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Clinical Observations

Functional Connectivity in Term Neonates With Hypoxic-Ischemic Encephalopathy Undergoing Therapeutic Hypothermia

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ABSTRACT

Background: We investigated whether therapeutic hypothermia and rewarming impact functional connectivity using electroencephalography (EEG) as a measure in neonates with hypoxic-ischemic encephalopathy. We hypothesized that EEG coherence and voltage correlations would be lower and phase lag greater in infants with hypoxic-ischemic encephalopathy than control subjects and that functional connectivity would evolve during therapeutic hypothermia with the greatest improvement occurring during rewarming.
Methods: This study was a retrospective study of 14 term neonates (greater than 37 weeks) with moderate hypoxic-ischemic encephalopathy who underwent therapeutic hypothermia and rewarming. Continuous EEG and video monitoring was conducted for 96 hours during therapeutic hypothermia and rewarming. The primary quantitative EEG measures of functional connectivity were coherence, phase lag, and voltage correlations. These EEG parameters were compared with a cohort of normal age-matched neonates.
Results: Neonates with hypoxic-ischemic encephalopathy had marked decreases in power, coherences, and voltage correlation and increases in phase lag when compared with control neonates. However, there were no significant changes in these measures between therapeutic hypothermia and rewarming.
Conclusions: Neonates with hypoxic-ischemic encephalopathy demonstrate significant abnormalities in functional connectivity compared with control subjects. These abnormalities persist through therapeutic hypothermia and rewarming and are not altered after rewarming. Although hypoxic-ischemic encephalopathy is associated with impaired functional brain connectivity, there is no evidence, using quantitative EEG measures, that therapeutic hypothermia or rewarming either improves or exacerbates these abnormalities in connectivity.

Introduction

Hypoxic-ischemic encephalopathy (HIE) is a significant cause of neonatal morbidity and mortality. Over the last decade, induced therapeutic hypothermia (TH) has become the standard of care in infants with HIE given its ability to significantly reduce neurological impairment and limit secondary cerebral damage.[1,2] Current protocols recommend that TH be initiated within six hours after birth, continued for around 72 hours, and then followed by slow rewarming.

Although TH has clear benefits in reducing morbidity and mortality after HIE, there are some concerns that hypothermia may also have adverse effects on brain development. For each degree of temperature reduction, cerebral blood flow and cerebral metabolic rate of oxygen ($CMRO_2$) decrease approximately 5%[3] with impaired or abolished cerebral blood flow autoregulation.[4] In addition, rewarming may exacerbate brain injury induced by the anoxic event, possibly through mitochondria injury.[5]

In this study, we assessed the effects of TH and rewarming on functional brain connectivity. Electroencephalography (EEG) oscillations arise from rhythmic changes in neuronal excitability and inhibition and the probability of action potential firing is dependent on the phase of the oscillation. The coupling between neuronal oscillations in different brain regions has been commonly assessed in terms of either the correlation of their relative phase of the waveform (phase coherence) (Fig 1) or, on a

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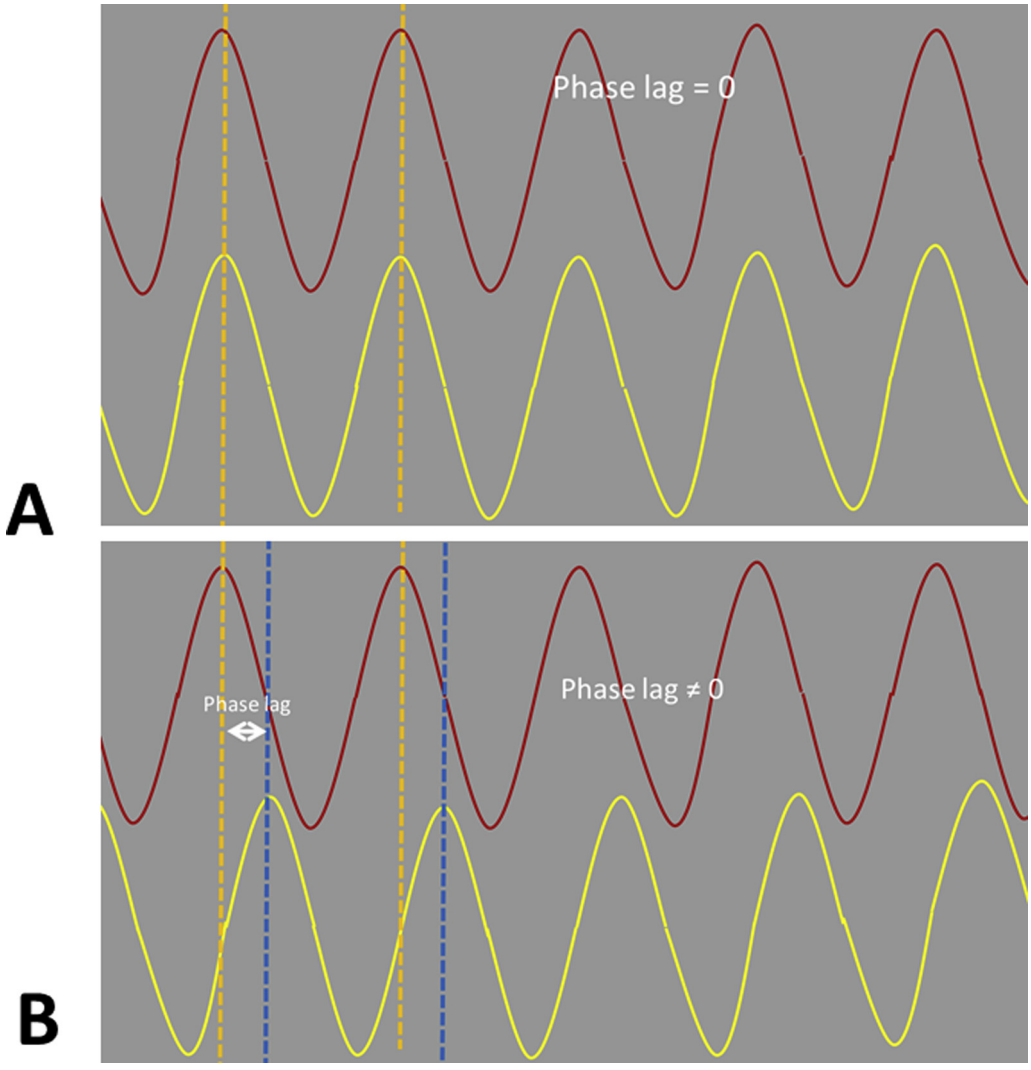

FIGURE 1. Example of coherence measure. (A) The waveforms are in phase and the phase lag is zero resulting in a high coherence. (B) The waveforms are not synchronous resulting in a phase lag >0 and a low coherence. The color version of this figure is available in the online edition.

slower timescale, voltage correlations, a comparison of symmetry of voltages over two brain regions. Phase coherence has a direct functional role in regulating neuronal communication between brain regions. Presynaptic action potentials that are aligned to the excitable phase of postsynaptic oscillations are more likely to result in depolarization and action potentials.[6,7] The phase alignment of oscillatory signals between sending and receiving neuronal populations located in two cortical areas therefore regulates the effective connectivity between these brain regions.[6,8] Voltage correlations, which is independent of phase, are measures of the amplitude (power) of oscillations in two areas and provide an indication of comodulation in the two regions by oscillatory activity.[9]

In this study, we asked whether functional brain connectivity, as measured by coherence, phase lag, and voltage correlations changed with rewarming in infants with HIE. In addition, we compared functional connectivity in age-matched infants with HIE with infants without a history of HIE. We hypothesized that EEG coherence and voltage correlations would be lower and phase lag greater in infants with HIE than control subjects and that functional connectivity would evolve during TH with the greatest improvement occurring during rewarming.

Methods

This study was a retrospective observational cohort study approved by the University of Vermont Institutional Review Board. Data were extracted from the medical records of 14 term neonates (greater than 37 weeks), born between 2013 and 2017, each of whom was diagnosed with moderate or severe HIE and subsequently underwent TH.

All neonates included in this study were treated with whole-body TH according to protocol: 33°C to 34°C of core body temperature initiated within six hours after birth, continued for 72 hours, followed by slow rewarming for more than at least four hours at a rate of 0.5°C per hour until their rectal temperature reached the desired range (36.5°C to 37°C). Medical records of eligible infants were assessed by a neonatologist (M.T.B.) for evidence of moderate or severe encephalopathy according to a modified Sarnat stage of 0 (normal) to 3 (profound encephalopathy).[10] On the basis of this stage, patients were subsequently classified as having clinically moderate (all evaluations $\leq$2) or severe HIE (at least one evaluation greater than 2). Infants with burst suppression, low voltage, (<10 μV) invariant records, or records without discernible state changes were not included in this study.

TABLE.
Demographic and Clinical Characteristics of the Study Population

Demographics	HIE infants
Gestational age (weeks)	40.15 ± 1.23
Birth weight (g)	3645 ± 690
Apgar 1 minute	2 (0, 2)
Apgar 5 minutes	4 (1, 6)
Apgar 10 minutes	5 (3, 7.5)
Sarnat score (initial)	2 (2, 2)
pH (<1 hour after birth)	7.108 ± 0.17
Base deficit (<1 hour after birth)	−15.05 ± 7.27
Seizures	2/14 (14.3%)

Abbreviation:
HIE = Hypoxic-ischemic encephalopathy
Data are presented as the mean ± S.D., median (interquartile range), or percentages.

The demographics of the 14 infants used in the study are provided in Table.

Eight term neonates who had 24-hour EEGs with video performed for possible seizures during the first four days of life were used as control subjects. These records were interpreted as normal by one of the authors (G.L.H.).

EEG recordings

Digital EEGs with video were obtained at or near the time of active cooling and continued for 96 hours. A full set of 21 electrodes was placed using the 10-20 electrode placement system. In addition, electrodes were placed at the lateral canthi of the eyes to record rapid eye movements (REMs) and chin to record the electromyogram. Electrocardiogram and respirations were also monitored. The recordings were done with a Pz reference electrode. Ten-minute segments of waking state, active (REM), and quiet (non-REM [NREM]) sleep were selected for analysis. Epochs with electrographic seizures, arbitrarily defined as 10 seconds or more of rhythmic epileptiform activity, were not used in the analysis.

Records were visually inspected by one of the investigators (J.M.) to eliminate recordings that were contaminated with artifact. EEGs were analyzed using NeuroGuide (Applied Neuroscience, Inc, Largo, FL, USA). Frequencies were analyzed using a Fast Fourier Transform (FFT) with the following parameters: epoch = two seconds at a sample rate of 128 samples/seconds = 256 digital time points and a frequency range from 0.5 to 30 Hz at a resolution of 0.5 Hz using a Cosine taper window as described previously.[11,12] Each two-second FFT is 101 rows (frequencies 0 to 30 Hz) × 19 electrode locations = 1919 elements in the cross-spectral matrix for each infant. FFT absolute and relative power for each of the 19 electrodes for delta (δ) (0 to 4 Hz), theta (θ) (4 to 8 Hz), alpha (α) (8 to 12 Hz), α1 (8 to 10 Hz), α2 (10 to 12 Hz), beta (β) (12 to 25 Hz), β1 (12 to 15 Hz), β2 (15 to 18 Hz), β3 (18 to 25 Hz), and high β (25 to 30 Hz) were calculated. FFT absolute power per hertz (1 to 30 Hz) and power ratios for each electrode (δ/θ, δ/α, δ/β, θ/α, θ/β, and α/β) were measured. FFT coherence for each electrode pair and FFT phase lag (degrees) between electrode pairs were then obtained. Intrahemispheric and interhemispheric pairwise combinations of electrodes were evaluated (171 pairs of electrodes). Split-half reliability coefficient was defined by the ratio of the variance of the even and odd seconds of the selected EEG record and referred to as the proportion of the obtained variance that is true variance. Test-retest reliability was determined by the ratio of the variance of the first five minutes of the recording with the second five minutes to assure there is no change in state. Only recordings in which the split-half reliability coefficient was greater than 0.95 and the test-retest was greater than 0.9 were used.

The FFT mean, variance, standard deviation, sum of squares, and squared sum of the real (cosine) and imaginary (sine) coefficients of the cross-spectral matrix were computed. Cross-spectral power (square root of the sums of the squares of the real and imaginary coefficients), autospectral power (diagonal of the cross-spectral matrix where the imaginary coefficient = 0 and power = sine square), coherence (square of the cross-spectrum divided by the product of the two autospectra), phase (arctangent of the ratio of the real to imaginary components for frequencies from 0.5 to 30 Hz), and voltage correlations (auto-spectral power = (A − B)/(A + B) × 200, where A = EEG channel 1 and B = EEG channel 2) were calculated.

Magnetic resonance imaging data

Of the 14 neonates with HIE, 13 had a brain magnetic resonance imaging (MRI) performed after the cooling was concluded. One of the authors (M.T.B.) coded the MRI based on the Rutherford scale of basal ganglia and thalamic lesions: 0 = no lesions; 1 = mild; 2 = moderate; and 3 = severe.[13]

Statistical analysis

Each of the infants with HIE had four days of recording, the first three days when undergoing cooling, and the fourth day at normal body temperature. Total power, relative power, coherence, phase lag, and voltage correlations for all 171 electrode pairs during each state (waking state, REM, and NREM) pairs were compared for days one to two, one to three, one to four, two to three, and two to four using the paired *t* test. Data from the control patients were compared for each state with day four recordings in the HIE infants using the *t* test. All statistical analysis was done using NeuroStat, the statistical package contained within NeuroGuide. Demographic data are presented as the mean ± S.D., median (interquartile range), or percentages.

Results

When visually analyzed no apparent changes were seen during the 96 hours of recording. Using quantitative EEG techniques, there were no differences in total power, coherence, phase lag, or voltage correlations across days for any of the three states. No significant differences in power, coherence, phase lag, or amplitude asymmetries were noted after rewarming when compared with days one, two, or three.

Compared with control subjects, infants with HIE had decreases in total power (Fig 2) and coherences and increases in phase lag (Fig 3) at all bandwidths. In both REM and NREM sleep patterns coherences were generally higher across all bandwidths in the control subjects than in the HIE group. Phase lag was lower in the control subjects compared with the HIE group, indicating a tighter coupling of waveforms across all frequencies in the control subjects. In addition, compared with control subjects there were significant differences in voltage correlations (Fig 4). Although the amplitude asymmetries are mixed, with some electrode pairs showing increased voltage correlations and others showing lower voltage correlations, in general, during both REM and NREM sleep patterns there was better comodulation of amplitude at electrode pairs in the control subjects than the HIE infants.

Discussion

The primary finding of this study is that in neonates with HIE, TH and rewarming results in no discernible effects on brain connectivity. Using coherence, phase lag, and voltage

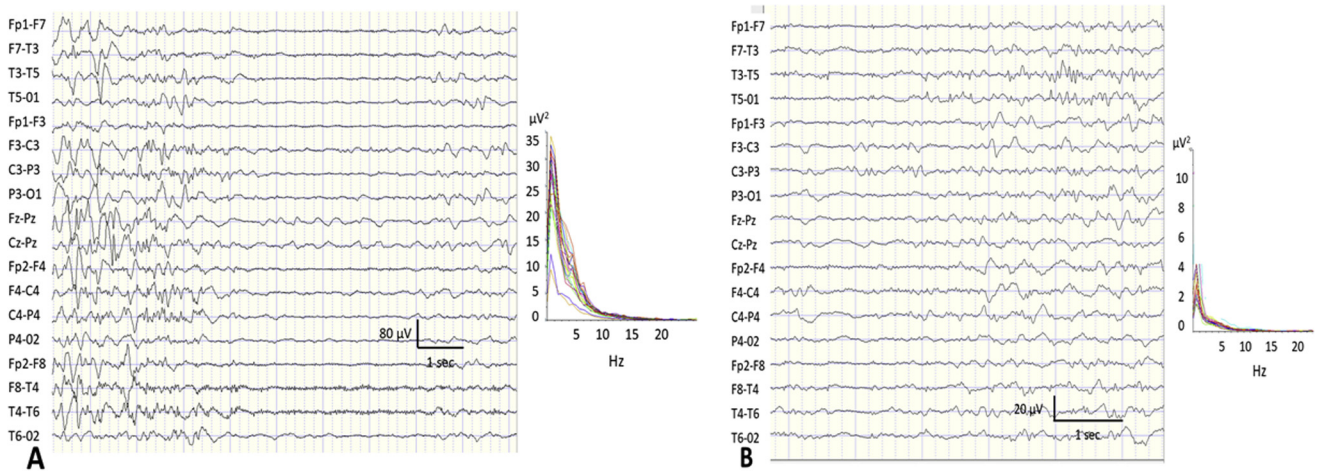

FIGURE 2. (A) Example of NREM (tracé alternant) sleep pattern in normal control infant. Absolute power in graph on right. The Fourier decomposes the EEG time series into a voltage by frequency spectral graph (power spectrum), with power being the square of the EEG magnitude, and magnitude being the integral average of the amplitude of the EEG signal, measured from positive peak to negative peak, across the time sampled. (B) Example of NREM sleep pattern in HIE infant. Absolute power in graph on right. Note the marked decreased in absolute power in the HIE child compared with the control infant. Color lines reflect a subset of the EEG channels. EEG = Electroencephalography; HIE = Hypoxic-ischemic encephalopathy; NREM = Non-rapid eye movement. The color version of this figure is available in the online edition.

correlations there were no significant group differences in the EEG connectivity measures through the four days of recordings. These findings suggest that TH does not impair functional connectivity, as measured by coherence, phase lag, and amplitude correlation to any significant degree in term infants with HIE undergoing TH.

Although TH had no discernible effects on the EEG in the neonates with HIE, there were differences in connectivity between infants with HIE and control infants. HIE was associated with impairments in coherence, particularly during sleep with reduced coupling of oscillatory activity between electrode pairs as measured by phase lag. These findings would suggest that HIE results in less coordinated firing of action potentials between different brain sites. In addition, as evidenced by the voltage correlation data, infants with HIE have reduced comodulation of oscillatory power at different brain regions. Although the significance of decreased voltage correlations after HIE is not entirely clear, overall the findings suggest that HIE results in dysregulation

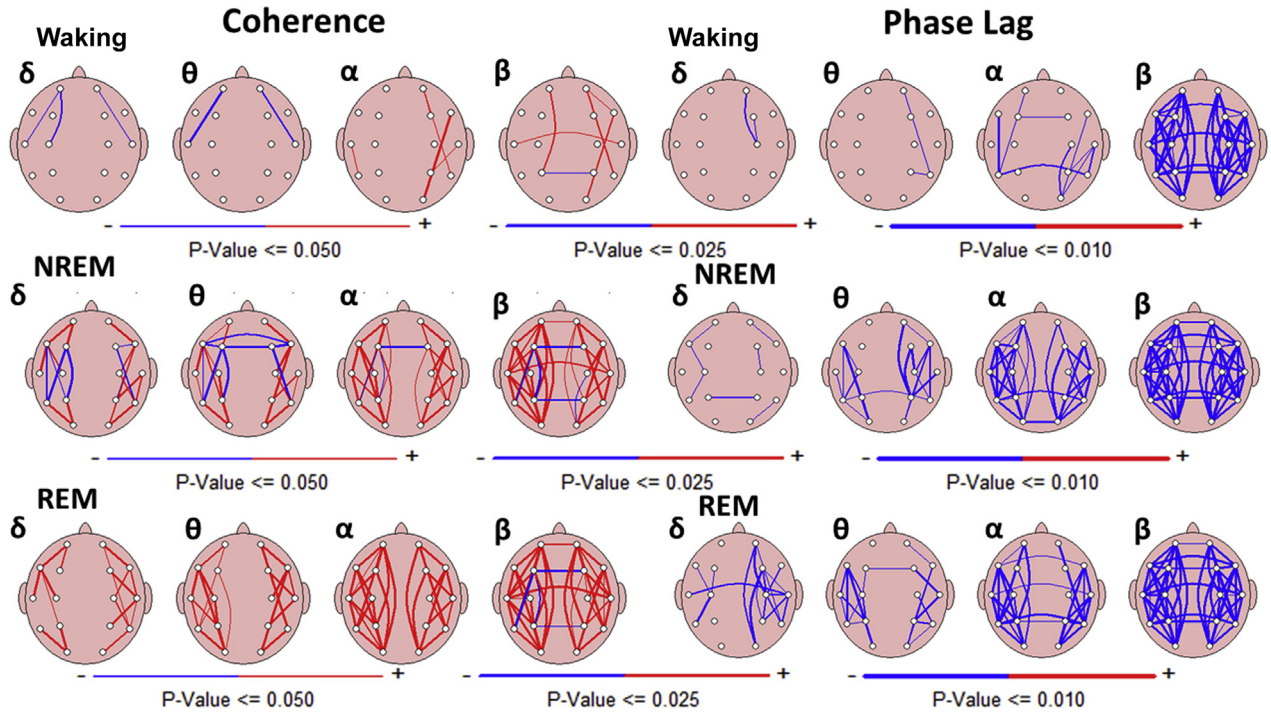

FIGURE 3. Comparison of coherence and phase lag between control subjects (n = 8) and infants with HIE (n = 14) in the waking, NREM, and REM states. Red and blue lines represent *P* values of the *t* test with the degree of significance related to the thickness of the line with the thicker the line the greater the *P* values. Red lines indicate coherences or phase lag greater in the control subjects than in the HIE infants whereas blue lines reflect lower coherences and phase lag in the control subjects than the HIE infants. In general, compared with control subjects, infants with HIE had lower coherences and increased phase lag suggesting that in the HIE infants there was reduced coupling of waveforms across functional networks. HIE = Hypoxic-ischemic encephalopathy; NREM = Non-rapid eye movement; REM = Rapid eye movement. The color version of this figure is available in the online edition.

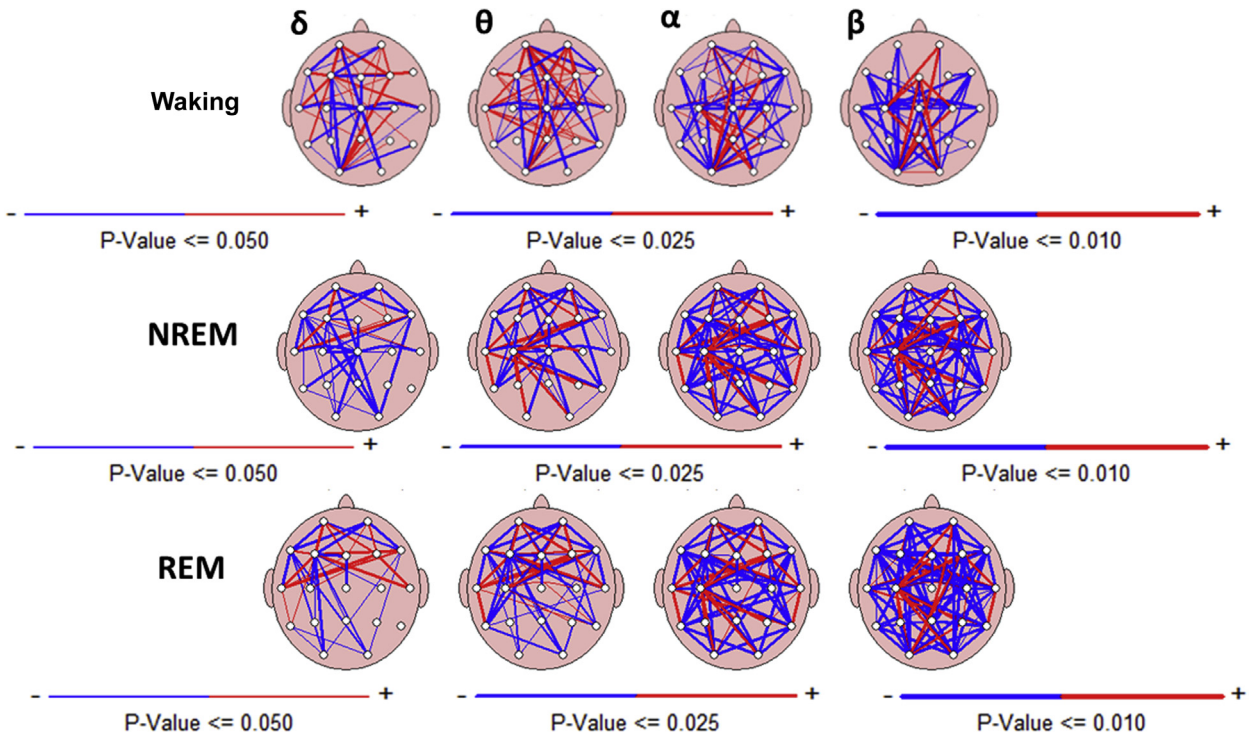

FIGURE 4. Voltage correlations in the control subjects (n = 8) and infants with HIE (n = 14) in the waking, NREM, and REM states. Red and blue lines represent *P* values with the degree of significance related to the thickness of the line with the thicker the line the greater the *P* values. Thus the blue lines would indicate lower voltage correlations in the HIE infants than the control subjects, whereas red lines would indicate increased voltage correlations in the HIE compared with the control subjects. Although variability was present, with some electrode pairs showing greater correlations and other brain regions showing lower correlations, in general, infants with HIE had reduced voltage correlations in all three states. HIE, hypoxic-ischemic encephalopathy; NREM, non-rapid eye movement; REM, rapid eye movement. The color version of this figure is available in the online edition.

of connectivity in the postnatal period. The long-term significance of these findings, if any, is currently uncertain.

Our findings do not indicate that rewarming results in any significant change in the connectivity measures. However, Birca et al.[14] performed quantitative analyses of EEG background from prewarming through hypothermia to postrewarming and found that warming was characterized by increased EEG discontinuity, more pronounced in newborns with severe compared with moderate HIE. They suggested that warming affects EEG background in HIE newborns undergoing transient hypothermia, but it was not clear whether this was a transient adaptive response or reflected an evolving brain injury. Of note, Birca et al.[14] did not assess functional connectivity using coherence measures. Although we found no quantitative changes in the EEGs during rewarming, most infants in our study had moderate HIE as evidenced by the Sarnat and MRI scores and the low incidence of seizures. We cannot rule out the possibility that in more severe infants rewarming could result in evolving brain injury.

It should be further noted that the lack of improvement or deterioration we saw in the transition between cooling and rewarming may be a function of the short time frame the infants were monitored after the rewarming. After complete rewarming we ended the recordings. Further studies would be warranted to evaluate functional connectivity changes over a longer period after rewarming. Whether TH hinders the development of connectivity cannot be answered in this study because our control group only had a single EEG and we did not measure changes in connectivity with serial studies in normal infants. Although it is known that coherences generally increase in the bilateral frontal-parietal networks during the first six months of life,[15] whether coherences would change during the first four days of life is not known. However, based on the rate of coherence changes with age,[12] it seems unlikely that there would be significant changes in coherence over the course of four days.

Conclusions

On the basis of the extensive EEG monitoring in a cohort of children with moderate HIE, we found no evidence that cooling or rewarming has substantial effects on functional brain connectivity.

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