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Spectral characteristics of the newborn rhesus macaque EEG reflect functional cortical activity

Ross E. Vanderwert¹, Pier F. Ferrari², Annika Paukner³, Seth B. Bower³, Nathan A. Fox¹, and Stephen J. Suomi³

¹University of Maryland ²Università di Parma ³Eunice Kennedy Shriver National Institute of Child Health and Human Development

Abstract

Brain electrical activity is one means of assessing neural development in awake, reactive infants. The development of the electroencephalogram (EEG) in the first week of infant rhesus macaque life is poorly understood though recent work has demonstrated the utility of using this measure to assess neural responses to biologically meaningful stimuli. Here we report on the emergence of EEG rhythms in one-week-old infant rhesus macaques under both light and dark conditions. Our data show that the 5 – 7 Hz frequency band responds reliably to changes in illumination. As well, we found EEG in higher frequencies (12 – 20 Hz) that significantly increase between dark and light conditions similar to the increase in the beta band of humans during cognitive tasks. These findings demonstrate similarities between infant human and infant monkey EEG and suggest approaches for future translational research in developmental psychobiology.

Keywords

Development; Electroencephalogram; EEG; Non-Human Primates; Alpha Rhythm

1. Introduction

Rhesus macaques have been an invaluable model for understanding the development of human cognitive and social behaviors and there has been increased interest in comprehending such changes in relation to the maturation of the non-human primate brain [1]. Evidence from a recent imaging study of typically developing rhesus macaques from birth through 4 years of age has shown remarkable similarities to the order and timing of human brain maturation [2]. While MRI provides important structural information, requiring the sedation of the animals, it precludes its application for understanding functional neural activity. The electroencephalogram (EEG) has been a powerful tool for understanding functional neural development in humans [3] and has significant promise for use in non-human primates.

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Corresponding Author: Ross E. Vanderwert, Human Development, University of Maryland, 3304 Benjamin Building, College Park, MD, 20742, rvanderw@umd.edu.

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Prior reports on the characteristics and development of the electroencephalogram (EEG) in rhesus macaques suggested remarkable similarities with the patterns of EEG in humans. In both human and rhesus adults, the dominant frequency in the EEG is around 10Hz and typically ranges from between 8 and 12Hz [4–7]. Moreover, humans and rhesus macaques share similar developmental trajectories, with the dominant frequency beginning around 4Hz in infancy and increasing to 10Hz in adults [8–12]. These early studies in human infants referred to this slow frequency activity in the EEG as “alpha” because of its resemblance to the classic adult alpha. However, in humans, the presence of a dominant “alpha” frequency has not been identified at birth, and some studies suggest that it only begins to emerge around 3 months of age [4–5,13].

EEG oscillations in the first week of life have been investigated in infant rhesus macaques. In the first developmental study of EEG in rhesus macaques, Kennard and Nims [11] measured EEG over the first two years, starting in the first week of life. During all of the testing sessions the infants were swaddled and their eyes were bandaged shut. Recordings were made once the infant had calmed after a period of struggling. They noted that in the first week the dominant frequencies in the EEG were slow wave activity ranging from 3 to 5 Hz, that those frequencies steadily increased over the first year, and that there was almost no activity in higher frequencies.

In contrast to the findings of Kennard and Nims [11], Robert de Ramirez de Arellano [12] measured rhesus macaque EEG activity from birth to 2.5 years. She noted that in the first week of life the EEG of awake infants was characterized by activity in the 5 to 10 Hz range superimposed on irregular slow waves ranging in the 1 to 3 Hz band. The methods used were nearly identical to those of Kennard and Nims [11]: infants were restrained in a chair and blindfolded, but in contrast to the previous study, the state of each monkey was noted during recordings. When the infants became drowsy during EEG acquisition, an increase in the amplitude of the 2 to 3 Hz band was observed [12]. This finding may partially explain why the dominant activity described by Kennard and Nims [11] was lower than the frequencies previously reported.

The two studies discussed above are the extant of the literature examining EEG in the first week of life in rhesus macaques. The descriptive nature of these studies and the lack of methodological information limit the interpretation of the findings and the potential comparison with human developmental studies. Furthermore, during data acquisition in both of these studies, the eyes of the monkeys were blindfolded, and there was no examination of the EEG while the infants sat quietly with their eyes open. In human infants in the second half of the first year of life and in children, the “eyes closed” condition causes an enhancement in the amplitude of the EEG frequency band thought to reflect alpha that is suppressed when the eyes are opened [4,14]. More recently, Stroganova, Orekhova, and Posikera [15] found in human infants that the enhancement of this frequency band could be achieved by placing infants in complete darkness. They examined the EEG of 8- and 11-month-old infants under light and dark conditions and showed a significant enhancement in the 5 to 7 Hz band for 8-month-olds and 6 to 8 Hz band in 11-month-olds. This effect was maximal over the occipital electrodes but was observed over parietal and precentral (FC3 and FC4) scalp locations as well. Marshall, Bar-Haim, and Fox [3] extended these results to 5-month-old infants. They found that the peak frequency in these young infants ranged from 4 to 7 Hz and could be identified across the scalp.

We recently examined the EEG activity of one-week-old infant rhesus macaques while observing biologically meaningful visual stimuli [16]. We identified activity in the 5 to 6 Hz band in the anterior electrodes that responded similarly to visual stimulation of facial gestures that were presented in front of the infant monkeys. The results of this study

suggested that in newborn monkeys the sensorimotor mu rhythm is present at birth and within the range of the rhythms described by Robert de Ramirez de Arellano [12].

The goal of this study was to fill in the gaps in our understanding of the EEG during the first week of life in infant rhesus macaques. Due to the fact that previous research on EEG in monkeys relied on visual examination without relating it to changes in the infant's state or environment [6,11,12], it was unclear under what conditions changes in EEG frequencies would be present in the first stages of postnatal development. Recent studies using computational methods to quantify EEG have suggested that there is functional EEG activity in frequencies around 5 to 6 Hz and investigated the responsiveness of this frequency band by examining the EEG under conditions of both light and darkness.

2. Methods

2.1 Subjects

Subjects were 39 infant rhesus macaques who were involved in ongoing experimental protocols that required separating the infants from their mother on day 1 post-partum. Ten infants were excluded from analyses due either to incomplete data ($N = 5$) or statistical outliers ($N = 5$). Of the remaining 29 infants, 7 provided data on all 4 testing days, 13 from 3 testing days, 8 from 2 testing days, and only 1 provided data from one testing day.

Animals were housed individually in plastic cages ($51 \times 38 \times 43$ cm) that contain an inanimate "surrogate mother," made of a polypropylene cylinder wrapped in a heating-pad and covered with fleece fabric. The incubator was maintained at a temperature of $\sim 27^{\circ}\text{C}$ and at 50%–55% humidity. Lights were on from 07:00 to 21:00. Infants could see and hear, but not physically contact, other infants. All animals were provided with a 50:50 mixture of Similac (Ross Laboratories, Columbus, Ohio, United States) and Rimilac (Bio-Serv, Frenchtown, New Jersey, United States) formulas. They were hand-fed until they were old enough to feed independently, usually by day 4. Formula was administered ad libitum until 4 mo of age.

2.2 Procedure

Subjects were tested on days 1–2, 3–4, 5–6, and 7–8 post-partum. During each testing period the infant was presented with three conditions of an imitation paradigm whilst EEG data were being collected (described by [16]) presented in random order. Following the imitation paradigm, one minute of EEG was collected while the lights were turned off. This "dark" condition was similar to the procedure described by Stroganova and colleagues [15]. The 40-second baseline period while the monkey was observing an inanimate object (a disk; for details see [16]) was used as a visual stimulation control condition. Video and EEG were recorded simultaneously while the infant had its eyes open. A video camera (Sony Digital Video Camcorder ZR600) was positioned 0.5 m behind the disk so that the infant's behavior could be easily identified. The start and end times of epochs where the infant was still and observing the disk were subsequently identified during behavioral analysis (frame-by-frame coding). These start and end times were then combined with the EEG analysis software and only epochs with artifact-free EEG were included in the analyses.

2.3 EEG Acquisition and Analysis

A custom lycra cap was made and fitted with 6 tin electrodes with their placement based on x-rays made of a typical infant rhesus macaques and a plaster mold of an infant skull. Two posterior electrodes were placed on scalp locations above the parietal-occipital lobes and two anterior electrodes over the frontal lobes (Figure 1). The zenith served as reference, and an electrode placed above the forehead served as ground. Each infant's head was shaved and

a mild abrading gel was applied to clean the scalp and improve impedances. Impedances were measured and kept below 20k Ω . Prior to recording EEG, a 50 μ V 10Hz signal was collected for calibration purposes. EEG was band pass filtered (0.1 to 100Hz), digitized with a 16bit A/D converter (\pm 5V input range) and sampled at 1000Hz. Signals exceeding \pm 250 μ V were removed. Epochs of clean EEG identified by the behavioral coding was submitted to a Fast Fourier Transform (FFT) using a 1 second Hanning window with 50% overlap, and spectral power (μ V²) was computed for single hertz bins from 2 to 20Hz. All data processing was performed using EEG Analysis System software, James Long Company.

Preliminary analyses showed no effects of day on any of the spectral power values so, for each infant, spectral power was averaged in each single hertz bin across all available testing days. The distribution of EEG spectral power is generally skewed left [17–18]; so the natural log of each single hertz bin was computed to better approximate a Gaussian distribution. We then computed the difference between dark and light conditions averaged across hemispheres. Figure 2 presents the difference in power between light and dark in the anterior and posterior electrodes. Based on visual inspection of the most sensitive frequencies and the previous literature, we created five bands: 2 – 4 Hz, 5 – 7 Hz, 8 – 11 Hz, 12 – 16 Hz, and 17 – 20 Hz by taking the natural log of the summed power in each single-hertz bin.

For the analyses of the frequency bands, relative power was computed for each electrode by dividing the absolute power in each band by the total power and multiplying by 100. Relative power has been used in a number of developmental EEG studies and has shown to have the greatest sensitivity to frequency composition [3,18–20]. For the band analyses we employed a 2 Condition (dark vs. light) \times 2 Region (anterior vs. posterior) \times 2 Hemisphere (left vs. right) repeated measures ANOVA.

3. Results

3.1 2 – 4 Hz

The results of the 2 – 4 Hz band analyses revealed a significant Condition \times Region interaction ($F(1, 28) = 4.409, p = .045$). Post hoc analyses of the interaction revealed that there were no differences in relative power in the 2 – 4 Hz band between dark and light conditions in the posterior region ($t(27) = .254, ns$), but there was greater power in the dark compared to the light in the anterior region ($t(27) = 2.122, p = .043$).

3.2 5 – 7 Hz

Analyses of the 5 – 7 Hz band revealed main effects for Condition ($F(1, 28) = 11.009, p = .003$) and Region ($F(1, 28) = 13.109, p = .001$) qualified by a Condition \times Region interaction ($F(1, 28) = 8.773, p = .006$). The main effects of Condition and Region showed that overall relative power in the 5 – 7 Hz band was greatest in the dark and in the posterior region. Follow-up analyses of the interaction revealed that the difference between light and dark conditions was greatest in the anterior region ($t(27) = 3.875, p < .001$), while this difference was at trend level in the posterior region ($t(27) = 1.745, p = .092$).

3.3 8 – 11 Hz

There was only a main effect of Region ($F(1, 28) = 4.912, p = .035$) in the 8 – 11 Hz band revealing greater power in the posterior compared to the anterior region.

3.4 12 – 16 Hz

There were significant effects of Condition ($F(1, 28) = 17.811, p < .001$) and Region ($F(1, 28) = 13.783, p < .001$) qualified by significant Condition \times Region ($F(1, 28) = 7.017, p = .$

013) and Condition x Region x Hemisphere ($F(1, 28) = 4.911, p = .035$) interactions in the 12 – 16 Hz band. Unlike the 5 – 7 Hz band where power was greater in dark than light conditions, power in the 12 – 16 Hz band was greater in light than dark conditions in the anterior region (A3: $t(27) = 4.335, p < .001$; A4: $t(27) = 4.769, p < .001$) and in the left posterior scalp location (P3: $t(27) = 2.110, p < .044$) but not the right posterior scalp location (P4: $t(27) = 1.137, ns$).

3.5 17 – 20 Hz

The results of the 17–20 Hz band were comparable to the power in 12 – 16 Hz band. The analyses revealed a significant effect of Condition ($F(1, 28) = 5.266, p = .029$) and Region ($F(1, 28) = 8.305, p = .008$), again qualified by a Condition x Region ($F(1, 28) = 11.242, p = .002$) interaction. Follow-up analyses of the interaction revealed that relative power from the anterior region was greater in light than dark conditions ($t(27) = 3.206, p = .003$) but there was no difference between dark and light conditions in the posterior region ($t(27) = .550, ns$). Figure 3 presents a summary of the effects found for each frequency band.

4. Discussion

The results of this study suggest that there are two broad frequency bands each of which show differential responses to light vs. dark stimulation. The frequency bands of 2 – 4 and 5 – 7 Hz showed increased power during the dark relative to the light conditions particularly in the anterior scalp locations. The frequency bands of 12 – 16 and 17 – 20 Hz showed the opposite pattern: increased power during light compared to dark conditions, again in the anterior scalp locations. These results expand upon the earlier study by Robert de Ramirez de Arellano [12], who showed through qualitative analyses a dominance of frequencies between 5 and 10 Hz in the EEG of week-old infant monkeys while their eyes were bandaged shut.

One of the major limitations of previous studies was blindfolding or bandaging the infants' eyes and neglecting to collect EEG under conditions in which the infants' eyes were open. In contrast, by comparing power in the EEG during both dark and light, we were able to identify activity in higher frequencies present in the infant macaque EEG. In frequencies between 12 and 20 Hz there was a significant enhancement of the signals in the light while the infant is processing visual information, a pattern similar to the increase in beta activity in humans during cognitive tasks [21]. Moreover, this effect was most prominent over the anterior region and may reflect activity in the frontal lobes. This finding is unique to both the developmental human and monkey EEG literature, which do not report any EEG activity above 12Hz in infants. Future studies will need to explore the practical importance of this high frequency activity.

The specificity of condition differences in both the low frequency bands (2 – 4 and 5 – 7 Hz) and the high frequency bands (12 – 16 and 17 – 20 Hz) to the anterior electrode sites suggest that these findings may reflect functional changes in infant attention as a result of visual stimulation. Research in human EEG finds that when subjects attend to stimuli there is suppression of power in low frequencies and increased power in higher frequencies, often interpreted as reflecting general attention processing [21,22] and this effect is typically observed across the scalp [3,15]. In our sample, the placement of the posterior electrodes was not directly over the occipital lobe where the greatest synchronization of the “classical” alpha in humans is usually observed. The posterior electrodes, in fact, were placed in a position that roughly corresponds to the parietal cortex. It is therefore possible that the sensitivity of these electrodes to light-dark changes was limited, and only marginally influenced, by the source that is usually in the occipital region of the scalp.

The human infant EEG literature suggests that an EEG rhythm responsive to visual stimulation may not be present until three months of age, however, the paucity of studies on very young neonates clearly suggests that more work is needed to fill this gap. The differences between human infants and infant macaques in the presence of this responsive EEG rhythm may be a result of the structural differences present between these two species at birth and in the first months of life. Infant macaques have a more mature brain at birth, both in total volume (roughly 64% of the adult size compared to 25% of adult size in human neonates [2]) and in myelination with macaque neonates resembling 3-month-old human infants [23]. Both of these features may contribute to the EEG [24] and explain the early emergence of this EEG rhythm responsive to visual stimulation in the rhesus macaques. Future comparative work is needed, however, in order to bridge the gap between human and monkey brain development.

Identification of an EEG rhythm that is responsive to changes in visual stimulation in the first week of life in rhesus macaque monkeys has practical importance for additional research in the EEG of infant monkeys. We have recently shown that activity in the 5 to 6 Hz band in infant rhesus changes as a function of active imitation or observation of motor behaviors. This sensorimotor EEG rhythm shares the same frequencies responds to the observation and execution of motor actions [25].

There are a number of limitations to this study. First, there were a limited number of electrode positions and differentiation between regions was difficult to achieve with such a limited number of channels. Previous EEG studies with infant rhesus utilized needle electrodes inserted into the scalp [11,12]. The custom EEG cap used in this study allowed for a much less invasive way to obtain EEG data but had a more limited number of electrode sites.

Second, during the dark condition the state of the infant was not known. Most often the dark condition occurred at the end of each EEG testing session when the infants may have been drowsy. Robert de Ramirez de Arellano [12] reported that, in the rhesus macaque at birth, drowsiness was characterized by an increase in slow 2 to 3 Hz activity. This finding may partially explain why we found significant differences in 2 – 4 Hz activity between light and dark conditions.

4.1 Conclusions

This study is the first examine resting EEG in the first week of life in infant rhesus macaques using both noninvasive techniques for the acquisition and computational approaches for the analysis of the data. Moreover, our study goes further and examines changes in the EEG associated with the presence and removal of visual stimuli. We found, under conditions of darkness, different and opposite patterns of activity in two broad EEG frequency bands. Under conditions of visual stimulation, we observed suppression in activity in low frequencies (2 – 4 and 5 – 7 Hz) and enhancement of activity higher frequencies (12 – 16 and 17 – 20 Hz). These findings highlight the presence of measurable EEG signals at birth reflecting functional cortical activity that relate to behavioral and cognitive processing.

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Highlights

- We measured EEG activity from newborn rhesus macaques (1 to 7 days old).
- We identified an alpha rhythm within the 5 – 7 Hz band similar to that of humans.
- We found EEG in higher frequencies probably associated with greater cognitive processing.
- Functional cortical activity at birth may relate to behavioral and cognitive processing.
- The findings point to the use of EEG to measure cognitive processes in newborns.

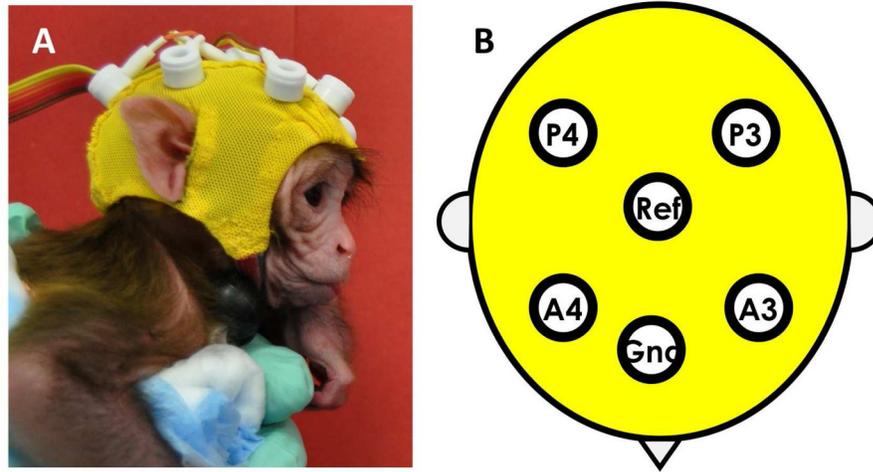


Figure 1. Infant EEG cap and electrode positions. (A) Example of the cap placement on an infant. (B) Electrode layout and position of electrodes in the cap. A = anterior; P = posterior; Ref = reference; and Gnd = Ground.

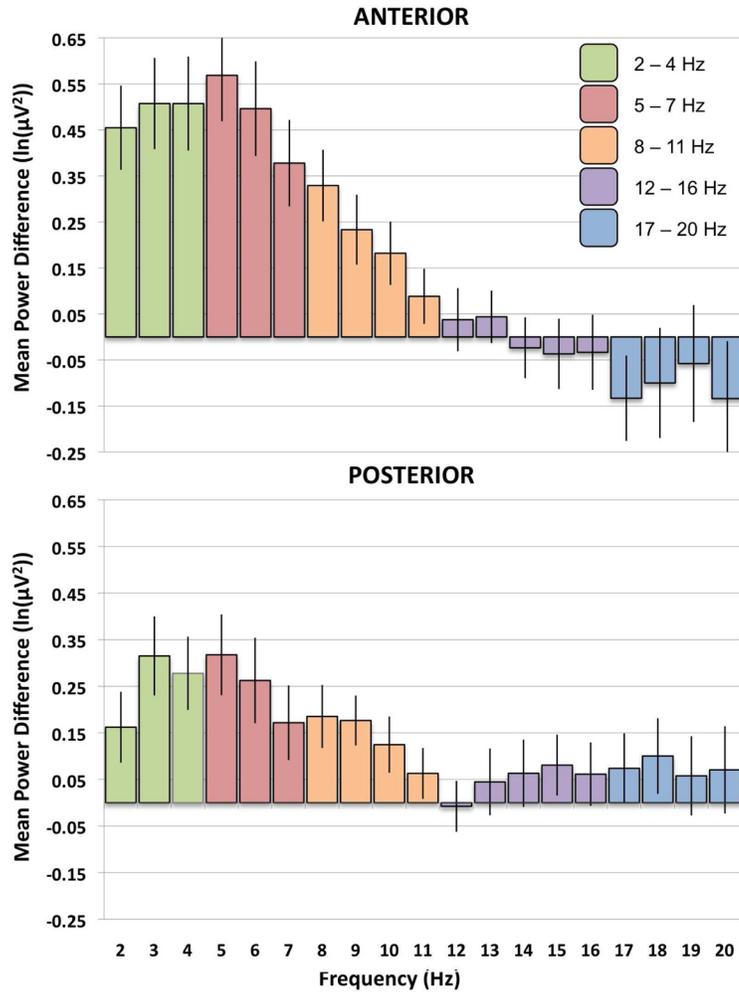


Figure 2. Mean change in power between light and dark conditions for the anterior and posterior electrodes for each frequency bin.

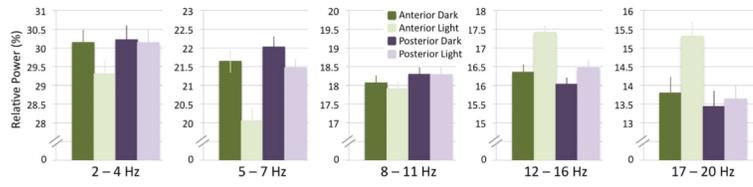


Figure 3.
Mean relative power values for each frequency band for both the dark and light conditions.