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A review of important EEG features for the assessment of brain maturation in premature infants.

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Running title: EEG—normal features in preterm infants.
Abbreviations

aEEG, amplitude electroencephalography; AS, active sleep; AW, active wakefulness; EEG, electroencephalography; GA, gestational age; IBIs, interburst intervals; NICU, neonatal intensive care unit; PTT, premature temporal theta; QS, quiet sleep; QW, quiet wakefulness; SAD, slow anterior dysrhythmia; SATs, spontaneous activity transients; STOPS, sharp theta on the occipitals of premature infants;

Abstract

This review describes the maturational features of the baseline electroencephalogram (EEG) in the neurologically healthy preterm infant. Features such as continuity, sleep state, synchrony and transient waveforms are described, even from extremely preterm infants and includes abundant illustrated examples. The physiological significance of these EEG features and their relationship to neurodevelopment is highlighted where known. This review also demonstrates the importance of multichannel conventional EEG monitoring for preterm infants as many of the features described are not apparent if limited channel EEG monitors are used.

Conclusion: This review aims to provide healthcare professionals in the neonatal intensive care unit with guidance on the more common normal maturational features seen in the EEG of preterm infants.

Key notes:

- This review focuses on the normal electroencephalographic features of preterm infants.
- Electroencephalography (EEG) provides useful information about neonatal brain health and prognosis.
- In preterm infants, multichannel EEG is essential to appreciate the complete repertoire of maturational features present.
INTRODUCTION

Despite increased survival of premature infants and some improvements for major
disabilities, disability rates remain high particularly in extremely low birthweight infants (1, 2).
It is simply very hard to mimic the intrauterine environment in the neonatal intensive care unit
(NICU). For babies born too soon, too small and too sick and who need intensive care,
information about the function of the brain is essential. The electroencephalography (EEG),
which measures cerebral electrical activity recorded from electrodes placed on the scalp in
predefined regions, provides critical real-time information about cerebral function. The EEG
can also provide real-time markers of cerebral dysfunction, even when it is secondary to
systemic disease and no macroscopic cerebral lesions are evident (3).

The neonatal EEG contains complex spatiotemporal information that can be difficult to
interpret. It is certainly more complex than the interpretation of other vital sign signals such
as heart rate or respiratory rate. As a result, in many NICUs around the world, a simpler
EEG methodology, using a restricted number of channels (1-2 channels) and a compressed
EEG display has been adopted (the amplitude integrated EEG or aEEG). Over the years,
this device has proved very useful in the hands of experienced users and provides a good
overview of the baseline EEG activity (4-6). However, because spatial information is limited
(1-2 channels) and the raw EEG trace is very compressed, important information (i.e,
specific waveforms and their evolution) about the EEG is lost and this is particularly true for
the premature infant (7,8). For appropriate assessment of the preterm EEG, multichannel
conventional EEG recording is essential (7, 9, 10). We have previously described an
effective technique for EEG application in preterm infants, using pre-packaged, sterile,
disposable, flat-surfaced EEG electrodes that delivers good quality EEG recordings with
minimum handling and minimising the risks of infection (9).
Accurate neonatal EEG interpretation requires a thorough appreciation of the appropriate maturational features for neonates of all gestational ages (GA) as baseline EEG patterns evolve in line with the rapid maturational changes taking place in the brain (11).

The EEG develops in the most immature neonates at 23/24 weeks GA through to full term age with 4 major trends: 1) increasing continuity, with defined periods of EEG quiescence for specific GAs; 2) the appearance of sleep cycling; 3) changes in synchrony between hemispheres; 4) and the appearance of several transient waveforms of prematurity. It is important to highlight that the last two trends (synchrony and appearance of specific transients) can only be detected and assessed in multichannel EEG recordings. Assessment of an infant’s EEG against these 4 parameters can indicate whether the maturity of the brain is appropriate for GA.

In this review we describe the EEG of preterm neonates, highlighting the rich variety of features that appear and disappear as the infant matures, and providing physiological insights into these features and clear illustrations of the patterns and waveforms encountered.

**DISCONTINUOUS VERSUS CONTINUOUS EEG ACTIVITY**

In preterm infants the baseline EEG is discontinuous. This discontinuous pattern is characterised by bursts of high amplitude delta-theta activity (sometimes superimposed by faster activity) intermixed with periods of quiescence (interburst intervals –IBIs-) (Fig. 1).

From a physiological perspective, this feature can be explained from animal experiments that have shown that cortical structures produce spontaneous, intermittent activity (12) that is a crucial endogenous driver for the development of brain connectivity before cortical networks are modulated by the exogenous stimuli/sensory input (13, 14). Furthermore, early in development, GABAergic transmission is not effectively inhibitory and may allow the generation of these endogenous events (15, 16).
Therefore, the electrical activity of early brain networks is characterised by 2 alternating modes of activity: the locally generated spontaneous activity transients (SATs or bursts) (17) and the periods of relative inactivity between them (IBIs) (14, 17).

With the maturation of normal inhibitory GABAergic transmission, spontaneous events are gradually abolished and ‘continuous’ oscillations emerge at different frequencies due to the increasing influence of exogenous sensory driven input (16). Consequently the overall amount of discontinuity decreases and continuity increases with GA (Fig. 2) (18).

**INTERBURSTS INTERVALS (IBIs)**

These are the periods of relative quiescence (low voltage activity) that occur in between consecutive bursts of activity. Their duration is one of the first parameters that is generally evaluated, as it has been shown to reflect brain maturation, being associated with the development of cortical folding (19). IBI duration decreases with GA (18) (Fig.1,3). Although there has been inconsistency in the duration of IBIs used by different authors in different cohorts (see Andre’ et al. (20) for a complete review), normative values in different GAs have been now reported as follows:

- ≤ 60 sec, between 23 and 27 weeks GA;
- ≤ 30 sec (40 sec might be accepted if occasional), 28-29 weeks GA;
- ≤ 20 sec, 30-31 weeks GA;
- ≤ 10-15 sec, 32-34 weeks GA;
- < 10 sec, 35-36 weeks GA (20).

As sleep cycling evolves (see below), discontinuity becomes primarily a feature of quiet sleep. For example at 35-36 weeks GA, the EEG should be continuous in active sleep and waking, and discontinuity only occurs in quiet sleep. Aside from the duration of IBIs, their amplitude also changes over time, becoming less suppressed with increasing GAs:
- <15 µV, 23-25 weeks GA;
- <30 µV, 23-25 weeks GA (20) (Fig. 1,3).

Finally, the morphology of burst activity changes with GA; the amplitude of these theta and delta activity bursts decreases with maturity (particularly in the fronto-temporal regions) and superimposed fast activity appears and increases with GA (11, 21).

**SLEEP WAKE CYCLING**

Clear agreement between the EEG state and the behavioural state is recognisable after 30 weeks GA (22), therefore in the older literature it has been reported that proper sleep-wake cycling is clearly visible only after 30 weeks GA (20), and that younger infants are in an indeterminate sleep state (20).

However, when full polygraphic recording is possible (electrooculogram, electromyogram, respiratory monitoring) for a sufficient time period (at least 60 min), differentiation of sleep is detectable in preterm infants <30 weeks GA (22-26). These infants show periods with eye movements and more continuous EEG activity alternating with periods without eye movements and discontinuous EEG activity (22, 23). The duration of two successive periods have been reported to vary from 9 min 40 sec to 55 min 20 sec in 10 preterm infants between 24 weeks 2 days - 26 weeks 4 days GA (23). Curzi-Dascalova et al. previously reported a mean (SD) sleep cycle duration of 39.7(18.6) min in premature infants between 27 and 30 weeks GA (24), whilst Scher et al. reported a mean (SD) cycle duration of 68(19) min in a slightly broader range of GAs (25-30 weeks) (25).

Sleep-wake cycling relies on the maturation of interconnected neural networks located throughout the cortex, diencephalon and brainstem and is recognisable in younger preterm infants because of the influence of deeper brain structures, before proper thalamo-cortical connectivity has developed (26-28).
In the normal preterm EEG, different sleep states and cyclicity is increasingly evident over time and at 35 weeks all sleep stages are clearly recognisable:

- Active wakefulness (AW) and quiet wakefulness (QW): characterised by continuous activity or activité moyenne; in AW with mainly movement and muscular artefacts.
- Active sleep 1 (AS 1): high-amplitude continuous tracing, preceding quiet sleep (QS).
- Active sleep 2 (AS 2): continuous lower-amplitude tracing with more rapid activity, which follows QS.
- QS: discontinuous or semi-discontinuous tracing (20).

For non-expert EEG reviewers, we would recommend becoming familiar with the differences between discontinuous activity (QS) (Fig. 4 A,D) and continuous activity (AS) (Fig. 4 B,C).

SYNCHRONY

Synchrony in the EEG is present when all EEG features occur simultaneously in homologous areas over both hemispheres.

Although interhemispheric synchrony has been shown to increase with increasing GA (20, 29, 30), synchronous bursts/IBI activity between the two hemispheres (paradoxical hypersynchrony) is present in preterm infants <30 weeks GA (29).

Indeed, high-amplitude bursts of activity are synchronous as early as 24 weeks GA, with 88% of bursts being synchronous between 24 and 27 weeks and almost 100% between 28 and 29 weeks GA (Fig. 5A)(22, 23).

High-amplitude delta wave bursts are often synchronous in the occipital areas as early as GA 26-27 weeks (20).

The high amplitude and synchronisation of EEG activity in very early preterm infants might be related to the fact that the cortex is still not fully developed, subcortical (thalamic) control predominates, and gradual synaptogenesis of the corpus callosum is ongoing (22, 29, 30).
Asynchronous activity after 30 weeks is physiological, if seen in less than 50% of the discontinuous EEG periods (29). It persists, with decreasing frequency until 36 weeks, when it is mainly seen at the beginning of QS (Fig. 5B) (20, 29). Thereafter, asynchrony decreases, disappearing at term age (29).

Synchrony is an important feature of EEG maturation, and reflects the development of the corpus callosum and, therefore, the interconnections between the two hemispheres (29, 30). However, the visual assessment of synchrony is difficult in the preterm EEG and ambiguity does exist (30, 31). It is even more difficult to assess in the aEEG, where compression of the EEG does not allow a clear evaluation of this feature. An automated assessment of synchrony would be a more objective and consistent measure of synchrony in the preterm EEG (30-32).

**DELTA ACTIVITY (0-3.5 Hz)**

**HIGH AMPLITUDE, DIFFUSE DELTA**

In earlier GAs (particularly <28 weeks) a common background feature is high amplitude (up to >300 µV), low frequency (0.3 - 1 Hz) mono- or diphasic, smooth activity (with superimposed faster activity on centro-temporal regions) organised as unilateral or bilateral bursts in centro-occipital or temporal regions (less on frontal), or as short sequences (<80 seconds) mainly in the occipital regions bilaterally (20, 22, 23).

**DELTA BRUSHES**

This is one of the most important feature of the preterm EEG. Delta brushes consist of a slow delta wave with fast rhythms superimposed (in the alpha-beta range) mainly on the ascending slope of the slow wave (20, 33, 34). They have been reported in all GAs, but have a peak between 32 and 35 weeks and tend to disappear between 38 and 42 weeks (20, 34). Their amplitude decreases with maturation and the frequency of both the slow component and the superimposed fast rhythms become faster up to term age (20, 22, 33, 34).
Delta brushes are initially seen diffusely over cortical areas, but subsequently become more predominant over central and temporal-occipital regions and finally in the occipital region only (around 36 weeks) (20, 22).

Delta brushes play a central role in early topographic mapping of the primary sensory areas. For example spontaneous waves of activity in the retina drive cortical delta brushes and mapping of the visual cortex before vision is established (35). Similarly, in the somatosensory cortex, spontaneously generated twitches and body movements in immature rats (36) and human preterm infants (37) produce somatosensory feedback, eliciting delta brushes and organise the broad layout of the cortical somatosensory topographic body map, necessary for sensorimotor coordination. On the EEG, delta brushes have been recognised as not only spontaneous activity or in response to endogenously generated movements, but as EEG waveforms that can also be induced by different sensory stimuli in infants less than 35 weeks GA (34, 37-41). Different stimuli evoke delta brushes in the relevant cortical regions: visual inputs evoke delta brushes in occipital regions (38), auditory inputs in temporal regions (41), tactile stimuli to hand and foot in lateral and medial regions of the contralateral central cortex respectively (37), and noxious stimuli to the heel can elicit delta brushes in the mid-temporal regions (39).

The somatosensory cortex develops extensively in the fetus both in human and nonhuman primates, and development of delta brushes from 6 months gestation reflects this process (37). With the gradual disappearance of delta-brushes in general and the progression of background activity from discontinuous to continuous, the sensory-elicited delta-brushes tend to disappear (around 35 weeks GA), and are replaced by mature evoked potential responses (38, 42). Therefore, delta brushes reflect the development of the sensory functions and offer a clear biomarker of brain maturation (41).
Overview of delta activity changes: Delta activity is a major characteristic of the preterm EEG that evolves as the infant matures. Several authors have reported that the amplitude of delta waves decreases with GA (20, 22, 33), particularly those ≤1Hz over occipital areas (43).

**Morphology:** from smooth or with superimposed theta-alpha rhythms to delta brushes (with superimposed faster rhythms -alpha/beta-). **Spatial organisation:** from diffuse, to centro-temporal to occipito-temporal to mainly occipital. **Amplitude:** It decreases with GA from >300µV to a range of 50-100 µV at term age. **Frequency:** from the lowest delta range (0-1 Hz) to the higher delta range (2-3.5 Hz). A visual summary of these changes is represented in Fig. 6.

**THETA ACTIVITY (4-7.5 Hz)**

**OCCIPITAL THETA ACTIVITY**

**STOPS:** This is the acronym used for “Sharp theta on the occipitals of premature infants”. STOPs are similar to premature temporal theta (PTT)-see below-, but are faster in frequency, lower in amplitude, more often unilateral, aside from the obvious difference in location. The incidence is higher in the younger ages, with a peak at 25 weeks (44).

**OCCIPITAL SAWTOOTH:** Regular rhythmic high amplitude 4±7 Hz activities that can occur in isolation in the occipital region or spread temporally and last 0.5 to 3.5 sec. The morphology is typically sinusoidal (45) (indicated with an arrow in Fig. 7 B).

**TEMPORAL THETA ACTIVITY**

**PTT or “TEMPORAL SAWTOOTH”:** Runs of rhythmic theta activity of 4.5-6 Hz, usually bilateral, often asynchronous. Its peak occurrence is at 29-31 weeks GA, and this activity disappears in active sleep (AS) at 32 weeks GA and in quiet sleep (QS) at 33-34 weeks GA (20, 46).
Overview of theta activity changes: Spatial organisation: From 24-28 weeks GA, theta activity is usually diffuse or localised in the occipital regions, then becomes more predominant in the temporal regions between 28 and 30 weeks (22). Therefore, premature occipital theta activity is prominent in infants with GA < 28 weeks, whereas PTT is prominent between 28 and 30 weeks (Fig. 7) (19). These EEG changes are also associated with changes in cortical folding development, which starts earlier in the occipital lobes and proceeds later to the temporal lobes (19). Amplitude: the amplitude of theta activity decreases with GA (being therefore lower for PTT during the peak at 31 GA) (20).

ANTERIOR TRANSIENTS

SLOW ANTERIOR DYSRHYTHMIA

This feature is characterised by short sequences of delta waves, with medium-high amplitude (50-100 µV), in frontal regions. It appears in AS 1, around 36 weeks GA (20)(Fig. 8A).

ENCOCHES FRONTALES

These are EEG transients that can be seen from 33-34 weeks. Immature frontal transients may be repetitive, often smooth, incomplete, and asymmetrical (unilateral or with difference in amplitude/frequency between the two hemispheres). Mature encoches frontales are seen from 35 weeks GA to term, and are diphasic with a small negative deflection followed by a wider and higher amplitude positive deflection. Usually synchronous, they can have a sharp morphology and present a medium-high amplitude (up to 200 µV) (20) (Fig. 8B). Some authors suggest that these transients are representative of healthy maturation (20).

CONCLUSION

In this review we have summarised the main physiological features of the preterm EEG (Fig. 9), their maturational changes and have provided some insight into their significance. These features of the preterm EEG can be used as a template with which to compare the EEG of
individual preterm infants and to determine if the EEG shows a normal developmental trajectory. Interpretation of the EEG of preterm infants, as with full term infants, should be interpreted in the context of any comorbidity that might alter, even transiently, the background EEG activity. Furthermore, preterm EEG interpretation needs to take into account any concurrent medication, such as morphine, surfactant, caffeine which have all been shown to influence the baseline EEG activity (47-50). Although multichannel EEG is predominantly used for neonatal seizure diagnosis in high risk infants (51), and this remains the main indication for its use in developing countries (52), it has the potential to provide valuable objective information about neonatal brain health in both term and preterm infants, including during the transitional period when the brain is particularly vulnerable (53). Furthermore, serial EEGs (including at equivalent term age) are useful for the assessment of brain health particularly if the neonatal course has been difficult, and/or if late-onset seizures are suspected (51). Serial EEGs are also helpful for outcome prediction (54, 55). This review highlights important features of the preterm EEG that can serve as valuable biomarkers of appropriate neurodevelopment, and has illustrated that many of these features are only evident when multichannel EEG monitoring is used.

COMPETING INTERESTS

None declared.

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**Figure 1**: infant 26 weeks GA. The image shows the typical discontinuous pattern of activity with bursts of high amplitude delta-theta activity and IBI of low activity (the time-point of the EEG is indicated with a line on the aEEG; Montage on top right; Sensitivity: 70 µV; High Cut Filter: 70 Hz; Low Cut Filter: 0.500 Hz; Time Base: 20 mm/sec).

**Figure 2**: A) infant 24+5 weeks GA; B) infant 31+5 weeks GA – active sleep. The image shows the clear difference of the EEG activity characterized in A) by discontinuous activity (mainly low voltage activity -IBI- and a brief low amplitude burst –arrow-) and in B) by continuous delta-theta activity of high-medium amplitude with faster rhythms superimposed (the time-point of the EEG is indicated with a line on the aEEG; Montage on top right; Sensitivity: 70 µV; High Cut Filter: 70 Hz; Low Cut Filter: 0.500 Hz; Time Base: 20 mm/sec). On the aEEG, A) discontinuous tracing, thick band because of the broad spectrum of amplitudes; B) continuous tracing, thinner band with a higher minimum voltage and a lower maximum voltage.

**Figure 3**: infant 31+5 weeks GA – quiet sleep, characterised by bursts of delta-theta activity of high-medium amplitude, with superimposed fast rhythms and IBIs with shorter duration and higher amplitude compared to previous ages (the time-point of the EEG is indicated with a line on the aEEG; Montage on top right; Sensitivity: 70 µV; High Cut Filter: 70 Hz; Low Cut Filter: 0.500 Hz; Time Base: 20 mm/sec).

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Figure 4: A-B) infant 24+5 weeks GA: A) discontinuous activity, B) brief periods of continuous activity. C-D) infant 31+4 weeks GA: clear cycling also recognizable on the aEEG A) active sleep characterized by continuous activity (thinner band on aEEG), B) quiet sleep characterized by discontinuous pattern (thicker band on aEEG) (the time-point of the EEG is indicated with an arrow on the aEEG; Montage on top right; Sensitivity: 70 µV; High Cut Filter: 70 Hz; Low Cut Filter: 0.500 Hz; Time Base: 20 mm/sec).

Figure 5: A) infant 25+5 weeks GA, synchrony of high-amplitude bursts (circle) and asynchrony of single delta waveforms (arrows); B) infant 35+5, persistence of some asynchrony between the two hemispheres at the beginning of quiet sleep (arrows indicate the asynchronous waveforms)(the time-point of the EEG is indicated with a line on the aEEG; Montage on top right; Sensitivity: 100 µV; High Cut Filter: 70 Hz; Low Cut Filter: 0.500 Hz; Time Base: 15 mm/sec).

Figure 6: A) infant 24+5 weeks GA: high amplitude, diffuse delta (circle), smooth or with superimposed theta rhythms; B) infant 28 weeks GA: lower amplitude delta, localized in centro-temporal regions, with superimposed fast rhythms (delta brushes) (arrows); C) infant 35 weeks GA: occipital delta brushes (arrows)(the time-point of the EEG is indicated with a line on the aEEG; Montage on top right; Sensitivity: 70 µV; High Cut Filter: 70 Hz; Low Cut Filter: 0.500 Hz; Time Base: 20 mm/sec).

Figure 7: A) infant 24+5 weeks GA: Theta activity (STOPS) prominent in occipital regions (arrow), B) infant 26 weeks GA: Theta activity in centro-temporo-occipital regions (STOPS - arrow- and PTT.-circle-);C) infant 28 weeks GA: PTT in temporal regions (circles)(the time-point of the EEG is indicated with a line on the aEEG; Montage on top right; Sensitivity: 70 µV; High Cut Filter: 70 Hz; Low Cut Filter: 0.500 Hz; Time Base: 20 mm/sec).

Figure 8: A) infant 35 weeks GA-active sleep:- Slow anterior dysrhythmia, brief sequence of rhythmic delta activity on both frontal regions (circles); B) infant 35+5 weeks GA -active sleep:- Encoches frontales on both frontal regions (circles) (the time-point of the EEG is indicated with a line on the aEEG; Montage on top right; Sensitivity: 70 µV; High Cut Filter: 70 Hz; Low Cut Filter: 0.500 Hz; Time Base: 20 mm/sec).

Figure 9: Maturation of preterm EEG features. Key: AS, active sleep; QS, quiet sleep; AW, active wakefulness; QW, quiet wakefulness; STOPS, Sharp theta on the occipitals of prematures; PTT, premature temporal theta; SAD, slow anterior dysrhythmia; GA, gestational age.
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