Sex differences in electrocortical activity in human neonates

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A company has been established for the commercialization of a routine EEG application of the digital system used in the study (SACS). One author (K.L.) owns shares in the company.

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Cerebral cortical activity in healthy, full-term human neonates (10 boys and 10 girls) was evaluated using spectral estimation of electroencephalogram frequency content with new equipment and analysis technique allowing the assessment of the lowest frequencies (i.e. infraslow waves). The activity was analysed under quiet sleep and active wakefulness taking sex into consideration.

During sleep, the mean amount of infraslow activity was 27\% larger in boys, whereas during wakefulness the average amount of higher frequencies was 17\% larger in girls. Both these differences indicate an earlier maturation of cortical function in girls than in boys.

**Keywords:** brain, DC, development, electroencephalogram, neonate, sex, spectral analysis

**Introduction**

The fact that the electroencephalogram (EEG) of the immature brain is dominated by low frequency activity has been recognized for a long time (for a review, see e.g. [1]). This knowledge is founded on registrations that for practical purposes, based on technical requirements of traditional recording equipment, were performed using a high-pass filter of typically 0.5 Hz. Studies indicating the physiologic importance of activity well below that level, however (often referred to as infraslow activity), have been presented. In animal experiments, this kind of spontaneous, endogenous activity has been demonstrated in various parts of the central nervous system (CNS) including the neocortex (for a review, see [2,3]). The activity is thought to reflect processes that are essential for normal development, taking part in the shaping of both local and long distance connections. During recent years, a few studies in human neonates have shown that infraslow activity can be recorded using equipment allowing these kinds of registration. The activity, with durations of about 1–5 s, can be seen in preterm infants, its amount diminishing as development proceeds [4,5]. This change is, as in animals, paralleled by the development of \( \gamma \)-aminobutyric acid type A (GABA\(_A\)) receptor-mediated inhibition through the change in function of the potassium chloride exchanger K\(^+\)-Cl\(^-\) cotransporter-2 (KCC2) [5,6]. One small study indicates that the quantification of the activity in sick neonates may have a prognostic capacity [7].

The aim of the present study was to study infraslow cortical activity in a homogeneous, well-defined group of healthy, full-term neonates in different activity states, using a large number of electrodes. This would enable us to investigate possible effects of sex and activity states on quantitative measures of this kind of neocortical activity. Combining all these features has not been done before and especially the evaluation of the lowest frequencies has the potential to increase knowledge concerning electrocortical function in human neonates.

**Methods**

**Study participants**

EEG recordings were performed in 20 healthy full-term neonates, 10 boys and 10 girls, born after an uneventful pregnancy and delivery. Their gestational age was 282 = 9 days, Apgar score 9.2/9.9/10 ± 0.6/0.3/0, weight 3744 ± 468 g and postnatal age at recording 32 ± 6 h (mean ± SD). None of these variables differed significantly between the sexes. The protocol was approved by the Human Ethics committee of the University of Göteborg and informed consent was given by the parents.

**Recordings**

Recordings were made during two behavioural states, easily defined clinically; quiet sleep (henceforth referred to as sleep) and active wakefulness (henceforth referred to as...
wakefulness). The system used for recording, Signal Archiving and Communication System (SACS Medical AB, Askim, Sweden), is a software system developed within the group that runs on a standard PC [8,9]. The duration of recordings was 108.4 ± 9.6 min (mean ± SD). The EEG was derived from 19 standard silver–silver chloride scalp electrodes 10 mm in diameter attached with paste. They were placed according to the 10–20 system at the positions F3, C3, O1, F4, C4, P4, O2, Fp1, F7, T3, T5, Fp2, F8, T4, T6, Fz, Cz and Pz. To optimize the recording for that of infraslow frequencies (cf. [10]), electrodes, connected to the amplifier and short circuited, were placed in the high sodium content contact paste used (Elefix, Nihon-Kohden Europe, Rosbach, Germany) for equilibration overnight and the skin was carefully peeled before electrode application. An electro-oculogram for the detection of eye movements was recorded from two surface electrodes, one above and one beside one eye, and an electrocardiogram (for artefact detection) was recorded from surface electrodes, one on each shoulder. The EEG signal was amplified using a REFA-72 amplifier (TMS International B.V., Enschede, The Netherlands), which uses a common average reference, and σ-δ modulation to produce a high-resolution 24-bit output at 256 Hz sampling rate with a 69 Hz bandwidth.

**Analysis**

The recordings were analysed visually (M.T.) using a common average reference montage to select artefact-free and stable EEG segments for analysis. The latter aspect was improved by allowing a 30-min equilibration with the electrodes in place on the head of the infant, before the selection of an EEG segment. To allow analysis of infraslow activity, 126 ± 36 s (mean ± SD) of EEG were exported from the SACS system in segments of no less than 30 s each and analysed with standard mathematical software (Matlab, The Mathworks Nordic Offices, Kista, Sweden). The mean was subtracted from each channel to eliminate the risk of leakage from a stable DC level, contaminating the spectral analysis. Thereafter, the EEG segments were windowed using a Hann window followed by Fourier transformation. As the EEG segments were of unequal length, the Fourier transforms resulted in different frequency resolutions. As we wanted to estimate power in different frequency bands, we used all the frequency domain data for each channel to estimate an autoregressive moving average model, yielding one parameterized spectral estimate per channel [11,12]. This procedure allows the user to define a frequency-dependent weight function. We defined these weights to be one for Fourier components corresponding to frequencies up to 30 Hz and zero elsewhere. Powers within two frequency bands were then estimated from the above-mentioned model: 0–0.5 and 0.5–30 Hz. Compared with more conventional methods of spectral estimation, the described procedure produces less variance [12] and allows a balanced estimation of segments of different lengths. The results for all electrodes were controlled by visual inspection of the raw data, the Fourier transformed data together with the parameterized spectral model, and the power within the selected frequency bands. An outlier was defined as a power value >3 SDs above the mean over subjects for an electrode. Such a value led to the exclusion of that particular electrode from analysis. The percentage of electrodes excluded per stage was 5 and 3% (sleep and wakefulness, respectively, not more than four electrodes in any infant). In recordings during wakefulness, eye movements were abundant leading to slow artefacts in frontopolar leads (Fp1, Fp2). Therefore, during this state, the activities recorded by these two electrodes were excluded for all infants. In addition, during wakefulness, stable EEG could not be found in six infants (boys/girls = 2/4), leading to the exclusion of these individuals from the analysis of this state.

**Statistical analysis**

The spectral estimates of the values of power in the two frequency bands from all electrodes were analysed separately for the two states using one-way analysis of variance (with the factor level and degree of freedom 1), taking sex into consideration.

**Results**

In all infants, infraslow activity could be detected by visual analysis. The activity was most prominent during sleep (Fig. 1). During this stage, the amount of this activity was 27% higher in boys than in girls (6535 ± 467 vs. 5153 ± 377 μV², mean ± SEM, F = 5.034, P = 0.025, Fig. 2). In wakefulness, the amount of infraslow activity diminished as expected in both sexes and no sex difference now prevailed. The amount of higher frequencies differed, however, being 17% higher in girls than in boys (216 ± 10 vs. 185 ± 8 μV², mean ± SEM, F = 6.556, P = 0.011).

**Discussion**

The present study is the first to systematically investigate and quantify the infraslow activity in human neonates using a full range of electrodes. Spectral estimation of EEG frequency content was used to compare well-defined, healthy, full-term neonates in terms of sex and activity stage. To simplify comparisons, only two frequency bands were estimated. The lower, 0–0.5 Hz, comprises the bulk of the activity (in this material 95 and 96% in sleep and wakefulness, respectively), that is, the approximate upper limit of infraslow activity [4,13]. This activity was contrasted with higher frequencies, in this study analysed in the frequency band 0.5–30 Hz. During sleep, larger amounts of activity of the lower band prevailed in boys than in girls. During wakefulness, the gender relationship was the opposite concerning the higher frequencies; that is, larger amounts in girls than in boys.

Spontaneous, endogenously driven activity has been demonstrated in the developing CNS of several species. This activity is seen on different levels including the neocortex and is characterized by infraslow waves [2,3,14–16]. These are, at least in newborn mice, almost exclusively coupled to quiet sleep [17]. The underlying processes are believed to be essential for normal development of the CNS [2]. The infraslow waves are due to the excitatory action of GABA via GABAA receptors, which in turn depends on the function of the KCC2 channel. Around birth, there is a switch in the function of this channel, changing the activity of GABA from excitation to inhibition [3,18]. During recent years, these experimental findings have been paralleled in humans. Thus, using EEG equipment allowing the recording of the lowest frequencies, infraslow waves have been demonstrated in sleeping human neonates [4]. Their prevalence decreases towards term, in parallel with
the appearance of the maturely functioning KCC2 channel [5]. A reduced amount of the activity, in sick neonates, may be associated with a poor prognosis [7].

The sex differences during both states – less infraslow activity during sleep and more high frequency activity during wakefulness in girls than in boys – indicate that the CNS of newborn full-term girls is more mature than that of boys. As the amount of infraslow waves is negatively related to the degree to which GABA release produces inhibition, the smaller amount in girls during sleep would indicate a higher capacity for inhibitory activity. This may be part of the reason for the larger vulnerability of the CNS of boys than that of girls in the perinatal period manifested both in an increased tendency for brain damage and in a higher mortality [19,20]. Moreover, many neurodevelopmental disorders are overrepresented in boys. Even after the same perinatal primary cortical damage, experimental evidence shows that secondary influences, related to functional disturbances, dominate in the male sex [21]. Possibly, sex variation in the processes behind the difference in maturation between the sexes, as seen in the present study, is part of the circumstances at play. The background may be related to various mechanisms, probably governed by sex-specific gene activation including sex hormones (see [22] for a review). Advanced CNS development in the human female sex has previously been demonstrated in terms of earlier appearing habituation to auditory stimuli (a basal form of learning) in fetuses [23], an earlier demonstrable, thicker corpus callosum in female fetuses [24] and shorter latencies of visual evoked potentials in female infants [25]. As the infraslow waves of the cortex are thought to be cortically generated [16], the findings of the present study are the first to show a sex difference of such activity in human neonates.

Conclusion

Using spectral estimation of EEG frequency content including the until recently disregarded infraslow activity, this study is the first to report on a sex difference in healthy, full-term human neonates, indicating an earlier development of cortical activity in girls than in boys. A practical consequence of this finding is that a possible effect of sex has to be evaluated in future studies using quantified measures of cortical activity, including those of the EEG.

References


Fig. 1 Example of electroencephalogram (EEG) recorded during quiet sleep in a 1-day-old girl. Two minutes of EEG, 10 s between vertical dotted lines. Infraslow activity is most easily seen as large amplitude, negative (upward) deflections over the posterior parts of the head.

Fig. 2 Power values (means and SEM) from spectral analysis of electroencephalogram in healthy neonates. Sex comparisons (analysis of variance, filled bars for boys) between two frequency bands during quiet sleep and active wakefulness, respectively. *P < 0.05.


