that explain 37.4% of variance; a second with frequencies of 2-10 Hz that explains 24.3%; and a third of 10-16 Hz that explains 21.02% of the variance. A
PCA applied exclusively to the 2-10 Hz band demonstrated the absence of independent sub-bands.

Figure 1, and Table 3 about here

Table 4 shows the results of the 3-way mixed ANOVAs (age x sleep stage x derivation) for each broad band. Only significant results after family-wise error correction for multiple comparisons are described. In the interests of clarity, they are described for each significant main effect and interaction together for the 3 bands.

Age main effect

An age main effect was significant only for the 2-10 Hz band. Post-hoc comparisons of the AP of this band indicated significant differences among all age groups (p<0.01) with greater power as age increased, except between 42-43 weeks of POST-C age (Figure 2A).

Sleep main effect

Sleep main effects were significant for all 3 broad bands. The AP was significantly higher in QS than AS in each one (Figure 2B). Age-by-sleep interaction was not significant.

Figure 2 and Table 4, about here

Derivation main effects and sleep-by-derivation interactions

Derivation main effects were significant for all 3 bands, as AP was higher at the central but lower at the temporal derivations in all three (not shown). Since sleep-by-derivation interactions were also significant for the 3 bands, they are described in more detail.
Sleep-by-derivation interactions for the 2-10 Hz band corroborated that AP was higher in QS than AS at all derivations, and also higher at the central derivations, but lower at the temporal ones (Figure 3), with significant differences among all derivations in both QS and AS, except between the left and right occipital and temporal derivations. For the 2-10 Hz band, there was a significant asymmetry between the left and right hemispheres at the central electrodes with higher power in the left central area in both sleep stages. The occipital and temporal derivations had higher power in the right hemisphere for the 17-30 Hz band, during QS and AS.

Sleep-by-derivation interactions for the 10-16 Hz band corroborated that AP was higher in QS than AS, except at O2. The AP of this band was significantly higher at both central derivations but lower at both temporal ones in QS; whereas in AS it was higher at O2 and the differences of O2 vs. the central derivations disappeared (Figure 3). The significant asymmetry observed in the 2-10 Hz band between the left and right central derivations also disappeared, but was maintained between temporal derivations in both sleep stages. Figure 3 shows the sleep-by-derivation interactions for the 17-30 Hz band, where AP at F4, C3 and C4 is higher in QS than AS, through there are no differences between sleep stages at O1, O2, T3 and T4. Significant asymmetries between O1, T3 and O2, T4, in both sleep stages, with higher power in the right hemisphere, are also seen.

Given that AP of the three bands identified by PCA was higher in QS than in AS, Relative Power was calculated (broad band absolute power/ 30 Hz absolute power) to explore if the
relative contribution of each band to total absolute power would give additional information on sleep stages EEG maturation. Relative power of each of the bands was submitted to three-way mixed ANOVAs. Previously, to ensure a normal distribution of data, the ln(x/1-x) transformation was used.

Results of ANOVAs of relative power of the three bands showed that relative power of the 2-10 Hz band was significantly higher in QS than in AS (F=401, p<0.0001); whereas, Relative Power of 10-16 Hz (F=505, p<0.0001) and 17-30 Hz (F=699, p<0.0001) bands was significantly higher in AS than in QS.

Correlation of AP with age

Table 5 about here

Correlation between POST-C age and AP was significant only for the 2-10 Hz band in both QS and AS (see Table 5 and Figure 4). In this band, AP increased with greater age at all derivations, but there were no significant differences in the correlations between derivations for either QS ($\chi^2=3.52$, p=0.75) or AS ($\chi^2=4.17$, p=0.50).

Figure 4 about here

IBIs of trace alternant

The principal component analysis applied to the AP of the EEG frequencies in the trace alternant IBIs identified only 2 components, which explained 75.8 % of total variance; one from 2-16 Hz that accounted for 36.8%, the other from 15-30 Hz that explained 39.02%. 
Results of the 2-way mixed ANOVAs with age (41, 42, 43, 44 weeks) and the derivations for each component (Table 3) showed significant differences for age and derivations with main effects for the 2-16 Hz band, but only a significant derivation main effect for the 15-30 Hz band.

The main effect of age showed that the AP of the 2-16 band differed significantly among 4 age groups, but not between weeks 42 and 43, and was higher with older age. Derivation main effects for AP showed similar significances for the two bands, as AP differed significantly among all derivations except between C3 and O2 and the left and right hemispheres (central, occipital and temporal derivations). AP was higher at central derivations and lower at temporal and frontal ones.

There was a significant correlation between the newborns’ POST-C age and AP for the 2-16 Hz broad band in each of the derivations of the trace alternant IBIs (r=0.44, p<0.0005). AP increased with age in this band at all derivations (not shown). Correlations for the 15-30 Hz band were not significant (r=0.10, p=0.25).

DISCUSSION

As hypothesized, this study found sleep EEG broad bands in newborns that differ from the Delta, Theta, Alpha and Beta bands conventionally described in awake adult humans, as has been demonstrated in sleep EEGs of both adult humans and rats. In addition, the method employed showed that it is possible to generate new information: three independent broad bands were identified in newborns that explained 82% of the variance found – from 2-10 Hz, 10-16 Hz and 17-30 Hz –, while only two broad bands were identified
during the immature IBIs of *trace alternant*: from 2-16 Hz and 15-30 Hz. Only the AP of the slow band (2-10 Hz) increased significantly during the first 5 weeks POST-C age, whereas the AP of the 3 broad bands was significantly greater in QS than AS.

**Broad bands**

The existence of these broad bands is a new finding in neonatal sleep EEG studies, which have traditionally used either the aforementioned conventional bands or 3 Hz narrow bands.\(^1,2,6,10,16\) The most interesting result was the detection of an independent band from 10-16 Hz which corresponds approximately to sleep spindle frequencies or the Sigma band of NREM in adult humans and cats.\(^32,33\) Identifying these frequencies as an independent band cannot be achieved using the traditional Beta band, which averages frequencies from 12-30 Hz, nor do they correspond to the fast frequencies of delta brushes since this pattern was not included in the quantitative analysis. This result of independent activity in Sigma frequencies suggests that it may correspond to a rudimentary oscillatory mode of the thalamo-cortical network within sleep spindle frequencies present as early as 41 weeks of POST-C age, but only during clearly-defined QS and AS, and not in the gradually-disappearing immature transitional IBI pattern of *trace alternant* during the first month of life.\(^3,11\) Sleep spindles are not visually-apparent in newborns until the age of 1-3 months, or 44 weeks.\(^3,8,11,13\) It may be that sleep spindles cannot be detected visually in the first month of life because a minimal amount of neuronal populations must be synchronized at the same frequency for this to appear,\(^14\) and the number of neurons that are synchronized within this frequency range at this stage of development is insufficient. The presence of this
independent Sigma band suggests that it could be considered a favorable sign of maturation.

PCA identified the slow band, 2-10 Hz, as a broad band that encompasses what are traditionally described as Delta, Theta and slow Alpha frequencies. A PCA applied exclusively to this 2-10 Hz band demonstrated the absence of independent Delta, Theta and Alpha bands; a finding consistent with sleeping EEGs in adults, which suggests that these frequencies are affected by the same modulatory influences during sleep.

Although the methodologies currently available do not permit determining the underlying mechanisms of the slow and Sigma bands, findings from cellular neurophysiology recorded simultaneously with intracranial field activity in adult cats have shown that Delta and sleep spindles in NREM sleep are generated in the thalamus, depending on the level of hyperpolarization attained in the thalamo-cortical relay neurons by the hyperpolarizing inhibitory GABAergic action that comes from the reticular thalamic nuclei and propagates to the cortex through the thalamo-cortical network. Thus, the rudimentary Sigma band in this stage of development suggests the incipient maturation of the thalamo-cortical network.

The fast 17-30 Hz band, meanwhile, corresponds to the fast frequencies of the conventional Beta band. The limit between the Sigma and high frequency bands at 17 Hz is consistent with the cut-off point at 17-18 Hz observed between the Sigma and Beta bands in adult humans. Frequencies above 17 Hz correlate with the Beta band during wakefulness and
REM sleep, while those below 17 Hz correlate positively with the Sigma band during NREM sleep, but negatively with Beta during wakefulness and REM sleep.\textsuperscript{35}

While at this stage of development, concordance among the physiological markers of QS and AS has already been attained,\textsuperscript{3,4} our results show that adult-like, state-dependent EEG activity—with higher delta absolute power in NREM sleep and lower in REM, and lower fast activity power in NREM than REM—has not developed, since the AP of the 3 bands is higher in QS than AS. The higher absolute power of all frequencies, slow as well as fast, in QS suggests a massive non-selective cortical response to incoming volleys, likely due to the poor maturation of cortical GABAergic inhibitory interneurons, which do not mature until around postnatal days 11-13 in rodents, and to the still rudimentary cortico-cortical short connections.\textsuperscript{24-26}

Since AP did not show higher power in AS than QS in fast frequencies, the relative power of the three bands was also compared to determine if the relative contribution of each band to total power, showed the same pattern of higher fast activity in AS than QS found in adult sleep. Significant differences between the two sleep stages showed that the relative power of 10-16 and 17-30 Hz over total power was higher in AS than in QS as it could be expected from the known earlier maturation of AS.\textsuperscript{3,4}

Age-dependent changes

The first month of life is an important period for the electrophysiological maturation of the cerebral cortex and the thalamo-cortical circuit. It is characterized by increased myelination, the establishment of new synaptic connections and short range cortico-cortical
connections,\textsuperscript{20,26} and the shift from depolarizing to hyperpolarizing action by the GABAergic system.\textsuperscript{24,25}

In terms of the age-dependent changes that occurred during the first 5 weeks postpartum in the sleep EEGs of the healthy full-term newborns studied, we found two main results: 1) that only the slow band from 2-10 Hz increased significantly with age, while the Sigma and Beta bands did not; 2) right-left asymmetries.

AP of the slow band (2-10 Hz) increased during the first 5 weeks of life. This finding is consistent with observations on longer life spans in pre-term and full-term newborns.\textsuperscript{6,11,16,17} The steady increase in the AP of the slow band (2-10 Hz) during the first 5 weeks of life that correlated positively with POST-C age and the presence of a rudimentary Sigma band are consistent with the gradual maturation of the GABAergic hyperpolarizing influence of the reticular nuclei neurons on relay thalamo-cortical neurons in the thalamus.

While the AP of the slow frequencies increased with age, that of the Sigma and fast bands did not do so in either QS or AS. The lack of differences with POST-C age in these frequencies in newborns is consistent with previous studies that reported decreases in the fast frequencies from pre-term to around 40 or 44 weeks.\textsuperscript{10,13,16} During the transition from pre-term to full-term, delta brushes that consist in slow waves crowned with fast frequencies abound. The Fourier analysis of these transients has shown a large amount of power around frequencies above 10 Hz within the burst that disappears if Fourier analysis is run after removing the delta brushes.\textsuperscript{16} This author found that power above 10 Hz decreases in both
sleep stages from 35-45 weeks, but increases from 46-52 weeks. The decrease correlates with the decreasing incidence of delta brushes.\textsuperscript{16}

The fast frequencies depend on the depolarizing effect of brain stem cholinergic and monoaminergic activating systems, which are functional by gestational week 35\textsuperscript{20,26} on the inhibitory action of cortical GABAergic interneurons\textsuperscript{34} and on the complex interaction of the brainstem and the thalamo-cortical network.\textsuperscript{21} Studies in rodents have shown that state-dependent or arousal modulation and the continuity of fast activity correlate with the maturation of the dorso-lateral geniculate nucleus of the thalamus and the thalamo-cortical network.\textsuperscript{16} This change also coincides with the shift from depolarizing to hyperpolarizing action in the GABAergic system. Thus, the lack of state-dependent modulation of AP of fast activity at this stage of development may result from the interplay of the immaturity of the thalamo-cortical network with that of the cortical GABAergic feedback circuitry and the still incomplete shift from the depolarizing to the hyperpolarizing effect of GABA.\textsuperscript{16,24}

Right-left asymmetries

Another contribution of this study is that it identified regional and interhemispheric power differences in healthy neonates with an anterior-posterior topographic dissociation between 2-10 Hz and 17-30 Hz bands that is related to POST-C age. The AP of the slow band (2-10 Hz) was more evident in C3 than C4 in both QS and AS and increased with POST-C age, as described previously,\textsuperscript{2,11,16} while the AP of the fast bands increased in the right occipital and temporal leads, in both sleep stages.
The higher right hemisphere power is consistent with previous results\textsuperscript{2,7,16} and suggests that the right hemisphere may develop more rapidly than the left. The achievement of the left central maximal AP in the 2-10 Hz band in healthy neonates at 40-41 weeks POST-C age and the presence of maximal AP of 17-30 Hz in the right occipital region at 45-46 weeks of POST-C age could constitute biomarkers of two milestones of normal electrophysiological development.

Limitations

There are several methodological questions that must be considered. First, our results are limited to the newborn period from 41-45 weeks of POST-C age in healthy full-term infants, so they need to be corroborated in a larger sample and then extended week-by-week to earlier and later weeks of maturational stages in order to ascertain in what week of age Sigma activity becomes apparent to visual inspection in the form of clearly-defined sleep spindles, and the clear full state-dependency of EEG characteristics for QS and AS is definitively established. Though there are many studies on sleep EEG in pre-term to 3-month-old newborns, most are based on EEG feature correlations with age, mixed pre-term and full-term neonates, analyzed only specific ages, or made no distinction between QS and AS.\textsuperscript{2,8-13} Our findings, in contrast, are based on healthy, full-term newborns with well-characterized POST-C ages and sleep stages. Our results are based on one sole method of analysis (PCA with VARIMAX rotation), which allowed us to generate new information on sleep EEG maturation. This findings suggest that it is worthwhile to explore the use of different methods of EEG analysis, as this may enrich our knowledge of sleep EEGs.
Also, the representativeness of these data could be questioned. Our AP power results are based on 15 to 26 EEG segments extracted from 1 hour of sleep recording after careful inspection for artifacts. Studies have demonstrated that 40 seconds of EEG meet the stationary and stability criteria required for Fourier analysis and do not significantly differ from longer times of analysis, at least in adults; however, further studies that analyze longer times are needed to corroborate the generalization of these results. Another possible limitation is that our sleep recordings were obtained during a short period of about 2 hours during the day, so they may not correspond to data obtained during night-time sleep. However, we would argue that for newborns, whose sleep cycle is only about 40 minutes, this recording time is sufficient for them to complete one sleep cycle. Also, since circadian regulations are not yet established, no circadian variations are to be expected. Clearly, our results need to be corroborated by analyzing longer sleep periods at different circadian times. Another point concerns the limited number of derivations recorded, which indicates the need for high-density recordings in future studies. Especially important is the lack of data from the left frontal derivation, which were lost due to technical problems with the digital files. However, we privileged the same number of newborns in each week rather than including this derivation in fewer newborns. Finally, despite these limitations, we believe that our study extends previous research and provides valuable information on the week-by-week maturation of sleep EEGs in the newborn period.

Conclusion

Broad band limits are important for analyzing sleep EEG activity. The results of the present study show that using spectral analysis and principal component analysis of absolute power
made it possible to identify an independent broad band within the Sigma frequencies. Our findings suggest that maturation of the oscillatory mode of the thalamo-cortical network within spindle frequencies in healthy, full-term newborns begins at least as early as 41 week of POST-C age. They further show the presence of broad bands distinct from those defined visually for awake adults. When studying well-defined sleep stages and POST-C ages, asymmetries become apparent, suggesting the differential development of the left central and right occipital and temporal regions during the newborn period.

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Abbreviations

EEG: Electroencephalographic activity
PCA: Principal component analysis
POST-C: Post-conceptional (age)
QS: Quiet sleep
AS: Active sleep  
w: Weeks  
IBIs: Interburst intervals of trace alternant  
EMG: Submental electromyogram  
EOG: Electrooculogram  
AP: Absolute power  
ANOVA: Analysis of variance  

Disclosure Statement

The authors have no conflicts of interest to declare. Financial Disclosure: none. Nonfinancial Disclosure: none.

REFERENCES


Figure legends

Figure 1. Power Spectra in Quiet and Active sleep stages. Color heat map of the absolute power ($\mu V^2$) by frequency (1-30 Hz) for each newborn, sleep stage and derivation in A. Average spectra for quiet (QS) and active sleep (AS) in B; vertical lines show the three broad band limits identified by principal component analysis in independent eigenvectors with percentages explained and factor-loading in the inset. Horizontal lines show significant differences between QS and AS.

Figure 2. Mean and standard error of the log-transformed absolute power of the three bands for age main effect in A (w: weeks of postconceptional age; horizontal lines indicate significant differences), and for sleep main effect in B (AS: active sleep, white; QS: quiet sleep, black). Asterisks indicate significant differences.

Figure 3. Mean and standard error of the log-transformed absolute power of the three bands for each derivation in Quiet sleep (in black) and Active sleep (in white). Horizontal lines indicate significant differences.

Figure 4. Pearson correlation coefficients between the log-transformed absolute power of the three bands and postconceptional age in weeks (w) for quiet sleep in A, and active sleep in B, at the left central derivation (C3).
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ABSTRACT

Spectral analysis of neonatal sleep is useful for studying brain maturation; however, most studies have analyzed conventional broad bands described for awake adults, so a distinct approach for EEG analysis may disclose new findings.

OBJECTIVES. To extract independent EEG broad bands using principal component analysis (PCA) and describe week-by-week EEG changes in quiet (QS) and active sleep (AS) during the first 5 weeks of postnatal life in healthy, full-term newborns.

METHODS. Polysomnography of spontaneous sleep was recorded in 60 newborns in 5 groups at 41, 42, 43, 44 and 45 weeks (n = 12 each) postconceptional age (POST-C). QS and AS stages were identified. Absolute power (AP) for 1 Hz bins between 1-30 Hz was subjected to PCA to extract independent broad bands.

RESULTS. PCA rendered three independent broad bands distinct from conventional bands. They explained 82.8% of variance: 2-10 Hz, 10-16 Hz and 17-30 Hz. ANOVAs (group x age x derivations) showed significant higher power at 2-10 Hz with greater age, higher power in QS than AS in all three bands, and significantly higher AP in the left central region, and in the right occipital and temporal areas, in both sleep stages.

CONCLUSION. A different method of analyzing sleep EEG generated new information on brain maturation. The Sigma frequencies identified suggest that sleep spindle maturation begins by at least 41 weeks of POST-C age. Inter-hemispheric asymmetries during sleep suggest earlier development of the central left region and the right occipital and temporal areas.

KEYWORDS: EEG spectral analysis; development; mechanisms of REM sleep generation; REM/NREM cycles; REM sleep; sleep spindles; newborns; preterm; active sleep; quiet sleep; slow wave sleep
SIGNIFICANCE

Analyses of sleep EEGs in neonates generate important information on brain maturation, but current studies use the same broad bands defined for awake adult humans. Applying principal component analysis to absolute power in clearly-defined, week-by-week, post-conceptional ages and polysomnography-defined sleep stages revealed an independent band of 10-16 Hz that corresponds to sleep spindle frequencies and suggests the presence of oscillatory activity in the thalamo-cortical network as early as the first week of life, though this is not yet visually-identifiable. This approach improves our understanding of EEG development during the first postnatal month and, with additional studies, may serve as a diagnostic tool for brain maturation.
INTRODUCTION

Neonatal electroencephalographic activity (EEG) during sleep has been considered a good biological marker of early brain development by permitting the objective characterization of brain function.\textsuperscript{1,2} Important changes in brain electogenesis occur neonatally and alterations in these ontogenetically-reproducible sleep EEG patterns can be identified.\textsuperscript{3,4}

Early detection of abnormalities in brain development in this period is essential for determining the commencement of early neuro-habilitation therapies.\textsuperscript{5} While some excellent analyses of waking EEG activity in neonates exist,\textsuperscript{6} EEG analysis during sleep is preferred because it is easier to obtain reliable, high-quality EEG recordings, since babies spend 16-18 h per day sleeping, their waking periods are brief, and they often move or cry. Also, wakefulness may be hard to differentiate reliably from active sleep.\textsuperscript{3,7}

Although visual analyses of neonatal EEGs accompanied by physiological variables are still the gold standard for identifying sleep stages,\textsuperscript{3,4,8-13} power spectra analyses of EEGs allow quantification of the frequency components of signals\textsuperscript{1,14} and can extract useful information on neonatal EEGs, thus permitting the neurobiological and electrophysiological integration that is required to better understand EEG maturation.\textsuperscript{15}

Spectral evolution of sleep EEGs has been amply described in large samples of newborns\textsuperscript{2,6,10,11,13,16,17} and has generated important results on the development of sleep EEGs, but most studies have been performed with the conventional EEG broad bands: Delta (0.5-3.5 Hz), Theta (4-7.5 Hz), Alpha (8-12 Hz), and Beta (13-30 Hz), defined through visual
inspection of awake adult humans. Broad band limits are important for understanding EEG activity during sleep but using a different method to obtain frequency broad bands based on the intrinsic values of the power spectra themselves, instead of a priori definitions, could reveal new information on neonatal EEG maturation. Using principal component analysis (PCA) of the power spectra, we found that while conventional broad bands are clearly-identified as independent during wakefulness, they differ during NREM and REM sleep. Thus, the broad bands might also differ from the conventional EEG bands of waking adults in the sleep patterns of newborns and yield new information on EEG maturation.

Quiet (QS) and Active sleep (AS) EEG evolution from pre- to full-term newborns has been described extensively, but approaches vary. While most studies have used correlation between age and EEG, not all reports distinguish sleep stages, some examined mixed term and pre-term newborns, and others failed to clearly divide the neonates into different age groups, or only focused on one age, such as one week, one month, three months or 40 days. Also, analyses have often been limited to only two EEG derivations with scant information on how maturation develops in the remaining regions, or have used bipolar leads. Because the first 28 days of the newborn period are marked by important maturational changes in sleep stages and brain maturation, weekly changes could be expected. However, the week-by-week postnatal evolution of EEG activity in groups of newborns at specific post-conceptional (POST-C) weeks of age in polysomnographically-identified sleep stages during the first 5 weeks post-delivery has not been described in detail.
The QS and AS sleep stages can be identified clearly in full-term neonate EEGs, but their EEGs are not yet fully state-dependent as in adults.\(^\text{21,22}\) Several signs of immaturity that gradually disappear with neonatal maturation persist in the first postnatal weeks.\(^\text{3,4,11}\) Complete concordance of physiological criteria to identify sleep stages has not been attained and the signs of QS and AS can be mixed, as occurs in transitional sleep, which is identified when electrophysiological features for these two sleep stages are discordant.

During the neonatal period, the EEG activity of QS, an early precursor of NREM sleep, alternates with a discontinuous pattern (\textit{trace alternant}) that consists in bursts of high-amplitude slow activity alternating with inter-burst intervals (IBIs) with minimal amplitudes. This pattern is frequent during QS from 1-3 weeks, but gradually disappears between weeks 3 and 6.\(^\text{8,9,12,23}\) Delta brushes, consisting of slow waves with fast superimposed frequencies, are frequent in QS up to 43 weeks of age.\(^\text{8,9}\) In AS, EEGs are continuous and low voltage irregular activity predominates, though the immature pattern of AS onset persists for about three months.\(^\text{3,4,8-11,23}\)

Brain growth and developmental changes are massive in neonates and include myelination, the multiplication of synapses in cortico-cortical neural circuits,\(^\text{20}\) inhibitory and feedback-controlling mechanisms in maturing intracortical and thalamic inhibitory circuits accompanied by the shift from depolarizing to hyperpolarizing action of GABA,\(^\text{16,24,25}\) and the establishment of new cortico-cortical and mature thalamo-cortical connections.\(^\text{26}\) These are all necessary conditions for the joint activation of cortical areas and allow a more concordant establishment of state-dependent electrical activity.
Given that broad band limits are important for analyzing EEG spectral characteristics, one objective of this study was to explore maturational changes in the sleep EEGs of newborns using a different method, such as principal component analysis (PCA), to extract information from spectral analysis. This method decomposes the original data—absolute power (AP)—into orthogonal frequency bands derived from the intrinsic structure of the data themselves, instead of using broad bands defined *a priori*. Considering that weekly changes in the EEGs of QS and IBIs of *trace alternant* of QS and AS are expected during the newborn period, our second aim was to describe sleep EEGs in 5 groups of healthy, full-term newborns at 41, 42, 43, 44 and 45 weeks of POST-C age in polysomnographically-defined sleep stages. We hypothesized that: i) the broad bands defined by PCA will differ from conventional ones and may provide new information on developing sleep EEGs; and ii) important changes will occur in EEG frequencies during sleep stages that will increase our knowledge of EEG evolution during this important developmental stage.

MATERIALS AND METHODS

Participants

Sixty healthy, full-term newborns were evaluated in a prospective, cross-sectional study divided by POST-C age at the time of study in 5 groups: 41, 42, 43, 44 and 45 weeks of age (n = 12, each). Table 1 shows the anthropometric and neonatal data and average and age ranges of the study groups.

All babies were born at the Children’s and Women’s Specialized Hospital (Querétaro, Mexico) between September 2013 and November 2017. Newborns were considered eligible
upon applying the following inclusion criteria: 1) singleton newborns with gestational age at birth of 38-41.5 weeks, defined by a first-trimester prenatal Doppler ultrasound study; 2) birth weight between the 10th and 90th percentiles according to local standards; 3) normal results in prenatal Doppler ultrasound studies; and 4) the absence of prenatal, perinatal or neonatal morbidity. Table 1 shows the anthropometric and neonatal data of the study groups.

Table 1 about here

The Hospital’s Ethics Committee approved the experimental protocol, which follows the principles of the World Medical Association’s Helsinki Declaration (2013). All parents gave their written consent to participate in the study.

Procedures

Sleep recording

Polysomnography of spontaneous sleep was recorded after feeding between 10 a.m. and 12 p.m., or 1:00 and 3:00 p.m., taking advantage of the polyphasic pattern of neonatal sleep. Gold cup electrodes were placed at Fp3, Fp4, C3, C4, O1, O2, T3 and T4, and EOGs were recorded on 2 channels through an electrode located 1 cm below the external angle of the right eye, and another placed 1 cm above the external angle of the left eye, each one referenced to linked mastoids. Sub-mental electromyogram (EMG), thoracic respiratory effort and electrocardiograms were also recorded following standard guidelines. The ground electrode was placed at Fpz. Electrodes were fixed in place with a size-4 tubular elastic mesh bandage that fit the head circumference of the neonate. Electrode impedances
were below 10 kΩ. Filters were set between 1-30 Hz for EEG and EOG, and between 10-100 Hz for EMG. All polygraphic signals were digitalized at a sampling rate of 200 Hz using a Medicid-3E EEG system (Neuronic Mexicana S.A., Mexico City).

Slow waves

Wakefulness, AS, QS and transitional sleep were identified visually off-line by a clinical neurophysiologist with over 20 years’ experience in newborn sleep scoring (LCR) in 30-second segments using standard criteria. QS was characterized by behavioral quiescence, closed eyes and regular breathing. Those EEGs were dominated by slow (1-4 Hz), continuous, symmetric, synchronous large waves with amplitudes of 50-150 µV and frontal or occipital predominance, alternating with three or more trace alternant episodes marked by alternations between large-amplitude, slow-wave bursts lasting 5-6 seconds, and interburst intervals (IBIs) or periods of smaller amplitude (25-50 µV).

AS was identified by irregular, low-voltage activity at frequencies between 5-8 Hz and amplitudes of 20-35 µV. Single or bursts of rapid eye movements were present with frequent facial movements and body activity interspersed with periods of quiescence. Respiration was typically irregular and very low-voltage EMG was present. Wakefulness, in turn, was identified when artifacts with diffuse low-voltage, strong motor activity, open eyes, irregular breathing and high-amplitude tonic and phasic EMG predominated.

Transitional sleep occurred in epochs in which 3 QS criteria co-occurred with 2 for AS. Phasic events like arousals and apneas were also identified.
The percentages of sleep stages were calculated over total sleep time. Interburst interval duration (IBIs) and the maximum discontinuity duration of trace alternant were also calculated. The percentage of wakefulness was calculated over total recording time. Apneas and arousals were quantified.

Quantitative EEG analysis

At least 15 artifact-free (median=16), 2.8-second EEG segments were selected visually for QS, AS and IBIs of trace alternant by a clinical neurophysiologist experienced in visualizing neonatal EEGs (LCR). A second neurophysiologist (JRG) subsequently confirmed that the epochs selected for analysis corresponded to QS, IBIs of trace alternant and AS. The power spectra of 40 sec of EEG activity (continuous or divided into 2 sec segments) do not differ significantly from the power spectra of 1 or 2 minutes and meet the stationary and stability criteria and clean signals required for quantitative analysis. Segments with sudden variations of the EEG signal, such as delta brushes, abrupt changes from baseline, transients with paroxysmal aspect, or contamination by rapid eye movements or electromyograms were excluded from the quantitative EEG analysis. Waking periods were not analyzed. The IBIs of trace alternant of QS were analyzed separately from continuous QS activity because these two patterns of QS EEG activity have opposed maturational trends, given that trace alternant disappears but continuous activity tends to increase with gestational age. Therefore, a separate analysis could yield additional information on sleep EEG maturation. Delta brushes were excluded from the quantitative analysis because they also disappear as age increases.
The artifact-free EEG segments were subjected to Fast Fourier Transform using the TrackWalker system (Neuronic Mexicana S.A.). The absolute power (AP) for 1 Hz narrow bands from 1-30 Hz was obtained for each newborn, derivation and sleep stage. The digital files from the Fp3 derivation were damaged in two patients in groups 41, 43, 44 and 45 weeks, so this derivation was excluded from the quantitative analysis.

AP spectra were subjected to principal component analysis to reduce variables by obtaining frequency bands with common variance. PCA is a multivariate statistical technique that decomposes data into constituent, uncorrelated orthogonal components derived from the intrinsic values—in this case, the AP of every 1 Hz bin—of the power spectra themselves, instead of broad bands defined a priori. Each derived component successively explains a maximum of data variance, but they can be totaled to recover the original observed AP spectra. After component extraction, the loading pattern may be rotated with VARIMAX rotation to maintain the orthogonal relationships between components, maximize high component loading and minimize low loading values. The original data consisted of 30 variables (AP of 1 Hz bins) for each newborn and experimental condition; that is, age, sleep stage and derivations that reflect a source of variance. Varimax rotation was applied.

To determine the frequency bands with common variance, Eigenvalues above 1 for eigenvectors and factor-loading above 0.60 were used to include or exclude a frequency in/from a factor or eigenvector. This limit was chosen based on the following criteria: 1) Those frequencies contiguous with the highest eigenvalues must be grouped together; 2) To avoid overlapping frequencies in two or more components, and 3) To avoid the exclusion of any frequency. In our case the clear cut limit was 0.6. Varimax rotation was applied.
Two PCA were run, one for high voltage slow activity of QS and AS together, a second for the IBIs of *trace alternant*, because maturation trends of high voltage QS activity and *trace alternant* follow opposed curves; that is, QS increases while *trace alternant* decreases with POST-C age.\(^3,4\)

The component scores derived for each experimental condition and subject can be treated with conventional statistical analysis; in this case, mixed three-way ANOVAs: age × sleep stage × derivations, or mixed two-way ANOVAs: age × derivations, in the case of the IBIs of *trace alternant*. The AP of the frequencies included in each new bands was then averaged for each newborn, derivation and sleep stage to illustrate results. Since PCA analysis grouped the 2-10 Hz frequencies in one independent band that spanned the traditional Delta, Theta bands and part of the Alpha band, we subjected the AP of these frequencies to PCA analysis (with the AP of 2-10 Hz as variables) to determine whether the variance within this frequency range could be separated into independent components.

Statistical analyses

The normality distribution of all variables was assessed by a Shapiro Wilk’s test. The AP of each broad band was subjected separately to 3-way mixed ANOVAs with POST-C age groups as the independent variable (41, 42, 43, 44, 45 weeks) and sleep stage (QS, AS) and derivations as within-subject variables. The AP of the broad bands from the IBIs were compared by 2-way mixed ANOVAs with age (41, 42, 43, 44 weeks) as the independent variable and derivations as the within-subject variable. Spectral energy values were log-transformed before statistical analysis. The confidence level was 99% (α≤0.01). Tukey’s
studentized t-tests were used for post-hoc, pair-wise comparisons after family-wise error
correction for multiple comparisons between age groups, derivations and interactions. When non-normality was found, a Kruskall-Wallis test was applied for comparisons. Finally, Pearson coefficient correlations between POST-C age and the average AP of each broad band and derivation of the 60 newborns were calculated for the sleep stages (QS, AS) and IBIs. For statistical analyses, the R program, 3.2.5 version was used.

RESULTS

The mean duration of the polysomnographies was 66.8 minutes (range: 50-87 min) with 95% lasting 60 min or more. At least one sleep cycle with AS and QS was observed in all studies. Table 2 shows the means, standard deviations and statistical results from sleep for the variables in the 5 study groups. The percentage of trace alternant and the number and maximum duration of the IBIs decreased, while the percentage of QS increased with greater POST-C age, as was expected. AS showed significant differences among groups, but no clear pattern associated with POST-C age. There were no differences in the percentages of wakefulness, transitional sleep or arousals, or in the amount and duration of apneas.

Table 2 about here

EEG quantitative analysis

Results of PCA applied to the AP values of the EEG frequencies in QS and AS rendered three independent broad bands that explained 82.8% of the variance found. Table 3 and Figure 1 show the frequency spectra and 3 broad bands for QS and AS identified: a first component for frequencies of 17-30 Hz that explain 37.4% of variance; a second with frequencies of 2-
10 Hz that explains 24.3%; and a third of 10-16 Hz that explains 21.02% of the variance. A PCA applied exclusively to the 2-10 Hz band demonstrated the absence of independent sub-bands.

Table 4 shows the results of the 3-way mixed ANOVAs (age × sleep stage × derivation) for each broad band. Only significant results after family-wise error correction for multiple comparisons are described. In the interests of clarity, they are described for each significant main effect and interaction together for the 3 bands.

Age main effect

An age main effect was significant only for the 2-10 Hz band. Post-hoc comparisons of the AP of this band indicated significant differences among all age groups (p<0.01) with greater power as age increased, except between 42-43 weeks of POST-C age (Figure 2A).

Sleep main effect

Sleep main effects were significant for all 3 broad bands. The AP was significantly higher in QS than AS in each one (Figure 2B). Age-by-sleep interaction was not significant.

Derivation main effects and sleep-by-derivation interactions

Derivation main effects were significant for all 3 bands, as AP was higher at the central but lower at the temporal derivations in all three (not shown). Since sleep-by-derivation interactions were also significant for the 3 bands, they are described in more detail.
Sleep-by-derivation interactions for the 2-10 Hz band corroborated that AP was higher in QS than AS at all derivations, and also higher at the central derivations, but lower at the temporal ones (Figure 3), with significant differences among all derivations in both QS and AS, except between the left and right occipital and temporal derivations. For the 2-10 Hz band, there was a significant asymmetry between the left and right hemispheres at the central electrodes with higher power in the left central area in both sleep stages. The occipital and temporal derivations had higher power in the right hemisphere for the 17-30 Hz band, during QS and AS.

Sleep-by-derivation interactions for the 10-16 Hz band corroborated that AP was higher in QS than AS, except at O2. The AP of this band was significantly higher at both central derivations but lower at both temporal ones in QS; whereas in AS it was higher at O2 and the differences of O2 vs. the central derivations disappeared (Figure 3). The significant asymmetry observed in the 2-10 Hz band between the left and right central derivations also disappeared, but was maintained between temporal derivations in both sleep stages. Figure 3 shows the sleep-by-derivation interactions for the 17-30 Hz band, where AP at F4, C3 and C4 is higher in QS than AS, through there are no differences between sleep stages at O1, O2, T3 and T4. Significant asymmetries between O1, T3 and O2, T4, in both sleep stages, with higher power in the right hemisphere, are also seen.

Given that AP of the three bands identified by PCA was higher in QS than in AS, Relative Power was calculated (broad band absolute power/ 30 Hz absolute power) to explore if the
relative contribution of each band to total absolute power would give additional information on sleep stages EEG maturation. Relative power of each of the bands was submitted to three-way mixed ANOVAs. Previously, to ensure a normal distribution of data for statistical purpose, the ln(x/1-x) transformation was used before statistical analysis. Results of ANOVAs of relative power of the three bands showed that relative power of the 2-10 Hz band was significantly higher in QS than in AS (F=401, p<0.0001); whereas, Relative Power of 10-16 Hz (F=505, p<0.0001) and 17-30 Hz (F=699, p<0.0001) bands was significantly higher in AS than in QS.

Correlation of AP with age

Table 5 about here

Correlation between POST-C age and AP was significant only for the 2-10 Hz band in both QS and AS (see Table 5 and Figure 4). In this band, AP increased with greater age at all derivations, but there were no significant differences in the correlations between derivations for either QS ($\chi^2$=3.52, p=0.75) or AS ($\chi^2$=4.17, p=0.50).

Figure 4 about here

IBIs of *trace alternant*

The principal component analysis applied to the AP of the EEG frequencies in the *trace alternant* IBIs identified only 2 components, which explained 75.8% of total variance; one from 2-16 Hz that accounted for 36.8%, the other from 15-30 Hz that explained 39.02%.
Results of the 2-way mixed ANOVAs with age (41, 42, 43, 44 weeks) and the derivations for each component (Table 3) showed significant differences for age and derivations with main effects for the 2-16 Hz band, but only a significant derivation main effect for the 15-30 Hz band.

The main effect of age showed that the AP of the 2-16 band differed significantly among 4 age groups, but not between weeks 42 and 43, and was higher with older age. Derivation main effects for AP showed similar significances for the two bands, as AP differed significantly among all derivations except between C3 and O2 and the left and right hemispheres (central, occipital and temporal derivations). AP was higher at central derivations and lower at temporal and frontal ones.

There was a significant correlation between the newborns’ POST-C age and AP for the 2-16 Hz broad band in each of the derivations of the trace alternant IBIs ($r=0.44$, $p<0.0005$). AP increased with age in this band at all derivations (not shown). Correlations for the 15-30 Hz band were not significant ($r=0.10$, $p=0.25$).

**DISCUSSION**

As hypothesized, this study found sleep EEG broad bands in newborns that differ from the Delta, Theta, Alpha and Beta bands conventionally described in awake adult humans, as has been demonstrated in sleep EEGs of both adult humans and rats. In addition, the method employed showed that it is possible to generate new information: three independent broad bands were identified in newborns that explained 82% of the variance found – from 2-10 Hz, 10-16 Hz and 17-30 Hz -, while only two broad bands were identified
during the immature IBIs of trace alternant: from 2-16 Hz and 15-30 Hz. Only the AP of the slow band (2-10 Hz) increased significantly during the first 5 weeks POST-C age, whereas the AP of the 3 broad bands was significantly greater in QS than AS.

Broad bands

The existence of these broad bands is a new finding in neonatal sleep EEG studies, which have traditionally used either the aforementioned conventional bands or 3 Hz narrow bands.\textsuperscript{1,2,6,10,16} The most interesting result was the detection of an independent band from 10-16 Hz which corresponds approximately to sleep spindle frequencies or the Sigma band of NREM in adult humans and cats.\textsuperscript{32,33} Identifying these frequencies as an independent band cannot be achieved using the traditional Beta band, which averages frequencies from 12-30 Hz, nor do they correspond to the fast frequencies of delta brushes since this pattern was not included in the quantitative analysis. This result of independent activity in Sigma frequencies suggests that it may correspond to a rudimentary oscillatory mode of the thalamo-cortical network within sleep spindle frequencies present as early as 41 weeks of POST-C age, but only during clearly-defined QS and AS, and not in the gradually-disappearing immature transitional IBI pattern of trace alternant during the first month of life.\textsuperscript{3,11} Sleep spindles are not visually-apparent in newborns until the age of 1-3 months, or 44 weeks.\textsuperscript{3,8,11,13} It may be that sleep spindles cannot be detected visually in the first month of life because a minimal amount of neuronal populations must be synchronized at the same frequency for this to appear,\textsuperscript{14} and the number of neurons that are synchronized within this frequency range at this stage of development is insufficient. The presence of this
The fast 17-30 Hz band, meanwhile, corresponds to the fast frequencies of the conventional Beta band. The limit between the Sigma and high frequency bands at 17 Hz is consistent with the cut-off point at 17-18 Hz observed between the Sigma and Beta bands in adult humans. Frequencies above 17 Hz correlate with the Beta band during wakefulness and
REM sleep, while those below 17 Hz correlate positively with the Sigma band during NREM sleep, but negatively with Beta during wakefulness and REM sleep.\textsuperscript{35}

While at this stage of development, concordance among the physiological markers of QS and AS has already been attained,\textsuperscript{3,4} our results show that adult-like, state-dependent EEG activity—with higher delta absolute power in NREM sleep and lower in REM, and lower fast activity power in NREM than REM—has not developed, since the AP of the 3 bands is higher in QS than AS. The higher absolute power of all frequencies, slow as well as fast, in QS suggests a massive non-selective cortical response to incoming volleys, likely due to the poor maturation of cortical GABAergic inhibitory interneurons, which do not mature until around postnatal days 11-13 in rodents, and to the still rudimentary cortico-cortical short connections.\textsuperscript{24-26}

Since AP did not show higher power in AS than QS in fast frequencies, the relative power of the three bands was also compared to determine if the relative contribution of each band to total power, showed the same pattern of higher fast activity in AS than QS found in adult sleep. Significant differences between the two sleep stages showed that the relative power of 10-16 and 17-30 Hz over total power was higher in AS than in QS as it could be expected from the known earlier maturation of AS.\textsuperscript{3,4}

Age-dependent changes

The first month of life is an important period for the electrophysiological maturation of the cerebral cortex and the thalamo-cortical circuit. It is characterized by increased myelination, the establishment of new synaptic connections and short range cortico-cortical
connections, and the shift from depolarizing to hyperpolarizing action by the GABAergic system.

In terms of the age-dependent changes that occurred during the first 5 weeks postpartum in the sleep EEGs of the healthy full-term newborns studied, we found two main results: 1) that only the slow band from 2-10 Hz increased significantly with age, while the Sigma and Beta bands did not; 2) right-left asymmetries.

AP of the slow band (2-10 Hz) increased during the first 5 weeks of life. This finding is consistent with observations on longer life spans in pre-term and full-term newborns. The steady increase in the AP of the slow band (2-10 Hz) during the first 5 weeks of life that correlated positively with POST-C age and the presence of a rudimentary Sigma band are consistent with the gradual maturation of the GABAergic hyperpolarizing influence of the reticular nuclei neurons on relay thalamo-cortical neurons in the thalamus.

While the AP of the slow frequencies increased with age, that of the Sigma and fast bands did not do so in either QS or AS. The lack of differences with POST-C age in these frequencies in newborns is consistent with previous studies that reported decreases in the fast frequencies from pre-term to around 40 or 44 weeks. During the transition from pre-term to full-term, delta brushes that consist in slow waves crowned with fast frequencies abound. The Fourier analysis of these transients has shown a large amount of power around frequencies above 10 Hz within the burst that disappears if Fourier analysis is run after removing the delta brushes. This author found that power above 10 Hz decreases in both
sleep stages from 35-45 weeks, but increases from 46-52 weeks. The decrease correlates with the decreasing incidence of delta brushes.\textsuperscript{16}

The fast frequencies depend on the depolarizing effect of brain stem cholinergic and monoaminergic activating systems, which are functional by gestational week 35\textsuperscript{20,26} on the inhibitory action of cortical GABAergic interneurons\textsuperscript{34} and on the complex interaction of the brainstem and the thalamo-cortical network.\textsuperscript{21} Studies in rodents have shown that state-dependent or arousal modulation and the continuity of fast activity correlate with the maturation of the dorso-lateral geniculate nucleus of the thalamus and the thalamo-cortical network.\textsuperscript{16} This change also coincides with the shift from depolarizing to hyperpolarizing action in the GABAergic system. Thus, the lack of state-dependent modulation of AP of fast activity at this stage of development may result from the interplay of the immaturity of the thalamo-cortical network with that of the cortical GABAergic feedback circuitry and the still incomplete shift from the depolarizing to the hyperpolarizing effect of GABA.\textsuperscript{16,24}

Right-left asymmetries

Another contribution of this study is that it identified regional and interhemispheric power differences in healthy neonates with an anterior-posterior topographic dissociation between 2-10 Hz and 17-30 Hz bands that is related to POST-C age. The AP of the slow band (2-10 Hz) was more evident in C3 than C4 in both QS and AS and increased with POST-C age, as described previously,\textsuperscript{2,11,16} while the AP of the fast bands increased in the right occipital and temporal leads, in both sleep stages.
The higher right hemisphere power is consistent with previous results\textsuperscript{2,7,16} and suggests that the right hemisphere may develop more rapidly than the left. The achievement of the left central maximal AP in the 2-10 Hz band in healthy neonates at 40-41 weeks POST-C age and the presence of maximal AP of 17-30 Hz in the right occipital region at 45-46 weeks of POST-C age could constitute biomarkers of two milestones of normal electrophysiological development.

Limitations

There are several methodological questions that must be considered. First, our results are limited to the newborn period from 41-45 weeks of POST-C age in healthy full-term infants, so they need to be corroborated in a larger sample and then extended week-by-week to earlier and later weeks of maturational stages in order to ascertain in what week of age Sigma activity becomes apparent to visual inspection in the form of clearly-defined sleep spindles, and the clear full state-dependency of EEG characteristics for QS and AS is definitively established. Though there are many studies on sleep EEG in pre-term to 3-month-old newborns, most are based on EEG feature correlations with age, mixed pre-term and full-term neonates, analyzed only specific ages, or made no distinction between QS and AS.\textsuperscript{2,8-13} Our findings, in contrast, are based on healthy, full-term newborns with well-characterized POST-C ages and sleep stages. Our results are based on one sole method of analysis (PCA with VARIMAX rotation), which allowed us to generate new information on sleep EEG maturation. This findings suggest that it is worthwhile to explore the use of different methods of EEG analysis, as this may enrich our knowledge of sleep EEGs.
Also, the representativeness of these data could be questioned. Our AP power results are based on 15 to 26 EEG segments extracted from 1 hour of sleep recording after careful inspection for artifacts. Studies have demonstrated that 40 seconds of EEG meet the stationary and stability criteria required for Fourier analysis and do not significantly differ from longer times of analysis, at least in adults; however, further studies that analyze longer times are needed to corroborate the generalization of these results. Another possible limitation is that our sleep recordings were obtained during a short period of about 2 hours during the day, so they may not correspond to data obtained during night-time sleep. However, we would argue that for newborns, whose sleep cycle is only about 40 minutes, this recording time is sufficient for them to complete one sleep cycle. Also, since circadian regulations are not yet established, no circadian variations are to be expected. Clearly, our results need to be corroborated by analyzing longer sleep periods at different circadian times. Another point concerns the limited number of derivations recorded, which indicates the need for high-density recordings in future studies. Especially important is the lack of data from the left frontal derivation, which were lost due to technical problems with the digital files. However, we privileged the same number of newborns in each week rather than including this derivation in fewer newborns. Finally, despite these limitations, we believe that our study extends previous research and provides valuable information on the week-by-week maturation of sleep EEGs in the newborn period.

Conclusion

Broad band limits are important for analyzing sleep EEG activity. The results of the present study show that using spectral analysis and principal component analysis of absolute power
made it possible to identify an independent broad band within the Sigma frequencies. Our findings suggest that maturation of the oscillatory mode of the thalamo-cortical network within spindle frequencies in healthy, full-term newborns begins at least as early as 41 week of POST-C age. They further show the presence of broad bands distinct from those defined visually for awake adults. When studying well-defined sleep stages and POST-C ages, asymmetries become apparent, suggesting the differential development of the left central and right occipital and temporal regions during the newborn period.

Acknowledgments

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Abbreviations

EEG: Electroencephalographic activity
PCA: Principal component analysis
POST-C: Post-conceptional (age)
QS: Quiet sleep
1. AS: Active sleep
2. w: Weeks
3. IBIs: Interburst intervals of trace alternant
4. EMG: Submental electromyogram
5. EOG: Electrooculogram
6. AP: Absolute power
7. ANOVA: Analysis of variance

Disclosure Statement

The authors have no conflicts of interest to declare. Financial Disclosure: none. Nonfinancial Disclosure: none.

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Figure legends

Figure 1. Power Spectra in Quiet and Active sleep stages. Color heat map of the absolute power (μV^2) by frequency (1-30 Hz) for each newborn, sleep stage and derivation in A. Average spectra for quiet (QS) and active sleep (AS) in B; vertical lines show the three broad band limits identified by principal component analysis in independent eigenvectors with percentages explained and factor-loading in the inset. Horizontal lines show significant differences between QS and AS.

Figure 2. Mean and standard error of the log-transformed absolute power of the three bands for age main effect in A (w: weeks of postconceptional age; horizontal lines indicate significant differences), and for sleep main effect in B (AS: active sleep, white; QS: quiet sleep, black). Asterisks indicate significant differences.

Figure 3. Mean and standard error of the log-transformed absolute power of the three bands for each derivation in Quiet sleep (in black) and Active sleep (in white). Horizontal lines indicate significant differences.

Figure 4. Pearson correlation coefficients between the log-transformed absolute power of the three bands and postconceptional age in weeks (w) for quiet sleep in A, and active sleep in B, at the left central derivation (C3).
<table>
<thead>
<tr>
<th>Age groups</th>
<th>41 weeks</th>
<th>42 weeks</th>
<th>43 weeks</th>
<th>44 weeks</th>
<th>45 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
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<td>38.68</td>
<td>39.5</td>
<td>39.1</td>
<td>39.3</td>
</tr>
<tr>
<td>Sd</td>
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<td>0.68</td>
<td>0.9</td>
<td>1.25</td>
<td>0.76</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
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<td>Range (in weeks, days)</td>
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<td>38.0 – 40.0</td>
<td>38.0 – 41.4</td>
<td>38.0 – 41.0</td>
<td>38.0 – 40.0</td>
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<td>Post-conception age</td>
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<td>43.1</td>
<td>44.1</td>
<td>45.6</td>
</tr>
<tr>
<td>Range (in weeks, days)</td>
<td>40.0 – 41.6</td>
<td>42.0 – 42.6</td>
<td>43.0 – 43.6</td>
<td>44.0 – 44.6</td>
<td>45.0 – 46.6</td>
</tr>
<tr>
<td>Postnatal age (days)</td>
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<td>24.3</td>
<td>25.5</td>
<td>36.8</td>
<td>44.7</td>
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<tr>
<td>Range</td>
<td>7 – 18</td>
<td>14 – 32</td>
<td>11 – 37</td>
<td>20 – 48</td>
<td>35 – 53</td>
</tr>
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<td>Birth weight (g)</td>
<td>3185</td>
<td>3183</td>
<td>3212</td>
<td>3236</td>
<td>3163</td>
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<tr>
<td></td>
<td>256.7</td>
<td>360</td>
<td>299</td>
<td>400</td>
<td>315</td>
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<tr>
<td>Head circumference</td>
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<td>37.2</td>
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<td>at the study (cm)</td>
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<td>0.81</td>
<td>1.08</td>
<td>1.19</td>
<td>1.29</td>
</tr>
<tr>
<td>5-minutes Apgar</td>
<td>9</td>
<td>9</td>
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### Table 2. Comparison of sleep EEG visual analysis findings in the newborns’ groups

<table>
<thead>
<tr>
<th>Age groups</th>
<th>41 weeks</th>
<th>42 weeks</th>
<th>43 weeks</th>
<th>44 weeks</th>
<th>45 weeks</th>
<th>Comparison</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>Sd</td>
<td>Mean</td>
<td>Sd</td>
<td>Mean</td>
<td>Sd</td>
</tr>
<tr>
<td>% Quiet Sleep</td>
<td>12.8</td>
<td>5.6</td>
<td>12.5</td>
<td>6.8</td>
<td>11.4</td>
<td>3.5</td>
</tr>
<tr>
<td>% Active Sleep</td>
<td>33.1</td>
<td>7.7</td>
<td>37.7</td>
<td>8.3</td>
<td>43.7</td>
<td>10.7</td>
</tr>
<tr>
<td>% Tracé alternant</td>
<td>33.2</td>
<td>6.7</td>
<td>34.28</td>
<td>7.6</td>
<td>27.9</td>
<td>7.8</td>
</tr>
<tr>
<td>% IBIs</td>
<td>4.96</td>
<td>2.6</td>
<td>2.6</td>
<td>1.09</td>
<td>2.95</td>
<td>1.07</td>
</tr>
<tr>
<td>Max IBIs Duration (s)</td>
<td>5.01</td>
<td>1.2</td>
<td>3.4</td>
<td>0.7</td>
<td>3.77</td>
<td>1.15</td>
</tr>
<tr>
<td>% Transitional Sleep</td>
<td>10</td>
<td>4.8</td>
<td>6.96</td>
<td>2.3</td>
<td>8.3</td>
<td>5.8</td>
</tr>
<tr>
<td>% Wakefulness</td>
<td>15.1</td>
<td>9.9</td>
<td>9.5</td>
<td>7.2</td>
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<td>11.9</td>
</tr>
<tr>
<td>% Arousals</td>
<td>4.26</td>
<td>1.6</td>
<td>4.08</td>
<td>0.94</td>
<td>4.14</td>
<td>2.08</td>
</tr>
<tr>
<td>N Apneas/ min sleep</td>
<td>0.75</td>
<td>0.82</td>
<td>0.61</td>
<td>0.43</td>
<td>0.58</td>
<td>0.49</td>
</tr>
<tr>
<td>Maximal duration of Apneas (s)</td>
<td>7.6</td>
<td>1.42</td>
<td>7.18</td>
<td>1.61</td>
<td>6.5</td>
<td>1.28</td>
</tr>
</tbody>
</table>

Abbreviations: IBIs: interburst intervals of tracé alternant; N: number. F: Fisher’s F value; H: Kruskall-Wallis’s χ² value; p: p-value.
Table 3. Results of principal components analysis of the Absolute Power of Quiet and Active Sleep

<table>
<thead>
<tr>
<th>Components</th>
<th>Eigenvalue</th>
<th>% of Variance</th>
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<tbody>
<tr>
<td>C1</td>
<td>11.2283</td>
<td>37.43</td>
</tr>
<tr>
<td>C2</td>
<td>7.3061</td>
<td>24.35</td>
</tr>
<tr>
<td>C3</td>
<td>6.3073</td>
<td>21.02</td>
</tr>
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</table>

Frequency (Hz) Rotated Vectors

<table>
<thead>
<tr>
<th>Frequency (Hz)</th>
<th>C1</th>
<th>C2</th>
<th>C3</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.13</td>
<td>0.335</td>
<td>-0.013</td>
</tr>
<tr>
<td>2</td>
<td>0.165</td>
<td><strong>0.876</strong></td>
<td>0.138</td>
</tr>
<tr>
<td>3</td>
<td>0.176</td>
<td><strong>0.872</strong></td>
<td>0.208</td>
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<tr>
<td>4</td>
<td>0.212</td>
<td><strong>0.869</strong></td>
<td>0.29</td>
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<tr>
<td>5</td>
<td><strong>0.187</strong></td>
<td><strong>0.825</strong></td>
<td>0.362</td>
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<tr>
<td>6</td>
<td>0.17</td>
<td><strong>0.818</strong></td>
<td>0.406</td>
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<tr>
<td>7</td>
<td>0.184</td>
<td><strong>0.724</strong></td>
<td>0.521</td>
</tr>
<tr>
<td>8</td>
<td>0.197</td>
<td><strong>0.696</strong></td>
<td>0.524</td>
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<td>9</td>
<td>0.252</td>
<td><strong>0.653</strong></td>
<td>0.597</td>
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<td>10</td>
<td>0.286</td>
<td><strong>0.636</strong></td>
<td><strong>0.63</strong></td>
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<td>11</td>
<td>0.311</td>
<td>0.549</td>
<td><strong>0.692</strong></td>
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<tr>
<td>12</td>
<td>0.378</td>
<td>0.444</td>
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<tr>
<td>13</td>
<td>0.432</td>
<td>0.475</td>
<td><strong>0.668</strong></td>
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<td>14</td>
<td>0.425</td>
<td>0.353</td>
<td><strong>0.74</strong></td>
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<tr>
<td>15</td>
<td>0.47</td>
<td>0.359</td>
<td><strong>0.711</strong></td>
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<tr>
<td>16</td>
<td>0.524</td>
<td>0.286</td>
<td><strong>0.705</strong></td>
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<tr>
<td>17</td>
<td><strong>0.651</strong></td>
<td>0.242</td>
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<tr>
<td>18</td>
<td><strong>0.697</strong></td>
<td>0.21</td>
<td>0.548</td>
</tr>
<tr>
<td>19</td>
<td>0.748</td>
<td>0.183</td>
<td>0.479</td>
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<tr>
<td>20</td>
<td><strong>0.828</strong></td>
<td>0.147</td>
<td>0.363</td>
</tr>
<tr>
<td>21</td>
<td><strong>0.824</strong></td>
<td>0.144</td>
<td>0.4</td>
</tr>
<tr>
<td>22</td>
<td><strong>0.887</strong></td>
<td>0.134</td>
<td>0.222</td>
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<tr>
<td>23</td>
<td><strong>0.888</strong></td>
<td>0.146</td>
<td>0.228</td>
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<tr>
<td>24</td>
<td><strong>0.896</strong></td>
<td>0.169</td>
<td>0.202</td>
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<tr>
<td>25</td>
<td>0.906</td>
<td>0.204</td>
<td>0.173</td>
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<tr>
<td>26</td>
<td><strong>0.905</strong></td>
<td>0.2</td>
<td>0.133</td>
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<td>27</td>
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<td>0.219</td>
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<td>28</td>
<td><strong>0.849</strong></td>
<td>0.278</td>
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<td>29</td>
<td><strong>0.834</strong></td>
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<td>30</td>
<td><strong>0.823</strong></td>
<td>0.344</td>
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Table 4. Comparison of the Absolute Power for the broad bands of EEG identified by PCA

<table>
<thead>
<tr>
<th>Bands</th>
<th>Age Groups</th>
<th>Sleep State</th>
<th>Derivations</th>
<th>Age x Sleep</th>
<th>Age x EEG</th>
<th>Sleep x EEG</th>
<th>AxBxC</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-10 Hz</td>
<td>10.4</td>
<td>0.0001</td>
<td>270</td>
<td>0.0001</td>
<td>155.1</td>
<td>0.0001</td>
<td>1.53</td>
</tr>
<tr>
<td>10-16 Hz</td>
<td>2.44</td>
<td>0.05</td>
<td>50.7</td>
<td>0.0001</td>
<td>88.03</td>
<td>0.0001</td>
<td>1.48</td>
</tr>
<tr>
<td>17-30 Hz</td>
<td>2.53</td>
<td>0.05</td>
<td>9.03</td>
<td>0.004</td>
<td>39.7</td>
<td>0.0001</td>
<td>1.66</td>
</tr>
<tr>
<td>2-16 Hz</td>
<td>5.72</td>
<td>0.002</td>
<td>-</td>
<td>-</td>
<td>73.4</td>
<td>0.0001</td>
<td>-</td>
</tr>
<tr>
<td>15-30 Hz</td>
<td>0.95</td>
<td>0.57</td>
<td>-</td>
<td>-</td>
<td>12.4</td>
<td>0.0001</td>
<td>-</td>
</tr>
</tbody>
</table>

Abbreviations: QS, Quiet sleep; AS, Active Sleep; IBIs, interburst intervals of trace alternant. F, Fisher’s F value. P, p-value. Significant differences after family-wise error correction are indicated in bold.
Table 5. Correlation values between newborns' postconceptional ages and Absolute Power (log) of the three broad bands by derivations, in QS and AS.

<table>
<thead>
<tr>
<th>EEG Derivations</th>
<th>2-10 Hz Band</th>
<th>10-16 Hz Band</th>
<th>17-30 Hz Band</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>QS</td>
<td>AS</td>
<td>QS</td>
</tr>
<tr>
<td>F4</td>
<td>0.43</td>
<td><strong>0.000</strong></td>
<td>0.34</td>
</tr>
<tr>
<td>C3</td>
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Abbreviations: QS, Quiet sleep; AS, Active sleep; r, Pearson correlation coefficient; p, p-value. Significant differences are indicated in bold.