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Research Article

Progressive Changes in EEG Features of Quiet Sleep in Late Preterm and Full-Term Newborns

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Abstract

The two main sleep stages –non-REM (NREM) and rapid eye movement sleep (REM)– with all the typical EEG features of sleep in adults are not present in neonatal development. Spectral analysis of quiet (QS) and active (AS) sleep (future NREM and REM sleep) EEGs offers an objective method for characterizing the evolution of EEG differences between these two stages in newborns when both EEG patterns and brain development are rapidly changing. To describe the progressive spectral EEG differentiation of QS and AS in late preterm and full-term healthy newborns, polysomnography of spontaneous sleep was recorded and subjected to spectral analysis and principal component analysis (PCA) to extract independent broad bands in 96 newborns, separated into eight groups (n=12 each): Pret35-37, Pret38-39, Pret40-41, Term40-41, Term42, Term43, Term44 and Term45 weeks of postmenstrual age at the time of the sleep recordings. Absolute power of QS and AS in the narrow bands (1-Hz bins) and of PCA broad bands was compared (Student-*t* tests between QS and AS, and mixed ANOVAs for the difference between QS and AS, with age and EEG derivations as factors, respectively). Narrow band and PCA analyses showed comparable results. Significant findings showed higher EEG power in QS than AS beginning at 2-4 Hz in Pret35-37, and including faster frequencies each week until adult NREM sleep features from 2-16 Hz were found to be significantly higher in QS at 44 weeks. These results show that spectral analysis of EEG provides new insights on sleep EEG maturation.

Keywords: *EEG spectral analysis, development, REM sleep, sleep spindles, newborns, preterm, active sleep, quiet sleep*

Introduction

Electroencephalograms (EEGs) of adult NREM sleep are characterized by high voltage oscillations in the delta frequency range (2-4 Hz) and transient activities, such as the K complex, slow waves <1 Hz, and sleep spindles. The spindles can be appreciated visually in EEGs as oscillations between 11 and 16 Hz [1] with increasing and decreasing voltages in the sigma band. There is evidence to suggest that sleep spindles may be important for cognitive activity [2,3], although results in infants have been less consistent [4,5].

Sleep spindles do not become observable visually in newborns until around 4-9 weeks after term [6]. For EEG oscillations to be visually-appreciated a large number of neurons must be synchronized at the same frequency [7]. Spectral analysis of EEG activity, however, can extract information on the energy or power of frequency oscillations contained in the epoch analyzed, even those that are not apparent under visual inspection [8,9]. Thus, it can provide new information on how sleep features emerge during early stages of development [10-12].

In a previous study of Corsi-Cabrera et al. [13], the sleep EEGs of 60 full-term newborns from 40 to 45 weeks of postmenstrual age (PMA) at the time of sleep recording, were subjected to spectral analysis. The power spectra of each

newborn, their EEG derivations, and sleep stages were then examined by principal component analysis (PCA). Using this approach, an independent band within sigma activity between 10 and 16 Hz was found, which suggests the rudimentary expression of sleep spindle activity well before it can be appreciated visually.

In that work, however, PCA was applied to the power spectra of the 60 full-term newborns together; that is, without separating them by weeks of postmenstrual age. For this reason, it was not possible to determine the exact week of postmenstrual age when sigma activity became differentiated as an independent band and emerged as a distinct feature of quiet sleep (QS) and, potentially, a precursor of adult NREM sleep compared to active sleep (AS), a precursor of REM sleep in adults. Given the importance of sleep spindles for brain functions during NREM sleep, and the need to advance our knowledge of sleep EEG maturation, the aim of the present study was to explore at what week of postmenstrual age sigma activity begins to be detected by PCA as an independent band characteristic of quiet sleep, as compared to active sleep, and whether it will be possible to identify it as an independent band earlier by including late preterm newborns in the study protocol.

Materials and Methods

Participants

96 healthy neonates born at the Children and Women's Specialized Hospital (Querétaro, Mexico) were included in the present study, divided into 8 groups, with 12 babies each, according to their PMA at the time of the study: three groups of preterm neonates aged 35-37, 38-39, and 40-41 weeks, and 5 groups of term neonates aged 40-41, 42, 43, 44, and 45 weeks.

All neonates were selected carefully according to the following inclusion criteria: for the full-term newborns, 1) gestational age at birth, 38-41.5 weeks, defined by a first-trimester, prenatal Doppler ultrasound study; 2) birth weight in the 10th-90th percentiles according to local standards; and 3) absence of pre-, peri- or postnatal complications according to a review of neurological and medical records. Additional inclusion criteria applied to the preterm neonates were: 1) gestational age at birth of 35-37.6 weeks, defined by the last date of menstruation. The anthropometric and neonatal data and the average age ranges of the groups are shown in Table 1.

Table 1. Neonatal and anthropometrical data of participants.

Age groups		Preterm 35-37	Preterm 38-39	Preterm 40-41	Term 40-41	Term 42	Term 43	Term 44	Term 45
GA (weeks)	mean	33	36	36	38.8	38.6	39.5	39.1	39.3
	SD	1.1	0.9	0.7	0.6	0.68	0.9	1.25	0.76
	range	31-35	35-37.6	35-37.6	38-40.4	38-40	38-41	38-41	38-40
PMA (weeks)	mean	36	39	41	41	42.2	43.1	44.1	45.6
	SD	1.1	0.4	0.52	0.6	0.25	0.19	0.19	0.62
	range	35-37.6	38-39.6	40-41.6	40-41.6	42-42.6	43-43.5	44-44.6	45-46.6
Birth W (g)	mean	1701	2358	2681	3185	3183	3212	3236	3163
	SD	138	331	300	256.7	360	299	400	315
HC (cm)	mean	31.5	34.6	35.6	35.3	36.5	37.2	37.6	38.6
	SD	0.7	1.3	0.9	0.63	0.81	1.08	1.19	1.29

GA: Gestational Age at birth; PMA: Postmenstrual Age; Birth W: Birth Weight; HC: Head Circumference at the study; SD: Standard Deviation

The experimental protocol was approved by the Hospital's Ethics Committee and follows the principles of the World Medical Association's Helsinki Declaration (2013). All parents gave their informed written consent to participate in the study.

Sleep recording

The spontaneous sleep of each newborn was studied by one polysomnography after feeding, between 10-12 p.m. or 1-3 p.m. Given the polyphasic pattern of neonatal sleep, all infants were studied while in a lateral position in an open crib during 60-90 minutes. For EEG recording, gold cup electrodes were placed at Fp3, Fp4, C3, C4, O1, O2, T3, and T4, referenced to linked mastoids, according to the 10-20 International System adapted for newborns [6,14,15]. Ocular movements (EOG) were recorded on 2 channels through an electrode placed 1 cm below the external angle of the right eye, and another placed 1 cm above the external angle of the left eye. Sub-mental electromyograms (EMG), thoracic respiratory effort, and electrocardiograms were recorded following standard guidelines [6]. The ground electrode was placed at Fpz. A tubular elastic mesh bandage that fitted the newborn's head allowed us to fix the electrodes in place. Electrode impedances were below 10k. Filters were set between 1-30 Hz for EEG and EOG, and 10-100 Hz for EMG. All polysomnographic signals were recorded using a Medicid-3E EEG system (Neuronic Mexicana S.A., Mexico City). A sampling rate of 200 Hz was used to digitalize all signals.

Sleep analysis

First, all QS and AS stages of the sleep cycle were identified offline in epochs of 30 seconds duration by a clinical neurophysiologist with experience in newborn sleep scoring (LCR), according to standard criteria [6,14]. Wakefulness, indeterminate sleep, *tracé discontinou and tracé alternant* were also identified.

An epoch was recognized as QS if the neonate remained behaviourally immobile with regular breathing and closed eyes. The EEGs showed a predominance of slow activity (1-4 Hz) with a large amplitude (50-150 μV), frequently interrupted by periods of generalized, widespread flattening below 25 μV, which were identified as *tracé discontinou/tracé alternant*. The typical EEG trait of preterm infants, delta brushes, were identified as bursts of activity between 8 and 25 Hz, crowning a slow wave [6,14].

AS was identified when conjugated rapid eye movements were observed in both EOG derivations, accompanied by low-voltage activity at frequencies between 5-8 Hz and amplitudes of 20-35 μV. Very low-voltage EMG was present, although small twitches in isolated muscle groups could occur. Breathing was typically irregular. Indeterminate sleep occurred in epochs where 3 QS criteria co-occurred with 2 for AS [6]. Phasic sleep events like arousals and apneas were also identified.

Waking periods were considered to be those in which the child had open eyes, high muscle tone in the extremities and chin due to spontaneous movements, crying, irregular and high heart and respiratory rates, and vocalizations or active feeding. These EEGs showed continuous synchronous irregular mixed frequency with superimposed frequent movement artifacts. Percentages of sleep stages and arousals were calculated over total sleep time. The maximum duration of *tracé discontinou/tracé alternant* discontinuities were also quantified. The percentage of wakefulness was calculated over total recording time.

Quantitative EEG analysis

Pre-processing

All QS and AS EEGs were segmented into epochs of 2.6 sec for Fourier analysis, meeting the stationary and stability criteria required for spectral analysis [16] and were visually inspected for artifacts. Epochs showing delta brushes, paroxysmal transients, electrooculographic or electromyographic artifacts were excluded so only artifact-free epochs were subjected to Fourier analysis. A senior neurophysiologist (JRG) independently confirmed that the clean epochs were of QS and AS stages.

It was necessary to eliminate electrocardiographic contamination (ECG) in the left frontal derivation as it could not be removed during the recording in one neonate in each preterm group and in two neonates in the Term-41 group.

These ECG artifacts were successfully removed off-line with independent component analysis, using the "runica" method offered in the EEGlab toolbox of Matlab [17].

EEG analysis

At least 23 EEG artifact-free epochs for each newborn were available in QS (mean 72.5 sec, range: 63-80 sec), and 24 segments for AS (mean: 72.5 s, range: 64-80 sec). The Fast Fourier Transform algorithm used by the TrackWalker system (Neuronic Mexicana S.A.) was applied to these EEG segments. EEG absolute power for 1 Hz narrow bands from 1-30 Hz was obtained for each newborn, EEG derivation, and sleep stage (QS and AS). Two strategies of analysis were used: 1) a narrow band spectral analysis to compare QS and AS in each 1-Hz bin; and 2) a broad band analysis using principal component analysis (PCA) to obtain frequencies that covaried and were orthogonally independent from other frequencies. The original data consisting of 30 variables (AP of 1-Hz bins) for each newborn, EEG derivation, and sleep stage were subjected to PCA, according to the methodology described in [13]. One PCA was run for each age group with QS and AS together.

This analysis makes it possible to reduce the number of variables and yields uncorrelated orthogonal components derived from the intrinsic values. After component extraction, the loading pattern was rotated with VARIMAX rotation to maintain the orthogonal relationships between components [18,19]. To determine the frequencies 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. Three or two broad bands, with variable limits for each age group of newborns were obtained (supplementary material). The absolute power of 1 Hz was separated by PCA in a different independent band in all age groups and was not considered in the statistical analyses.

The area under the spectrum curve for each broad band rendered by PCA was calculated for each age group, sleep state, and derivation [20]. To quantify the week-by-week changes in the difference between QS and AS EEG power, the difference between QS minus AS was calculated for each broad band and age group. This construct allowed us to quantify the rapid change of EEG power in QS with respect to AS, while minimizing the influence of specific factors of each newborn that can affect EEGs, such as head size, gyri geometry, and transmission distance. These factors are individual characteristics of each neonate, but are common to both sleep states, so they are eliminated by subtraction.

Statistical analyses

Spectral energy values were log-transformed before statistical analysis to ensure a normal distribution. For the narrow-band analysis, the EEG power of each Hz bin was compared between QS and AS with Student-*t* tests for dependent samples for each age group. The confidence level was 99% ($\alpha \leq 0.01$). Bonferroni experiment-wise correction was applied, and $\alpha \leq 0.0002$ was established to indicate significance.

In order to compare the age groups and EEG derivations, the differences of QS minus AS absolute power for each broad band identified by PCA were subjected, separately, to a 2-way mixed analysis of variance (ANOVA) with age group as the independent variable (35-37, 38-39, Pret41, and Term41, 42, 43, 44, 45 weeks) and EEG derivations as the within-subject variables. The confidence level was 99% ($\alpha \leq 0.01$). Tukey's studentized t-tests were used for post-hoc, planned comparisons between age groups and derivations. Only significant results are described. MatLab [17] and the R program, 3.2.5 version, were used for the graphs and statistical analyses.

Results

Sleep variables

The mean duration of the EEG studies was 64 minutes (range 50-90 min) with 90% lasting 60 min or more to allow the recording of AS and QS in all neonates. Table 2 shows the means and standard deviations of the sleep variables. The percentage of QS remained stable in the preterm and full-term neonates aged 41-43 weeks but doubled its value in those aged 44 and 45 weeks. The percentage of AS ranged from 29-43%, with no definite changes with age.

As expected, the percentage of *tracé discontinou* decreased gradually in the groups of preterm infants, while *tracé alternant* increased gradually between weeks 38 and 42 PMA, stabilized at 43-44 weeks, and decreased to less than half in the 45-week group. The percentages of wakefulness, transitional sleep, and arousals, and the number and duration of apneas were relatively stable in all groups.

Table 2. Neonatal and anthropometrical data of participants.

Age groups		Preterm 35-37	Preterm 38-39	Preterm 40-41	Term 40-41	Term 42	Term 43	Term 44	Term 45
Quiet Sleep %	**mean**	12.5	13.4	11.2	12.8	12.5	11.4	22.7	38.3
	SD	4.4	9.2	3.9	5.6	6.8	3.5	13.1	17.1
Active Sleep %	**mean**	40.6	41.3	42.9	33.1	37.7	43.7	29.7	33.2
	SD	11.7	16.7	11.4	7.7	8.3	10.7	9.1	9.2
***Tracé discontinou* %**	**mean**	34.09	7.29	5.52	4.96	2.6	2.95	2.38	1.23
	SD	11.7	4.2	2.5	2.6	1.09	1.07	1.66	2.23
***Tracé alternant* %**	**mean**	1.29	19.1	23.4	33.2	34.28	27.9	26.1	12.9
	SD	3.1	11.8	9.07	6.7	7.6	7.8	13.6	17.7
Transitional Sleep %	**mean**	4.55	8.29	8.7	10	6.96	8.3	12.06	8.65
	SD	3.2	4.8	5.4	4.8	2.3	5.8	7.4	2.6
Wakefulness %	**mean**	8.9	16.7	18.8	15.1	9.5	14.7	15.9	9.2
	SD	5.1	7.8	14.8	9.9	7.2	11.9	10.7	7.9
Time in arousals %	**mean**	3.4	4.5	5.6	4.26	4.08	4.14	4.97	3.84
	SD	1.7	2.2	3.8	1.6	0.94	2.08	3.4	0.75
N apneas/min sleep	**mean**	1.27	0.79	0.41	0.75	0.61	0.58	0.41	0.66
	SD	0.87	1	0.21	0.82	0.43	0.49	0.51	1.03
Maximum duration of apnea (s)	**mean**	7.7	7.2	7.21	7.6	7.18	6.5	7.08	6.1
	SD	1.7	2.16	2.11	1.42	1.61	1.28	2.25	3.4
N apneas/min sleep: Number of apneas per minute in sleep; SD: Standard Deviation									

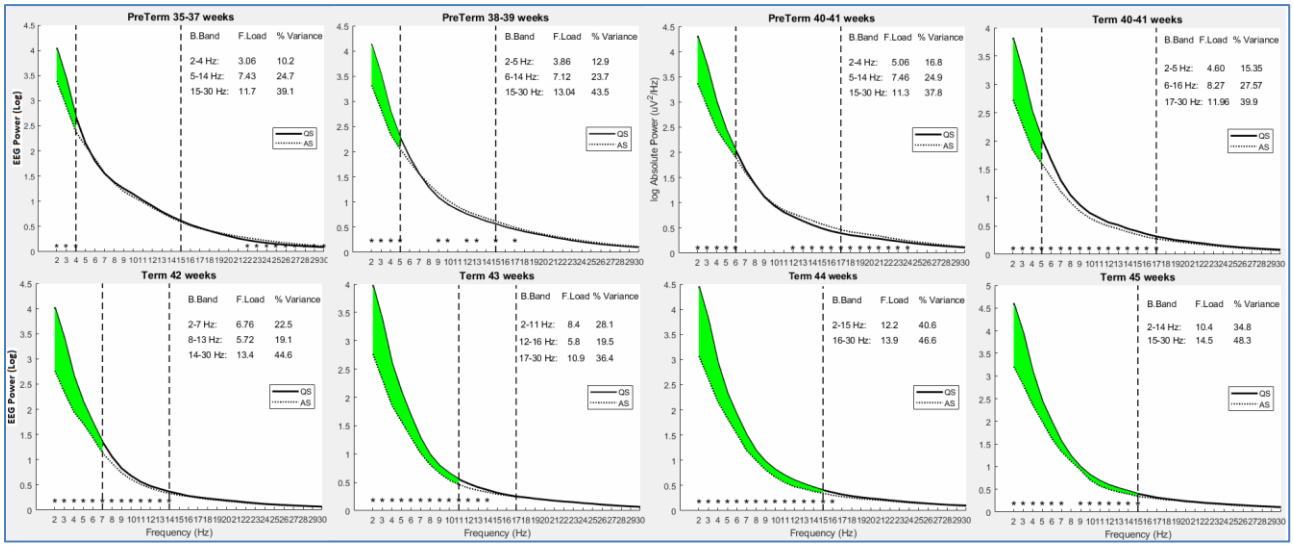

Figure 1. EEG spectra, log-transformed from 2-30 Hz for each newborn age group. Quiet sleep (QS) in continuous line, active sleep (AS) in dashed lines. Asterisks show significant Student-t test differences between QS and AS after Bonferroni correction for each frequency of the narrow band analysis. Dashed vertical lines show the three independent broad band limits identified by PCA, with percentages of variance explained and factor-loading in the inset. Significant difference of age as main factor in the slow band (ANOVA age x derivation) between QS and AS is highlighted in green. The difference in the slow band increases with the age of the groups, and higher frequencies were incorporated to include those features of future adult NREM sleep.

Quantitative EEG analysis

Narrow band differences between QS and AS

As shown in Figure 1, significant results of the Student-*t* tests (asterisks) for the EEG power of the narrow bands in QS and AS showed significantly higher values in the former than the latter in the slow frequencies, but increasingly involved faster frequencies as the age of the groups increased. It appears that the differences between the two sleep stages include higher frequencies during the transition from preterm to term ages, until frequencies of 7-17 Hz were included. In the full-term newborns after 40-41 weeks, the frequencies that showed significant differences in EEG power between QS and AS remained relatively stable in all full-term groups. For the preterm newborns of 35-37, 38-39, and 40-41 weeks, significantly higher EEG power in AS than QS was shown in frequencies around 13-25 Hz. In the full-term neonates, in contrast, EEG power was always higher in QS than AS.

Broad band analysis

Three broad bands, one slow, one intermediate, and one fast, with different limits were identified by PCA for each preterm group, and for three full-term groups: Term41, 42, and 43. In the Term44 and 45 groups, PCA rendered only two broad bands, one slow that included the frequencies of the intermediate band of the younger groups, the other fast. The limits of the broad bands are shown in Figure 1 by the vertical bars in each group. The total variance explained was above 75% in all groups. As can be seen, the upper limit for the slow broad band increased with age. The 1-Hz bin was excluded because it explained only 1% of the variance. The slow band began at 2 Hz and ended at 4 Hz in the Pret35-37 group, at 5 Hz in the 38-39 group, and at 6 Hz in the 40-41 group. In the full-term newborns, the upper limit of the slow band ended at 5 Hz in the Term40-41 group, at 7 Hz in 42, at 11 Hz in 43, and at 15 Hz in the 44 and 45 groups, indicating that the differences between the two sleep stages came to include higher frequencies in the transition from preterm to term ages until sigma frequencies of 7-17 Hz, corresponding to adult sleep spindles, were observed. As Figure 1 shows, the limits of the slow band coincide approximately with the significant results of the narrow band analysis with Student-*t* tests for each frequency bin. The fast band showed similar limits for all PMA age groups beginning around 17 Hz in each one.

Table 3. Results of 2-way ANOVAs for the EEG power differences between QS and AS for the broad bands identified by PCA

EEG broad bands	Factor Age group		Factor Derivations		Interaction Age x Derivations	
	F	p	F	p	F	P
Slow	10.3	**0.0001**	11.1	**0.0001**	3.63	**0.0001**
Intermediate	1.21	0.31	1.88	0.07	1.31	0.11
Fast	1.98	0.06	4.21	**0.0003**	2.01	**0.0002**

Table 3 shows the results of the 2-way mixed ANOVAs (age group x derivation) for the difference of AP of QS minus AS of the EEG broad bands identified by PCA. Age and derivation main effects, as well as age x derivation interaction were significant for the slow band. Figure 1 shows a significant age main effect (shaded area), while Figure 2 presents the results of the post-hoc comparisons for the main effect of age in the slow band. EEG power was higher in QS than AS, and there was a gradual increase among the three preterm groups that was significant between 35-37 compared to Pret40-41, and for Pret38-39 compared to Term40-41. There were also significant differences between the three preterm groups and the five full-term groups, but non-significant differences among the Term42, 43, 44, and 45 groups.

Figure 3A shows the derivation main effect in the slow band. AP was higher at both central and frontal derivations with respect to the right occipital and temporal derivations. The QS-AS difference was also higher in the left than the right occipital region. At the left central derivation, AP was higher compared to the right occipital and both temporal derivations.

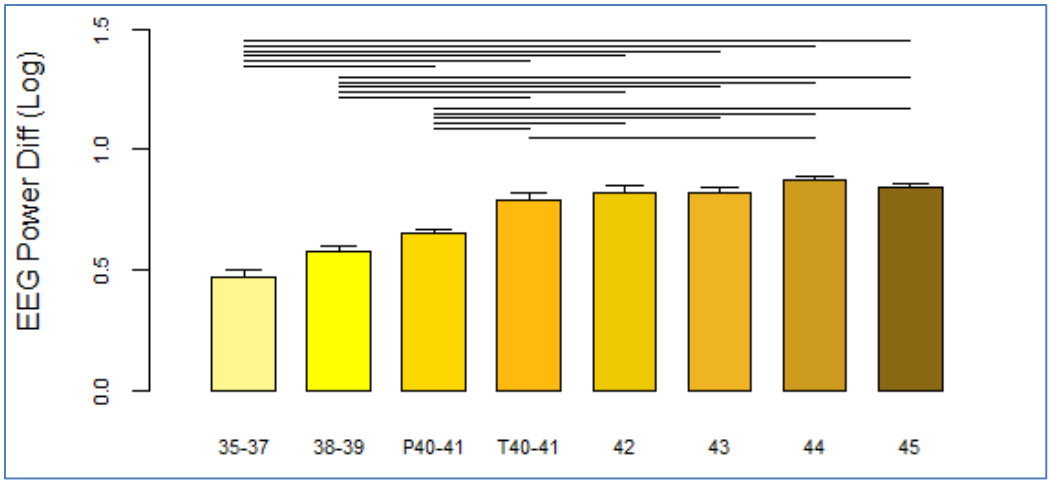

Figure 2. Mean and standard error of the log-transformed EEG power difference between QS and AS in the slow band. There is a significant main effect of age, with differences between the preterm and full-term groups. Horizontal lines indicate significant differences between age groups (P40-41: Preterm 40-41 weeks; T40-41: Term 40-41 weeks).

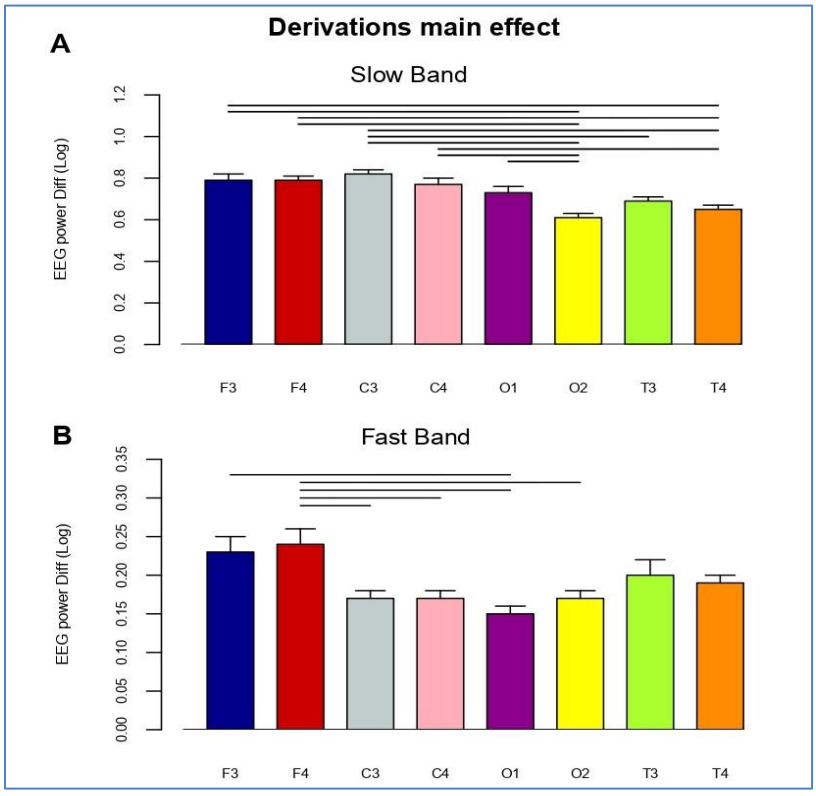

Figure 3. Mean and standard error of the log-transformed EEG power difference between QS and ASfor the slow (A) and fast (B) bands, in each derivation (main effect of Derivations of the mixed ANOVA age x derivations). Horizontal lines indicate significant differences.

There were no significant differences for the intermediate band. The fast band showed a significant derivation main effect, as well as age x derivation interaction. According to the post-hoc multiple comparisons of the derivation main effect, the right frontal derivation showed the greatest AP difference as compared to both the central and occipital leads, and the left frontal compared to the left occipital leads (Figure 3B). Although age x derivation interactions were significant for the slow and fast bands, a larger number of participants in each group would be required to make reliable statistical comparisons among groups at each derivation.

Differences between premature and full-term newborns at term age

Special attention must be paid to the results obtained between the only two groups that were comparable because they had the same PMA: Pret40-41 and Term40-41. The only difference between these two was that one group was born before term while the other completed full-term age. The planned comparisons of these two groups showed that the AP difference at F3 was significantly lower in the Pret40-41 group than in Term40-41. As Figure 4 shows, the topographic pattern of AP difference differed in the two groups. In the Pret40-41 group, asymmetry was observed between F3 and F4 with significantly higher EEG power in F4 than F3 and O2. These changed in the Term40-41 group, where the highest AP difference covered a larger area and predominated in the bilateral frontal and central regions, and a significantly greater AP difference was found for the left frontal derivation with respect to the ipsilateral temporal derivation. Given that these differences between groups of similar age could be related to the application of ICA to eliminate ECG artifacts in frontal regions, these comparisons were repeated after excluding all the cases in which ICA correction was performed. The significant changes already described were replicated in this new analysis (p<0.01).

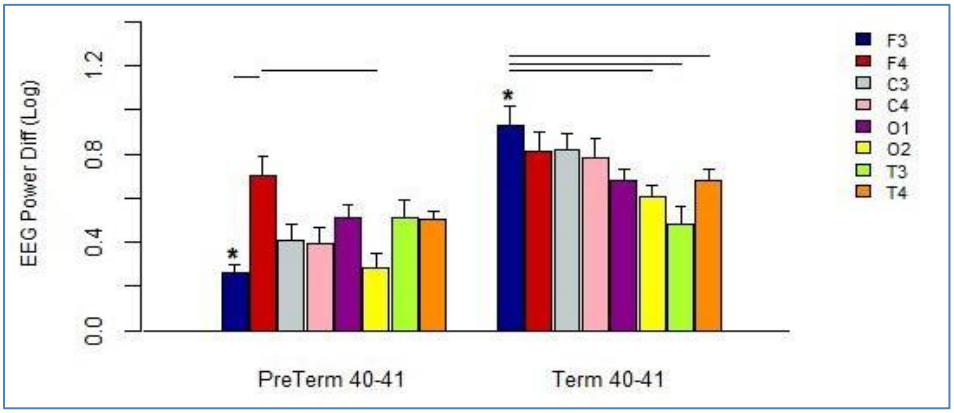

Figure 4. Comparison of the preterm vs. full-term newborn groups at 40-41 weeks of postmenstrual age. Mean and Standard error (log-transformed) of EEG power difference between QS and AS for the slow band in each derivation. Horizontal lines indicate significant differences between derivations; asterisks show significant differences between groups.

Discussion

The aim of this study was to explore the progressive changes of the EEG features of QS compared to AS in low-risk late preterm and healthy full-term newborns from 35 to 45 weeks of postmenstrual age. Narrow band and broad band analyses identified by PCA showed comparable results. The most important finding of the spectral analyses was that the differentiation between the QS and AS EEGs emerged as a process that gradually incorporated more EEG frequencies and showed significantly higher power in QS than in AS each week. That process began with significant differences in the 2-4 Hz range in the 35-37 weeks group until differences, also significant, in AP between QS and AS appeared that included frequencies from 2-16 Hz at 44 weeks which resembled EEG features of NREM sleep observed in adults. Thus, the EEG features distinctive of QS occurred first in slow frequencies in the range of delta activity (2-4Hz), but later were detected in the range of sleep spindle activity.

Principal component analysis identified three broad bands that differed from the traditional ones, with different band limits in each week of postmenstrual age: one slow, one intermediate, and one fast. The slow band, that at 37 weeks of postmenstrual age included only frequencies from 2-4 Hz recruited progressively higher frequencies with each week of age, until at 44 POST-C age the slow band incorporated sigma frequencies in the range of sleep spindle activity at the expense of the disappearance of the intermediate band as an independent band. At that point, these frequencies became part of QS EEG differentiation. Although sleep spindles are not evident under visual inspection until 2-3 months of age [6,21], the present results of the spectral EEG analysis showed that activity within sleep spindle

frequencies are expressed rudimentarily and may be detected as part of QS EEG features from 44 weeks of gestation. The emergence of QS EEG differentiation was more evident in anterior (frontal and central) than posterior derivations, and at left than right occipital ones.

Principal component analysis makes it possible to identify frequencies that covary with each other but vary independently from other frequencies. This suggests that the frequencies which gather are acting under the same modulatory influence [19]; that is, that at 44 weeks delta, theta, and sleep spindle frequencies come under the same modulatory influence, just as occurs during NREM sleep in adults [22,23].

The clock-type delta and spindle frequencies that characterize adult NREM sleep are generated by the synchronous activation of large groups of cortical neurons, determined by the change from the tonic to the oscillatory mode of the thalamic-cortical network. Experimental research with cats [22,24,25] and rats [26] has demonstrated that both frequencies are generated by the same thalamo-cortical relay neuron due to the hyperpolarizing action of the GABAergic neurons of the reticular nucleus of the thalamus on relay neurons and their imposition on cortical neurons. The frequency of oscillation, delta, or spindle frequencies depends on the level of hyperpolarization attained in the thalamo-cortical relay neurons (close to -70 to -90 mV in NREM sleep). The larger the hyperpolarization, the slower the oscillatory frequency [27,28].

The gradual increase in QS-AS EEG differences with age in the slow band identified by PCA analysis can be attributed to the fact that an increasing number of thalamo-cortical and cortico-cortical connections are established at older ages, causing an increasing number of cortical neurons to enter synchronously in the oscillatory burst mode, thus increasing the EEG power of slow activity [24,25]. The possibility of cortically expressing finer and faster gradations of frequencies, such as those characteristics of the gradual transition between phases N2 and N3 of adult NREM sleep, will depend on the progressive establishment of new thalamic-cortical and cortico-cortical synapses, and on the maturation of the GABAergic inhibitory circuits in the thalamic reticular nucleus and cerebral cortex [26,29-31].

However, two types of delta frequencies have been found by intracranial recordings in cats: the clock-type delta that originates in the thalamus, and a second at the cortical level [22]. Unfortunately, scalp recording is not capable of determining the origin of EEG oscillations, so it was not possible in this experiment to ascertain if the activity in the 2-4 Hz range in the preterm groups was of cortical or thalamic origin. The delta frequencies detected in the preterm neonates may be of cortical or thalamic origin, or both. In contrast, the study does show that delta and spindle frequencies, which in adults are part of the same physiological state –NREM sleep– become part of QS EEG differentiation as of 44 weeks of PMA.

The intermediate band from 6-15 Hz and 7-17 Hz was identified by PCA as an independent band showing significant differences between QS and AS in preterm neonates from 35 to 41 weeks of PMA, respectively. Some isolated faster frequencies also showed significant differences in the narrow band analysis. It is not possible with the present methodology of scalp recording to determine the origin and meaning of this activity in the preterm groups, so further research is needed. At this stage of development, the brain undergoes fast changes in, for example, the subplate, a fetal transitory structure located immediately below the cortical plate that is essential in its organization but disappears between weeks 37 and 43 of postmenstrual age [32,33].

Despite the great care taken to exclude any delta brush apparent to eye inspection from the quantitative analyses, the higher power within these frequencies in the preterm groups could be related to the presence of delta brushes, which show their characteristic rapid oscillations between 8-25 Hz crowning the slow wave and are typical of premature sleep in both QS and AS [6,14]. EEG power in the preterm groups in these oscillations may have been detected by the Fourier analysis, though its low amplitude prevented detection by visual analysis. The higher AP in AS compared to QS in the fast frequencies is likely due to the fact that fast activity is more prominent in the former than the latter, and that the

presence of the fast activity inherent in AS and of fast activity in the delta brushes may have aggregated. This is consistent with results reported by Myers et al. [34], who found a 60% contribution of delta brushes to EEG power between 8-25 Hz in preterm neonates.

The fast band showed no significant differences among sleep states or the newborn groups, suggesting that it does not play a role in the electroencephalographic differentiation of QS and AS, at least in ages between 37 and 45 weeks. However, fast activity was more prominent in the right frontal derivations.

The specific comparison between the Pret40-41 and full-term (Term40-41) groups at the same postmenstrual age showed no significant differences in the differentiation of QS and AS EEGs despite the difference in gestational age at birth. This suggests that prematurity does not play a role in QS-AS EEG differentiation. However, the QS-AS differentiation was significantly higher in frontal and central derivations in the Term40-41 group, whereas the differences in the Pre40-41 group were limited to the right frontal region.

Our results should be considered preliminary, exploratory data. Unfortunately, we did not have a large enough number of sleep recordings in low-risk preterm neonates younger than 35 weeks of PMA, which would have allowed us to analyze the emergence of the differentiation of QS-AS with EEG quantitative analysis from an early age, nor serial recordings of the same neonates. We are well aware that accelerated brain maturation occurs in this stage of development, so additional research is needed, based on narrower age differences and a higher number of newborns in each group. Future week-by-week studies using quantitative EEG analysis in older infants (2-3 months) are also required to track the maturation of QS EEGs and incipient sigma activity until the population of neurons synchronized at these frequencies is sufficient for sleep spindles to be appreciated by visual inspection of EEG recordings.

The study of differences in QS and AS EEGs and their rapid change in these ages may be useful for clinical evaluation, especially in preterm infants who are at a higher risk of certain functions being compromised in the future. But this can only be confirmed by analyzing sleep EEGs in a larger number of healthy neonates and in newborns with demonstrated brain damage.

Conclusion

Both approaches, narrow and broad band identified by PCA analysis, add new information on the maturation of the differentiation of QS-AS EEGs between 37 and 45 PMA. QS differentiation in these stages of brain maturation is marked by higher power in QS than AS in slow frequencies, and the recruitment of an increasing number of frequencies until the sigma range is covered. These results show that the main EEG bands –delta, theta, and sigma– features of NREM sleep in adults can begin to be identified as early as 44 weeks of gestation as a distinct pattern of future NREM sleep.

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Disclosure Statement

The authors have no conflicts of interest to declare.

Contribution to the field statement

Neonatal EEG spectral analysis allows the quantitative characterization of sleep EEG maturation. Typical features of NREM sleep, such as high voltage oscillations in the delta frequency range and transient activities like sleep spindles, develop rapidly in the first months of life and have been related to subjects' future cognitive ability. The knowledge of

this development may be useful for clinical evaluation, especially in preterm newborns who have a high risk of certain functions being compromised in the future. However, there is little information about how sleep features emerge in early postnatal stages. This cross-sectional study, conducted with 96 late preterm and full-term healthy newborns, applied principal component analysis to EEG power recorded in neonates at postmenstrual ages that ranged from 35 to 45 weeks. Results show that neonatal sleep EEG differentiation in these stages of brain maturation is manifested in higher power during quiet sleep (NREM precursor) than active sleep (REM precursor) for slow frequencies, and the recruitment of an increasing number of frequencies until delta, theta, and sigma activity, typical features of adult NREM sleep are covered at 44 weeks of postmenstrual age.

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