Published in final edited form as:

J Neurosci Methods. 2016 February 01; 259: 135–142. doi:10.1016/j.jneumeth.2015.11.025.

A Novel Technique for Quantitative Bedside Monitoring of Neurovascular Coupling

R. B. Govindan^a, An Massaro^b, Taeun Chang^c, Gilbert Vezina^d, Adré du Plessis^a

^aDivision of Fetal and Transitional Medicine, Fetal Medicine Institute, Children's National Health System, 111 Michigan Ave NW, Washington, DC 20010, USA

^bDivision of Neonatology, Children's National, 111 Michigan Ave NW, Washington, DC 20010, USA

^cDivision of Neurology, Children's National, 111 Michigan Ave NW, Washington, DC 20010, USA

^dDivision of Diagnostic Imaging and Radiology, Children's National, 111 Michigan Ave NW, Washington, DC 20010, USA

Abstract

Background—There is no current method for continuous quantification of neurovascular coupling (NVC) in spontaneous brain activity. To fill this void, we propose a novel method to quantify NVC using electroencephalogram (EEG) and near-infrared spectroscopy data.

New Method—Since EEG and NIRS measure physiologic changes occurring at different time scales, we bring them into a common dynamical time frame (DTF). To achieve this, we partition both signals into one-second epochs and calculate the standard deviation of the EEG and the average value of the NIRS for each epoch. We then quantify the NVC by calculating spectral coherence between the two signals in the DTF. The resulting NVC will have a low resolution with all of its content localized below 1 Hz.

Results—After validating this framework on simulated data, we applied this approach to EEG and NIRS signals collected from four term infants undergoing therapeutic hypothermia for neonatal encephalopathy. Two of these infants showed no evidence of structural brain injury, and the other two died during the course of the therapy. The intact survivors showed emergence of NVC during hypothermia and/or after rewarming. In contrast, the two critically ill infants, who subsequently died, lacked this feature.

Comparison with Existing Methods—Existing methods quantify NVC by averaging neurovascular signals based on certain events (for example seizure) in the EEG activity, whereas our approach quantifies coupling between spontaneous background EEG and NIRS.

Address for correspondence: Dr. R. B. Govindan, Division of Fetal and Transitional Medicine, Fetal Medicine Institute, Children's National Health System, 111 Michigan Ave, NW, Washington, DC 20010, USA, rgovinda@childrensnational.org.

Conflict of Interest

None to declare

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Conclusion—Real-time continuous monitoring of NVC may be a promising physiologic signal for cerebral monitoring in future.

Keywords

Electroencephalogram; Near-infrared spectroscopy; Neurovascular coupling; Spectral coherence; Hypoxic ischemic encephalopathy

1. Introduction

Neurovascular coupling (NVC) is a neural activation support mechanism involving synergistic interactions between the metabolic and vascular systems. The concept of NVC dates back to 1890, when Roy and Sherrington first proposed that the brain possesses an intrinsic mechanism by which the vascular supply can be varied locally in response to local variations in neural activity [1]. With the advent of modern imaging techniques, it is possible to quantify the NVC by simultaneous measurement of cerebral hemodynamic changes and electrocortical signals. Some commonly used techniques to measure cerebral hemodynamics include functional magnetic resonance imaging (fMRI), optical imaging spectroscopy, and LASER Doppler flowmetry (LDF). LDF is an invasive technique reserved for in vivo measurements in animal models, whereas the optical technique is used in animal and human studies. fMRI measures cerebral hemodynamic changes through blood oxygen-level dependent (BOLD) signals. Though these techniques offer high spatial resolution, they are not transferable to bedside application in their current state. Another optical technique with many applications in cerebral hemodynamic monitoring is near-infrared spectroscopy (NIRS). The portable and noninvasive features of NIRS make it suitable for long-term bedside cerebral hemodynamic monitoring [2, 3, 4, 5, 6, 7]. Animal models have shown that the cerebral hemoglobin difference [HbD, oxygenated hemoglobin (HbO₂) minus deoxygenated hemoglobin (Hb)] is a reliable surrogate for cerebral blood flow (CBF) [8]. The standard technique to measure electrocortical activity is electroencephalography (EEG), which is used in routine clinical practice for critically ill patients because it can be performed at the bedside.

Previous studies quantified NVC by associating cerebral hemodynamic changes with certain spontaneous changes in brain activity, such as the discontinuous patterns in the EEG of preterm infants [9]. Simultaneous changes in EEG and NIRS have also been observed during electrocortical seizures in infants [10]. In addition, NVC has also been studied using auditory [11, 12, 13] or visual stimuli [14]. However, conducting an auditory or visual response paradigm is difficult at the bedside in an intensive care unit setting. Further, reliable techniques are needed to identify the spontaneous events in the brain signals of the infant to time lock and average the vascular signals. Thus, in this work we decided to quantify NVC in the spontaneous brain activity and NIRS signals.

Our understanding of the integrity of NVC and its relevance to clinical care and long-term outcome remains impeded by the lack of a real-time quantitative and continuous technique to measure the cerebral hemodynamic responses to electrocortical activation. This void in our understanding is more glaring in cases where such information might have the greatest

clinical utility, namely at the bedside of critically ill patients. To fill this void, we propose a novel approach using spectral coherence to quantify NVC in resting brain activity. We validate our approach using data from a numerical simulation. Finally, we demonstrate the application of this approach to quantify the NVC using EEG and NIRS signals obtained from four infants that were undergoing therapeutic hypothermia for neonatal encephalopathy.

Hypoxic ischemic encephalopathy (HIE) occurs in about 4 to 6 out of every 1000 births [15]. It is a clinical syndrome characterized by disturbed neurological function and/or seizures [15]. Newborns with HIE have a high propensity to develop cerebral palsy. Characteristic EEG background patterns have been well described in the setting of neonatal HIE and established as reliable predictors of neurodevelopmental outcome [16]. In particular, burst suppression patterns (BSP), characterized by bursts of neural activity interspersed with quiescent periods, have shown to be predictive of adverse outcomes in HIE infants [17]. In the foregoing discussion, we will call BSP -or discontinuity, its improved variant - 'fluctuating rhythmic patterns' (FRP). Simultaneous EEG and NIRS monitoring may help to characterize the vascular responses to FRP and define its prognostic value in newborn infants with HIE.

2. Materials and Methods

2.1. Converting EEG and NIRS to a common time scale

Because NVC is defined as the association between CBF and electrocortical activity, we used HbD to characterize vascular changes and EEG to measure the electrocortical activity. HbD quantifies slow dynamics (vascular changes) in the order of seconds, while EEG quantifies fast dynamics (brain function) in the order of a few milliseconds. To reliably quantify the covariation between EEG and NIRS, we converted HbD and EEG to a common time scale which we called the dynamical time frame (DTF). To achieve this, we partitioned the HbD and EEG signals into one-second epochs and calculated the standard deviation of the EEG and average HbD for each epoch. We computed spectral coherence between HbD and EEG in the DTF to quantify NVC. Note that the sample rate of HbD and EEG in the DTF is 1 Hz. Thus, DTF not only brings both EEG and HbD into a common time scale but also allows us to investigate the dynamical interaction between them. We would like to mention that the standard deviation of HbD for one-second epoch as calculated for EEG did not allow correct quantification of the NVC in our physiological signals. Hence, we used the mean value of the HbD in each one-second epoch to convert HbD into a common time scale.

2.2. Quantification of NVC

The normal impulse-response time for NVC is on the order of 5–10 seconds. To quantify NVC, we used 40 minutes of HbD and EEG signals in the DTF. We partitioned the signals into one-minute, non-overlapping epochs. Prior to the calculation of the coherence spectrum, the data in 1-minute epoch were convolved with a rectangular window in the time domain to avoid spectral leakage. Coherence was calculated using Welch periodogram approach [5, 18, 19]. In short, this approach involved calculating the cross-spectrum between EEG and HbD ($S_{EEG;HbD}$), and the power spectra of EEG (S_{EEG}) and HbD (S_{HbD}) in one-minute epochs

and averaging those quantities over all epochs to get the estimates of the same. Coherence was defined as: $C(\omega) = \frac{|\overline{S_{EEG, HbD}(\omega)}|^2}{\overline{S_{EEG}(\omega)} \cdot \overline{S_{HbD}(\omega)}}$, with ω being the frequency in Hz, and the overline indicates an estimate of that quantity [18].

The confidence level derived under the hypothesis of independence between EEG and HbD at the 100 α % level is given by $1 - (1 - \alpha)^{1/(M-1)}$, with M being the number of non-overlapping epochs used in the estimation of spectral quantities for the coherence estimation [18]. $C(\omega)$ is a normalized measure. It takes on a value of one in cases of perfect synchrony between two signals and a value of zero in cases of complete asynchrony. $C(\omega)$ was considered statistically significant at a frequency of ω if $C(\omega)$ was greater than the confidence limit (which we will henceforth call threshold). In our analysis, we used $\alpha = 0.999$ to avoid false positives.

If $C(\omega)$ was statistically significant, we calculated phase spectrum $\phi(\omega)$ [18] as follows: $\phi(\omega) = \arg\{S_{EEG, HbD}(\omega)\}$. The explicit expression that relates phase spectrum and frequency can be written as $\phi(\omega) = \delta\omega + c$, where δ is the delay between EEG and HbD.

2.3. Stochastic Model

We considered the following stochastic model to test the assumption that the NVC can be quantified by transforming the EEG and HbD signals into a DTF. We assumed the sample rate to be 1000 Hz and simulated two Gaussian-distributed white noise sequences, (x, y) for forty minutes to model EEG and HbD, respectively. In almost all HIE infants, we observed FRPs in the EEG and introduced these patterns in the simulated EEG to quantify the coupling. Based on what we observed in several hours of EEG from multiple subjects, we assumed that the FRP occurs with a periodicity of 15–20 seconds. To model the time of occurrences of FRP (τ) we generated Poisson distributed random numbers with a mean value of 20 and converted this to time units by calculating its cumulative sum and multiplying by the sample rate of 1000 Hz. In our simulation, the duration of the burst was kept as one second with a randomness defined by q_i by Poisson distributed random numbers with a mean value of one. To model the percentage (P) of coupling between the two sequences, we generated another sequence (Z) from a uniform distribution with the same number of random numbers as the number of FRP in the simulated EEG.

We fixed a value for P and established coupling between EEG and HbD in the following way. We inserted FRP in the EEG signal (sequence x) at time instances dictated by τ as follows: we multiplied the EEG segment in the time period from τ_i to $\tau_i + q_i$ by the standard deviation of the EEG in that segment and by a constant value of 5 (arbitrary selection). The standard deviation factor was used along with the constant to account for the dynamical variation in the signal. To express this mathematically, we denoted the values of x from the period τ_i to $\tau_i + q_i$ as a sequence Sx_{τ_i} . The sequence Sx_{τ_i} was modified as follows: $Sx'_{\tau_i} = Sx_{\tau_i} \cdot \sigma(Sx_{\tau_i}) \cdot 5$, with $\sigma(\cdot)$ being the standard deviation of the values of x in the sequence Sx_{τ_i} , and Sx'_{τ_i} being the modified Sx_{τ_i} . We inserted Sx'_{τ_i} in the time periods from τ_i to $\tau_i + q_i$ in the time series x .

As done with the time series x , we modified the time series y if the value of Z at time τ_i was less than the predefined value of P . If the value was less than the predefined value of P , we added a constant value of 5 to HbD (sequence y) in the time period from τ_i+10 to τ_i+10+q_i . To mathematically express this modification, we denoted the values of y in the time period τ_i to $\tau_i + q_i + 10$ as sequence Sy_{τ_i} . The sequence Sy_{τ_i} was modified as follows: $Sy'_{\tau_i} = Sy_{\tau_i} + 5$. That is, to each member of the sequence Sy_{τ_i} a value of 5 was added and Sy'_{τ_i} is the modified Sy_{τ_i} . To this end, the sequence Sy'_{τ_i} was inserted into the time series y in the time periods from $\tau_i + 10$ to $\tau_i + q_i + 10$. Note that there is a delay of 10 seconds between the FRP introduced in EEG and the corresponding changes introduced in HbD. Although the times series x and y were independent random numbers, the simultaneous changes introduced to x and y enabled coupling between them. For a modification done to x at a chosen location, P dictated whether or not a change should be made to time series y at this location; hence, the degree of coupling should increase with increasing values of P . In our simulation, we varied P from 0 to 1 in steps of 0.05. For each P value we generated 100 different realizations. For EEG and HbD obtained from each realization, we quantified the NVC using the procedures described in Sections 2.1–2.2. The MATLAB code used to simulate the EEG and HbD is given in Appendix-I.

2.4. Clinical Data

This study included newborns who were part of an ongoing prospective longitudinal study evaluating physiological biomarkers of brain injury in babies with HIE. Continuous recordings of EEG (Nihon Khoden, America, Inc, Irvine, CA), cerebral NIRS (NIRO 200, Hamamatsu Photonics, Hamamatsu, Japan) (HbO₂, Hb and Tissue Oxygenation Index), arterial oximetry (Masimo Corporation, CA, USA), electrocardiogram, and blood pressure from an indwelling arterial line (Philips IntelliVue MP70, MA, USA) were collected in a time-locked manner.

Our data acquisition system had two computers. One computer (PC1) was dedicated for EEG acquisition and configured similar to the other computers in the hospital used for clinical EEG collection. The EEG acquisition system was interfaced with the clinical server using Persyst software (Persyst Development Corporation, AZ, USA). The EEG signals were directly transferred to the clinical server, without further processing, for subsequent offline review and analysis. Our data acquisition system was installed on PC2 and it used an analog-to-digital card (National Instruments, Austin, TX) to sample the signals. In PC2, software written in LabView (National Instruments, Austin, TX) acquired and stored the collected data into independent files every five minutes. In parallel, in PC2, we retrieved the analog EEG signals while the EEG system (PC1) was transferring the signals to the clinical server and merged them with the rest of our signals in a time-synced manner. Time synchronization between EEG and NIRS was accomplished on-line during acquisition, as following: the clocks in the EEG acquisition computer, NIRS device and the data acquisition computer were synchronized, by referring them to the hospital's central clock. The data acquisition computer received triggers from the NIRS device and EEG signals to monitor their arrival times. Based on the time of arrival of the triggers, the lag/lead of the NIRS signal with respect to EEG signal was identified and adjusted accordingly to establish time-synchronization between them. The original sample rates of EEG and NIRS were 250 Hz

and 5Hz, respectively. However, using their analog signals, we sampled them at 1KHz in our acquisition setup. A high sample rate of 1KHz is needed to precisely sample the fetal or neonatal R-wave [20]. Since all of the signals are time-locked and sampled simultaneously in our setup, the rest of the signals were also sampled at 1KHz. We developed processing routines with a graphical user interface in MATLAB (Mathworks, Inc, MA, USA) that read data from the files saved on the acquisition computer and calculated different physiological metrics. One of the modules of the processing routines performed NVC calculation and displayed the NVC index (every 40 minutes). We have filed a patent of our monitoring device and it is currently under review.

The newborns underwent therapeutic hypothermia for encephalopathy according to the National Institute of Child Health and Human Development Protocol [21]. Infants were cooled using the Blanketrol II cooling unit (Cincinnati Sub-Zero, Cincinnati, OH, USA) for 72 hours followed by rewarming over 6 hours by 0.5°C/hour. EEG was recorded using 21 Ag/AgCl electrodes. For each infant, the EEG electrode placement followed the International 10–20 system. The EEG was made reference free by constructing neonatal bipolar or commonly called double banana montage [22]. NIRS optodes were placed on the right and left cerebral hemispheres in the Fronto-Temporal regions. Using the signals acquired by the clinical EEG montage at Fp1, C3, Fp2 and C4 locations, the montage calculated Fp1-C3, Fp2-C4 on-line. We used Fp1-C3 and Fp2-C4 to study the correlation with NIRS collected from the left and right cerebral hemispheres, respectively. These EEG electrodes were selected due to their close proximity to the NIRS optodes. In this work, all processing was done off-line using MATLAB.

We selected four infants to study the NVC; two of them (Subject 1 and Subject 2) survived with no evidence of MRI brain injury and the other two (Subjects 3 and 4) died during the course of our monitoring. Henceforth, we will refer to Subjects 1 and 2 as infants with favorable outcome and to Subjects 3 and 4 as infants with adverse outcome. The study was approved by the Children's National Institutional Review Board and informed written consent was obtained from the parents of the infants.

We partitioned EEG and HbD into 40-minute, non-overlapping epochs and characterized NVC using the approach described in Sections 2.1–2.2. Prior to the calculation of NVC, a notch filter at 60 Hz was used to attenuate the power line noise. Furthermore, in the DTF, epochs of EEG with standard deviation $<5\text{mcV}$ or $>100\text{mcV}$ were discarded as artifacts. Using the calculated NVC, we also quantified the NVC-index, which is the ratio of the number of (40-minute) epochs that displayed significant coherence to the total number of epochs over a period of six hours. Since the NVC analysis was carried out for every 40 minutes, the resulting trajectory of the NVC had a low time resolution.

3. Results

3.1. Numerical Simulation

Figure 1a shows the EEG with FRP simulated data using the model (see Section 2.3) for one particular realization. The HbD signals simulated for different values of parameter P (which incorporates the degree of coupling between EEG and HbD) are shown in Figures 1b and 1c.

Because approximately three FRP per minute were inserted, we expected about 120 patterns in 40 minutes. The EEG simulated using our model displayed 118 patterns. For a chosen value of P , approximately $P \times 118$ instances in HbD would show changes from the baseline values. For example, for $P = 0.05$, there should be a change in the HbD from the baseline and HbD data shown in Figure 1b at approximately six different instances. Similarly, HbD data shown in Figures 1c exhibited changes from the baseline activity at 118 instances dictated by P . Note that the duration of the FRP is about one second and is not apparent in Figure 1 due to the coarser time resolution chosen for plotting the data.

Results of NVC analysis for all model data are shown in Figure 2. The coherence between EEG and HbD in the DTF increases as a function of the coupling parameter P as expected for this model. For a given P , the maximum value of coherence between EEG and HbD in the DTF in 0.0167–0.4 Hz was calculated. We used the statistically significant coherence values and computed mean and standard deviation. These values are shown in Figure 2a as a function of P . There was no coherence between EEG and HbD for $P < 0.1$, indicating that there were not enough common events between EEG and HbD for low values of P . For each value of P , the mean and standard deviation of the delays from all of the realizations that displayed significant coherence are shown in Figure 2b. For $P < 0.3$, the variation in the delay is large, whereas for $P > 0.3$ the variation in the delay is small. The estimated delay coincided with the delay used in the model.

3.2. Clinical Data

Both infants with favorable outcome were male, born at 39 weeks gestation, and presented with moderate (Sarnat stage 2) encephalopathy [23]. Subject 3 was a male infant born at 38 weeks gestation who also presented with moderate encephalopathy. Subject 4 was a 39 week gestational age female who presented with severe (Sarnat stage 3) encephalopathy. Subject 3 died at around 32 hours of life. Subject 4 died at 89 hours of life. Both infants with adverse outcomes exhibited electrographic seizures while infants with favorable outcomes did not have seizures during the entire study period. Physiological monitoring started at approximately 8 hours of life. The total study duration ranged between 22–88.62 (median: 70.4) hours.

For Subject 1, the EEG and HbD signals in the DTF from 88:37 to 88:40 hours since birth are shown in Figure 3a and from 90:07 to 90:10 hours since birth are shown in Figure 3b. We decided to present 3-minutes of tracings so that the FRPs in the EEG and the corresponding changes in the HbD could be seen clearly. Coherence spectra calculated for 40 minutes of data during the early part of the study and after 90 minutes into the study are shown in Figures 3c and 3d, respectively. As shown in Figure 3c, there was no significant coherence between EEG and HbD. Though EEG displayed FRPs in both Figures 3a and 3b, in Figure 3a HbD did not exhibit any significant associated changes and hence the EEG and HbD from this period exhibited no significant coherence. However, in Figure 3b, the FRPs in the EEG were associated with changes in the HbD signals and EEG and HbD displayed significant coherence for this period. Further, significant coherence at < 0.1 Hz indicates that the frequency of FRP occurrence is about 15–20 seconds.

In Figure 4 we show NVC results of all four infants as a function of age (hours of life). For Subjects 1–4, the NVC quantified from the left hemisphere is shown in Figures 4(a,c,e,g) and from the right hemispheres is shown in Figures 4(b,d,f,h). In each figure, the correlation r between coherence and its probability p are displayed in the inset. The Pearson's correlation coefficient was calculated using coherence from all of the epochs. The results from both hemispheres were comparable within a narrow limit of 0.05 (except for Subject 4). In Subjects 1 and 2, there was a significant increase in the NVC during hypothermia and normothermia. Though the correlation detected for Subject 2 on the left hemisphere is marginally significant, the trend is positive. In Subject 3, there was no detectable NVC over the entire period, whereas in Subject 4, there were a few instances (6 epochs on the left hemisphere and 7 epochs on the right hemisphere) at which a significant NVC was detected. In both the left and right hemispheres of Subject 3 and in the right hemisphere of Subject 4, the correlation between coherence and time was not significant. In the left hemisphere of Subject 4, the correlation between coherence and time is significant; however, the trend is negative, indicating the NVC is decreasing with time.

We further confirmed the evolution of NVC with time observed for the infants with favorable outcomes and a lack of such a correlation in the infant (subject 4) with adverse outcomes as follows: we compared the values of the NVC during hypothermia (group 1) with the values of NVC during rewarming and normothermia (group 2). Here, the null hypothesis was that there is no difference in the NVC values of the two groups. Further, we combined the results from subjects 1 and 2 since they had favorable outcomes, and we did not include subject 3 in this analysis since it died before reaching the rewarming phase of the study. The p -values obtained for the infants that had favorable outcomes were 0.03 and 0.005 for left and right hemispheres, respectively. For subject 4, which had an adverse outcome, the p -values were > 0.05 for both left and right hemispheres. The ranges of different phases of the therapy including hypothermia (HT), rewarming (RW) and normothermia (NT) were indicated below Figure 4h. The NVC-index calculated from left and right hemispheres is shown in Figure 5a and Figure 5b, respectively. In Figure 5, the NVC-index is higher in infants with favorable outcomes compared to the infants with adverse outcomes.

4. Discussion

Simultaneous monitoring of NIRS and EEG offers a potentially powerful technique for quantitative monitoring of NVC at the bedside of sick infants. In adults, NIRS has been shown to be an excellent tool to study NVC [24, 25]. Several techniques have been developed to simultaneously measure changes in vascular and electrocortical activity in critically ill infants [14, 26, 27, 28, 29, 30, 31, 32, 9]. However, most of these techniques involved either quantifying the vascular changes to the electrical activity induced in the brain (visual evoked response) [16] or averaging the neurovascular signals with reference to the events in the brain signals (e.g. discontinuous patterns [9] or seizures [10]). In this work, we focus on quantifying NVC in the spontaneous brain activity and vascular signals. We demonstrate the emergence of NVC that would be expected in newborns without significant brain injury after perinatal HIE. We also show a weak or deteriorating NVC in two infants with adverse outcomes.

The study of NVC in critically-ill infants has offered additional insights into cerebral physiology that cannot be gained with either EEG or NIRS alone. For example, earlier work has shown changes in NIRS measurements 2–10 seconds before a generalized spike-wave discharge in childhood epilepsy [10]. Similarly, transient changes in NIRS measurement related to electrocortical seizure have been described in young infants [26]. The feasibility of localizing EEG sources in neonates has been demonstrated by simultaneous NIRS and EEG measurements [30].

In quantifying the NVC, we assumed that neuronal changes cause changes in the mean values of the hemodynamic states, and we used coherence to quantify this association. We tested this feature in our phenomenological model wherein the FRP in the neuronal signal was modeled as a significant change in the standard deviation of the signal from the baseline activity. The hemodynamic changes corresponding to the FRP were modeled as a change in the mean value of the signal from the baseline. The correct estimation of the coupling and delay incorporated in the model using our proposed scheme supports our assumption about the NVC.

The static threshold used in this work to assess the significance of the coherence is not new and it has been used in many works [6, 7, 5]. To check the reliability of the static threshold, we also performed a data-driven bootstrap approach by block-shuffling the data [19] and estimating the threshold. The estimated 99.9 percentile threshold matched the one computed from the analytical formula within a narrow limit of ± 0.02 . Based on this finding, and also in order to make the real-time processing computationally efficient, we used a static threshold in this work.

In premature infants, NIRS and EEG recordings during bursts of neuronal activity- a pattern typical of quiet sleep - have been used to evaluate NVC [9]. The current study showed a consistent pattern of oxygenation changes associated with the cortical activation, which is different in premature infants with and without brain injury. This change in the NIRS has been argued to be due to the vascular processes inducing prolonged vasodilation [33, 34]. Based on this evidence, significant coherence between EEG and HbD in infants with favorable outcome may indicate the changes in the local CBF to meet the demands of the neural activation, and lack of such a change in infants with adverse outcomes may indicate impaired NVC. In the hypothermia setting, emergence of sleep-wake cycling (SWC) and normalization of background activity during hypothermia and rewarming has been shown to be associated with the favorable neonatal outcome [35]. In our study, the emergence of NVC in infants with favorable outcome may indicate the recovery of cortical activity which then engages the NVC system.

In this study, we demonstrated the feasibility of quantifying NVC continuously in HIE infants undergoing therapeutic hypothermia for neonatal encephalopathy. A study of age-related hemodynamic changes using visual stimulation has shown a nonlinear (quadratic) relation between hemodynamic changes and neuronal effects [36]. Since our work focuses on the NVC in the spontaneous changes in the brain activity, we did not use a nonlinear model to test our assumption about the NVC.

In the high-risk populations, such as the one studied here, the physiological data are highly susceptible to artifacts. Artifacts from non-brain sources have been argued to adversely affect the quantification of the NVC [36]. Additionally, the coherence in the very low-frequency is highly susceptible to artifacts including breathing, movement, etc. In addition to the high α used in this work to avoid spurious coherence, a well-defined automated scheme is needed to pre-screen these data for artifacts. In this work, we assumed the transformed EEG and HbD signals were stationary processes and we used coherence to quantify the coupling between them. Future work would also inspect the phase relationship between EEG and HbD to understand the phase modulations between them.

In estimating the coherence, we convolved the signals with a rectangular window prior to the calculation of Fourier transform. In the future, the coherence estimation can be improved by convolving with a bell-shaped window in time domain, which can smooth the side lobes and minimize the spectral leakage. We have used the HIE population as a model to demonstrate the feasibility of our approach to perform neurovascular-coupling analysis at the bedside but our approach can be used to monitor other critically ill cohorts including congenital heart disease and premature infants monitored in the intensive care units for vital signs.

Our preliminary results show a possible relationship between the NVC evolution and disease progression. However, we need a larger population to validate this finding. Studies in a broader spectrum of illness severity together with long-term neurodevelopmental outcome evaluations are currently underway. Several factors can impede the quantification of the NVC. For example, electrographic seizures have been shown to modulate the vascular responses [10]. The loss of NVC in infants with adverse outcome may also be due to the presence of seizures. Further studies with a larger cohort are needed to show whether EEG-NIRS coherence at the low-frequency range is a biomarker for poor clinical outcome in HIE infants. Though we did not find significant NVC in infants with adverse outcomes that displayed electrographic seizures, future work will identify these data segments using reliable techniques and exclude them from further analysis.

Although a 40-minute window is adequate for NVC analysis to infer varying physiology of the critically ill infants, the analysis at current time point includes 39 minutes of data from the past. Hence, the results should be regarded as close to clinically informative time. We have a working prototype developed in MATLAB to perform NVC analysis in real-time. Since the analysis is performed every 40 minutes, the resulting time resolution of the NVC trajectory is coarse in the current version. In the future, we are planning to re-engineer our tools in machine-level language to improve the processing speed. Once this is achieved, we will be able to perform the analysis using a slide window of 0.5–1 minutes and improve the time resolution of the NVC trajectory.

5. Conclusion

We propose for the first time a technique that allows continuous monitoring of NVC at the bedside of critically ill term newborns. The importance of continuous monitoring of this physiologic entity is emphasized by the observed distinguishing NVC patterns in newborns with HIE undergoing hypothermia.

Acknowledgments

Role of the Funding Source

This study was supported by an internal special purpose fund in the Division of Fetal and Transitional Medicine at Children's National as well as by the Award Numbers UL1RR031988 and KL2 RR031987 from the NIH National Center for Research Resources. We had full access to all of the data used in this study and we take complete responsibility for the integrity of the data and the accuracy of the data analysis.

We would like thank Dr. Maria Powell, Ms. Sophie Wohlers, and Ms. Marina Metzler for their editorial assistance and Mr. Tareq Al-Shargabi for his technical comments on the manuscript.

Abbreviations

NVC	Neurovascular coupling
DTF	Dynamical time frame
FRP	Fluctuating rhythmic pattern
HbD	Oxygenated (HbO ₂) and deoxygenated hemoglobin difference
BSP	Burst suppression pattern
NIRS	Near-infrared spectroscopy
LDF	LASER Doppler flowmetry
HIE	Hypoxic ischemic encephalopathy

Bibliography

1. Roy C, Sherrington C. On the regulation of the blood supply of the brain. *J Physiol.* 111890; :85–108.
2. Brady KM, Lee JK, Kibler KK, Easley RB, Koehler RC, Shaffner DH. Continuous measurement of autoregulation by spontaneous fluctuations in cerebral perfusion pressure: comparison of 3 methods. *Stroke.* 39(9)2008; :2531–7. [PubMed: 18669896]
3. Brady KM, Lee JK, Kibler KK, Smielewski P, Czosnyka M, Easley RB, Koehler RC, Shaffner DH. Continuous time-domain analysis of cerebrovascular autoregulation using near-infrared spectroscopy. *Stroke.* 38(10)2007; :2818–25. [PubMed: 17761921]
4. du Plessis AJ. Near-infrared spectroscopy for the in vivo study of cerebral hemodynamics and oxygenation. *Curr Opin Pediatr.* 7(6)1995; :632–9. [PubMed: 8776012]
5. Govindan RB, Massaro AN, Andescavage NN, Chang T, du Plessis A. Cerebral pressure passivity in newborns with encephalopathy undergoing therapeutic hypothermia. *Front Hum Neurosci.* 82013; :266.
6. O'Leary H, Gregas MC, Limperopoulos C, Zaretskaya I, Bassan H, Soul JS, Di Salvo DN, du Plessis AJ. Elevated cerebral pressure passivity is associated with prematurity-related intracranial hemorrhage. *Pediatrics.* 124(1)2009; :302–9. [PubMed: 19564313]
7. Soul JS, Hammer PE, Tsuji M, Saul JP, Bassan H, Limperopoulos C, Disalvo DN, Moore M, Akins P, Ringer S, Volpe JJ, Trachtenberg F, du Plessis AJ. Fluctuating pressure-passivity is common in the cerebral circulation of sick premature infants. *Pediatr Res.* 61(4)2007; :467–73. [PubMed: 17515873]
8. Tsuji M, duPlessis A, Taylor G, Crocker R, Volpe JJ. Near infrared spectroscopy detects cerebral ischemia during hypotension in piglets. *Pediatr Res.* 44(4)1998; :591–5. [PubMed: 9773851]

9. Roche-Labarbe N, Wallois F, Ponchel E, Kongolo G, Grebe R. Coupled oxygenation oscillation measured by nirs and intermittent cerebral activation on eeg in premature infants. *Neuroimage*. 36(3)2007; :718–27. [PubMed: 17482837]
10. Roche-Labarbe N, Zaaimi B, Berquin P, Nehlig A, Grebe R, Wallois F. Nirs-measured oxy- and deoxyhemoglobin changes associated with eeg spike-and-wave discharges in children. *Epilepsia*. 49(11)2008; :1871–80. [PubMed: 18631367]
11. Devor A, Dunn AK, Andermann ML, Ulbert I, Boas DA, Dale AM. Coupling of total hemoglobin concentration, oxygenation, and neural activity in rat somatosensory cortex. *Neuron*. 39(2)2003; :353–9. [PubMed: 12873390]
12. Dunn AK, Devor A, Bolay H, Andermann ML, Moskowitz MA, Dale AM, Boas DA. Simultaneous imaging of total cerebral hemoglobin concentration, oxygenation, and blood flow during functional activation. *Opt Lett*. 28(1)2003; :28–30. [PubMed: 12656525]
13. Sheth S, Nemoto M, Guiou M, Walker M, Pouratian N, Toga AW. Evaluation of coupling between optical intrinsic signals and neuronal activity in rat somatosensory cortex. *Neuroimage*. 19(3)2003; :884–94. [PubMed: 12880817]
14. Cooper RJ, Everdell NL, Enfield LC, Gibson AP, Worley A, Hebden JC. Design and evaluation of a probe for simultaneous eeg and near-infrared imaging of cortical activation. *Phys Med Biol*. 54(7)2009; :2093–102. [PubMed: 19287076]
15. Sarnat H, Sarnat M. Neonatal encephalopathy following fetal distress. *Arch Neurol*. 331976; :696–705. [PubMed: 987769]
16. Howard RS, Holmes PA, Koutroumanidis MA. Hypoxic-ischaemic brain injury. *Pract Neurol*. 11(1)2011; :4–18. [PubMed: 21239649]
17. Sinclair DB, Campbell M, Byrne P, Prasertsom W, Robertson CM. Eeg and long-term outcome of term infants with neonatal hypoxic-ischemic encephalopathy. *Clin Neurophysiol*. 110(4)1999; :655–9. [PubMed: 10378734]
18. Halliday DM, Rosenberg JR, Amjad AM, Breeze P, Conway BA, Farmer SF. A framework for the analysis of mixed time series/point process data—theory and application to the study of physiological tremor, single motor unit discharges and electromyograms. *Prog Biophys Mol Biol*. 64(2–3)1995; :237–78. [PubMed: 8987386]
19. Govindan RB, Raethjen J, Kopper F, Claussen JC, Deuschl G. Estimation of time delay by coherence analysis. *Physica A: Statistical Mechanics and its Applications*. 350(2–4)2005; :277–295.
20. Stinstra J, Golbach E, van Leeuwen P, Lange S, Menendez T, Moshage W, Schleussner E, Kaehler C, Horigome H, Shigemitsu S, Peters MJ. Multicentre study of fetal cardiac time intervals using magnetocardiography. *BJOG*. 109(11)2002; :1235–43. [PubMed: 12452461]
21. Shankaran S, Laptook AR, Ehrenkranz RA, Tyson JE, McDonald SA, Donovan EF, Fanaroff AA, Poole WK, Wright LL, Higgins RD, Finer NN, Carlo WA, Duara S, Oh W, Cotten CM, Stevenson DK, Stoll BJ, Lemons JA, Guillet R, Jobe AH. Whole-body hypothermia for neonates with hypoxic-ischemic encephalopathy. *N Engl J Med*. 353(15)2005; :1574–84. [PubMed: 16221780]
22. Shellhaas RA, Chang T, Tsuchida T, Scher MS, Riviello JJ, Abend NS, Nguyen S, Wusthoff CJ, Clancy RR. The american clinical neurophysiology society's guideline on continuous electroencephalography monitoring in neonates. *J Clin Neurophysiol*. 28(6)2011; :611–7. [PubMed: 22146359]
23. Sarnat H, Sarnat M. Neonatal encephalopathy following fetal distress. *Arch Neurol*. 331976; :696–705. [PubMed: 987769]
24. Aslin RN, Mehler J. Near-infrared spectroscopy for functional studies of brain activity in human infants: promise, prospects, and challenges. *J Biomed Opt*. 10(1)2005; :11009. [PubMed: 15847575]
25. Obrig H, Wenzel R, Kohl M, Horst S, Wobst P, Steinbrink J, Thomas F, Villringer A. Near-infrared spectroscopy: does it function in functional activation studies of the adult brain? *Int J Psychophysiol*. 35(2–3)2000; :125–42. [PubMed: 10677642]
26. Cooper RJ, Hebden JC, O'Reilly H, Mitra S, Michell AW, Everdell NL, Gibson AP, Austin T. Transient haemodynamic events in neurologically compromised infants: a simultaneous eeg and diffuse optical imaging study. *Neuroimage*. 55(4)2011; :1610–6. [PubMed: 21255658]

27. Dehaes M, Aggarwal A, Lin PY, Rosa Fortuno C, Fenoglio A, Roche-Labarbe N, Soul JS, Franceschini MA, Grant PE. Cerebral oxygen metabolism in neonatal hypoxic ischemic encephalopathy during and after therapeutic hypothermia. *J Cereb Blood Flow Metab.* 34(1)2014; :87–94. [PubMed: 24064492]
28. Enfield LC, Cantanhede G, Westbroek D, Douek M, Purushotham AD, Hebden JC, Gibson AP. Monitoring the response to primary medical therapy for breast cancer using three-dimensional time-resolved optical mammography. *Technol Cancer Res Treat.* 10(6)2011; :533–47. [PubMed: 22066594]
29. Lin PY, Roche-Labarbe N, Dehaes M, Carp S, Fenoglio A, Barbieri B, Hagan K, Grant PE, Franceschini MA. Non-invasive optical measurement of cerebral metabolism and hemodynamics in infants. *J Vis Exp.* 73(73)2013; :e4379.
30. Roche-Labarbe N, Aarabi A, Kongolo G, Gondry-Jouet C, Dumpelmann M, Grebe R, Wallois F. High-resolution electroencephalography and source localization in neonates. *Hum Brain Mapp.* 29(2)2008; :167–76. [PubMed: 17390314]
31. Roche-Labarbe N, Carp SA, Surova A, Patel M, Boas DA, Grant PE, Franceschini MA. Noninvasive optical measures of cbv, sto(2), cbf index, and rcmro(2) in human premature neonates' brains in the first six weeks of life. *Hum Brain Mapp.* 31(3)2013; :341–52.
32. Roche-Labarbe N, Fenoglio A, Aggarwal A, Dehaes M, Carp SA, Franceschini MA, Grant PE. Near-infrared spectroscopy assessment of cerebral oxygen metabolism in the developing premature brain. *J Cereb Blood Flow Metab.* 32(3)2012; :481–8. [PubMed: 22027937]
33. Hamel E. Perivascular nerves and the regulation of cerebrovascular tone. *J Appl Physiol.* 100(3)2006; :1059–64. [PubMed: 16467392]
34. Koehler RC, Gebremedhin D, Harder DR. Role of astrocytes in cerebrovascular regulation. *J Appl Physiol.* 100(1)2006; :307–17. [PubMed: 16357084]
35. Massaro AN, Tsuchida T, Kadom N, El-Dib M, Glass P, Baumgart S, Chang T. aeg evolution during therapeutic hypothermia and prediction of nicu outcome in encephalopathic neonates. *Neonatology.* 102(3)2012; :197–202. [PubMed: 22796967]
36. Fabiani M, Gordon BA, Maclin EL, Pearson MA, Brumback-Peltz CR, Low KA, McAuley E, Sutton BP, Kramer AF, Gratton G. Neurovascular coupling in normal aging: a combined optical, erp and fmri study. *Neuroimage.* 85(Pt 1)2014; :592–607. [PubMed: 23664952]

Appendix-I. MATLAB code to simulate coupling between EEG and HbD

```
function [x,y]=eeg_hbd(i)
p=1:-0.05:0; % Probability to modify HbD (P)
for k1=1:length(p)
rand('seed',i);
r=poissrnd(20,300,1); % Periodicity of Fluctuating rhythmic pattern (FRP)
r1=cumsum(r); % Time instances of FRPs
rr=poissrnd(1000,300,1); % Duration of FRPS
rr=rr/1000;% conversion of unit of duration to seconds
rand('seed',i+100)
rr1=rand(300,1); % Condition that determines the insertion of FRP in HbD(Z)
randn('seed',i)
E=randn(1000*60*40,1); % Generation of EEG time series (x)
e=E;
randn('seed',i+200)
H=randn(1000*60*40,1);% Generation of HbD time series (y)
h=H;
```

```

in=10;% Time delay between EEG and HbD
for k=1:length(r)
if (r1(k)*1000+1)+floor(rr(k)*1000)+in*1000>length(E)
break;
end
ii=r1(k)*1000+1;jj=ii+floor(rr(k)*999);
iil=ii+in*1000;jj1=iil+floor(rr(k)*999);
e(ii:jj)=e(ii:jj)*std(e(ii:jj))*5; %Insertion of FRP in EEG
if rr1(k)>p(k1)
h(iil:jj1)=h(iil:jj1)+rr(k)*5; % Modification of HbD
end
end
x(:,k1)=e;y(:,k1)=h; clear E h; % At the end of simulation there will be 21
columns of x and 21 columns of y
end
return

```


Highlights

- A novel method using EEG and NIRS to characterize continuous neurovascular coupling
- Method validated using a stochastic model
- Method applied to infants on therapeutic hypothermia for neonatal encephalopathy
- Method found neurovascular coupling in infants with no detectable brain injury

Figure 1.

Data simulated using the stochastic model. (a) EEG simulated with FRP with a periodicity of approximately 20 seconds with duration of approximately one second for a period of 40 minutes. HbD simulated for different values of the parameter P (b) 5% and (c) 100%.

Figure 2.

Quantification of NVC for data simulated using the stochastic model. (a) Coherence, and (b) delay are shown as a function of the model parameter P . Error-bars represent an average $\pm$ one standard deviation of the quantity over all realizations (see Section 3.1 for details). The dashed line in (a) represents the threshold for coherence. In (b), the delay incorporated in the model (10-seconds) is shown by a dashed line. Coherence is a dimensionless quantity whereas delay is in seconds.

Figure 3.

EEG and HbD from Subject 1 in the DTF for 3 minutes. EEG and HbD from Subject 1 (a) from 88:37 to 88:40 hours since birth and (b) from 90:07 to 90:10 hours since birth. The corresponding coherence spectra calculated from 40 minutes of data from those periods are shown in (c) 88:37 to 89:17 hours since birth and (d) 90:07 to 90:47 hours since birth, respectively. In (c), there is no significant coherence between EEG and HbD, whereas in (d), there is a significant coherence between EEG and HbD at 0.05 Hz. Dashed lines in (c) and (d) indicate threshold for the coherence estimate.

Figure 4.

Results of NVC analysis for the HIE infants. For Subjects 1–4, the maximum coherence between EEG and HbD in 0.0167–0.4 Hz as a function of hours of life is shown in (a),(c),(e) and (g) for left hemisphere and in (b), (d), (f) and (h) for right hemisphere. The horizontal dashed line indicates threshold. The correlation coefficient r between coherence with time and its probability p are given in the inset of each plot. Recall that the Subjects 1 and 2 had favorable outcome and the Subjects 3 and 4 had adverse outcome. In our monitoring, the range (minimum and maximum) of different phases of the therapy, hypothermia (HT), rewarming (RW), and normothermia (NT) calculated from all of the subjects is indicated at the bottom of Figure 4h.

Figure 5.

The NVC-index calculated for a) left hemisphere and b) right hemisphere. The black and gray lines indicate the results obtained for infants with favorable and adverse outcomes, respectively.