Published in final edited form as:

J Pediatr. 2018 May ; 196: 38–44. doi:10.1016/j.jpeds.2017.12.071.

Autonomic Dysfunction in Neonates with Hypoxic Ischemic Encephalopathy Undergoing Therapeutic Hypothermia Impairs Physiologic Responses to Routine Care Events

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Abstract

Objective—To evaluate whether infants with HIE and evidence of autonomic dysfunction have aberrant physiological responses during NICU care events which could contribute to evolving brain injury.

Study Design—Continuous tracings of heart rate (HR), blood pressure (BP), cerebral near infrared spectroscopy (NIRS), and video EEG data were recorded from hypothermia-treated newborns with HIE. Videos between 16–24 hours of life were reviewed to identify distinct care events: (1) diaper changes (DC); (2) pupil exams (PE); (3) endotracheal tube manipulation (ET); (4) painful procedures (PP). Pre-event heart rate variability (HRV) was used to stratify patients into groups with Impaired versus Intact autonomic nervous system (ANS) function. Post-event physiological responses were compared between groups with nearest mean classification approach.

Results—Data from 99 clinical events in 25 newborns with HIE were analyzed. The Intact Group showed increased HR/BP after stimulating events (DC and PP), while the Impaired Group showed no change or decreased HR/BP. With vagal stimuli (PE and ET), the Intact Group demonstrated the expected mild decrease in HR while the Impaired Group showed minimal change in HR. Additionally, BP in the Intact group remained stable/increased whereas BP

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Potential Conflicts of interest / Disclosures: All authors have nothing to disclose.

decreased in the Impaired group after PE. NIRS measures increased after DC in the Impaired group but remained stable or decreased in the Intact group.

Conclusion—HRV metrics identify infants with impaired ANS function at risk for maladaptive responses to NICU care events. These data support HRV as a real-time continuous physiological biomarker capable of guiding neuroprotective care of high risk newborns.

Keywords

newborn; hypothermia; neonatal intensive care unit; autonomic nervous system; heart rate variability

Introduction

Neonatal hypoxic ischemic encephalopathy (HIE) remains a significant cause of mortality and long-term neurodevelopmental disability among term infants,^{1–4} despite the widespread use of therapeutic hypothermia (TH) which has been shown to improve outcomes.^{2, 5–7} Noninvasive and readily accessible tools are needed to evaluate the risk of evolving brain injury in real time, to identify those newborns who are not responding adequately to TH and who may benefit from adjuvant neuroprotective strategies.

The quantitative analysis of heart rate variability (HRV) has been previously described as a powerful means of bedside physiologic assessment of critically ill infants.^{8–21} Depressed HRV generally reflects autonomic dysfunction,^{18, 22} and has been studied as a physiologic marker that is associated with increased risk of death, neurodevelopmental impairment, and MRI evidence of brain injury among infants with HIE.^{23–27} It is unclear whether disturbed autonomic function (as reflected by depressed HRV) is purely a biomarker of evolving brain injury, or whether failure of autonomic nervous system (ANS) regulation plays a contributory role in the development of secondary brain injury in newborns with HIE.

This study aims to evaluate whether there is a mechanistic link between ANS dysfunction and severity of brain injury in newborns with HIE. To that end, HRV metrics were used as a means of real-time detection of impaired autonomic function. Physiologic responses to common NICU interventions such as diaper changes, pupillary exams, endotracheal tube manipulations, and nociceptive procedures were compared between those with and without intact ANS function at the time of intervention. We hypothesized that infants with impaired ANS function would exhibit aberrant circulatory and cerebrovascular responses to stressful events during neonatal critical care, and that these aberrant responses could contribute to the pathogenesis of overall brain injury in infants with HIE. Secondly, we examine the relationship between the cumulative duration of impaired ANS function and brain injury assessed by MRI.

Methods

Study Population and Data Collection

Term neonates were enrolled in this study between December 2012– July 2015 according to the National Institutes of Child Health and Human Development (NICHD) Neonatal

Research Network inclusion criteria for therapeutic hypothermia.³ Included infants were > 36 weeks gestational age, > 1800 gm at birth, demonstrated metabolic acidosis and/or low Apgar scores, and had signs of moderate to severe clinical encephalopathy according to modified Sarnat criteria.^{3, 28} Infants were treated with whole-body hypothermia for 72 hours. The study was approved by the Children's National Health System Institutional Review Board. Informed consent was obtained from the parent of each study participant.

Demographic and presenting clinical characteristics were extracted from the medical records of the birth hospital and study institution. Continuous tracings of heart rate, cerebral NIRS, and video EEG data were recorded as soon as possible after admission and analyzed using the methods described below.

Continuous Recording of Cerebral and Systemic Hemodynamic and Oxygenation Changes

Continuous cerebral oxygenation was monitored using the NIRO-200NX spectrophotometer device (Hamamatsu Photonics, Hamamatsu, Japan) which identifies changes in the concentrations of cerebral oxyhemoglobin (HbO₂) and deoxyhemoglobin (Hb). NIRS monitoring was performed over the right and left fronto-temporal regions. From these measurements, the hemoglobin difference (HbD = HbO₂-Hb) was calculated as a surrogate marker of cerebral blood flow.^{29, 30} Total hemoglobin (HbT = HbO₂+Hb) was also calculated to reflect total cerebral blood volume.^{31, 32} Mean arterial pressure (MAP) from an indwelling arterial line and EKG recordings were recorded from the NICU bedside cardiorespiratory monitor (Phillips IntelliVue MP70, MA, USA) in a time-locked manner at a rate of 500 Hz and up-sampled to 1kHz with customized software developed in LabView (National Instruments, TX, USA). Data were expressed as change from baseline by subtraction of the mean value in the 0.2 minutes preceding the NICU care event of interest.

EKG Signal Pre-Processing, HRV Metrics and Calculation of the Autonomic Dysfunction Index

EKG signal pre-processing was performed using MATLAB (Mathworks, Inc., MA, USA) as previously described.^{33–35} HRV metrics were calculated according to established methods using traditional statistics and advanced signals processing approaches in frequency and time domains.^{34, 36} The following metrics were quantified: standard deviation of RRI (SDNN), low (LF) and high (HF) frequency relative spectral power, short-alpha exponents (α_s) and root mean square short and long time scales (RMS_s, RMS_L) using detrended fluctuation analysis (DFA). DFA alpha exponents in the long time scale (α_L) were not utilized as this metric was not related to outcomes in our prior work.²⁵ The LF power domain generally corresponds to sympathetic innervation and baroreflex function whereas the HF domain predominantly reflects the parasympathetic (vagal) tone of the ANS.^{22, 37, 38} The sum of power in the frequency bands covering 0.05–0.25 Hz³⁹ and 0.3–1 Hz³⁹ were used to quantify LF and HF power respectively, and were divided by the total power (0.05 to 2 Hz). The α exponent quantifies the autocorrelations in the RRI for different time scales (short (α_s): 15–30 beats and long (α_L): >40 beats).^{34, 40} The root mean square fluctuation in short (RMS_s) and long (RMS_L) time scales were also calculated. The RMS fluctuation quantifies the variability in the RRI in the corresponding time scales. α_s , RMS_s and RMS_L quantify the sympathetic tone of the ANS.^{34, 40}

We used previously defined thresholds for HRV metrics^{25, 40}: $\alpha_s < 1.1356$, $RMS_S < 0.0062$, $RMS_L < 0.03227$, $LF < 0.21$, and $HF > 0.34$. If any of the five analyzed HRV metrics fell below threshold in the 10-minute window immediately preceding the caregiving event, the infant was classified as “ANS Impaired” during that caregiving event. Otherwise, infants were classified as “ANS Intact,” if all HRV metrics were above thresholds (indicating normal HRV) in the data window preceding the event.

An Autonomic Dysfunction Index was calculated for each infant to describe the percentage of time during 20–24 hours of life with impaired ANS function. The ADI was defined as the number of 10-minute epochs classified as impaired (i.e. 1 HRV metric beyond threshold) divided by the total number of 10-minute epochs during monitoring.

Identification of Interventions

Continuous video EEG recordings (Nihon Khoden, America, Inc., Irvine, CA, USA) were reviewed by a study investigator (H.E.C.) blinded to physiological and outcome data. Video data between 16 and 24 hours of life, a known period of peak secondary energy failure following HIE⁴¹ as well as peak discriminatory power of HRV²⁵, were reviewed to identify the precise timing of 4 distinct care events: diaper changes (DC), pupil exam (PE), endotracheal tube suctioning/manipulation by a nurse or respiratory therapist (ET), and nociceptive skin-breaking painful procedures such as heelsticks or intravenous or intra-arterial line placement (PP).

Magnetic Resonance Imaging

MRI was performed according to our institutional protocol at target age 10–12 days of life on 3T scanner (Discovery MR750, GE Healthcare, Milwaukee, WI). Images were scored by an experienced neuroradiologist (G.V.) blinded to clinical and physiological data according to a validated scoring system.⁴² Infants were classified into groups based on degree of brain injury by MRI: 0 = normal, 1 = mild injury (Basal ganglia (BG) score < 3, Watershed (WS) score < 4), 2 = moderate to severe injury (BG 3, WS 4), 3 = died.

Statistical Analysis

Differences in baseline characteristics between groups were assessed by Wilcoxon Rank-Sums Tests and Chi-square tests where appropriate. Statistical analysis was performed using SAS 9.3 (SAS Institute Inc, Cary, NC, USA).

A nearest mean classification approach was used to compare the intervention-evoked response between ANS groups using leave-one-out cross-validation method.⁴³ Briefly, cross-validation prediction results were achieved by leaving out one subject's data from one of the groups and computing a grand average for each group for the physiological signal of interest with remaining subjects. This analysis was done within a series of 1-minute windows post-intervention. Each subject's data was compared to the grand average of the ANS-intact and ANS-impaired groups respectively and classified according to the mean square difference between the subject and group grand averages. A contingency table was then constructed to calculate the sensitivity, specificity, and accuracy of the classification. An accuracy value of >0.5 was considered as a significant separation in the grand averages

between ANS groups. This analysis was performed for each minute from 0 to 5 minutes following the intervention to identify significant differences in group responses and the range of latency in the response. These analyses were performed using MATLAB (Mathworks, Inc., MA, USA).

Results

Overview of Study Population

A total of 27 patients had video data available for review during the time period of interest. The median number of hours of video data reviewed for each infant was 6.58 (range 2.58–8) hours. We analyzed a total of 99 clinical events. The median number of events per subject was 4 (range 1–9). There were 26 diaper changes, 26 pupil exams, 24 endotracheal tube manipulations, and 23 nociceptive procedures that occurred during the study period. Two patients had none of the codeable care events designated *a priori* during the time period of interest. For the 25 infants included in the study, the average gestational age was 38.1 (+/– 1.9) weeks, average birth weight was 3.22 (+/– 0.71) kg, and 20% had severe encephalopathy at presentation. Electrographic seizures were observed in 28%. One patient had an electrographic seizure during an identified care event; this patient's physiologic data were excluded from analysis for that event. Three infants died after withdrawal of care for poor neurodevelopmental prognosis. Of the surviving infants, 12 (48%) had normal MRI, 7 (28%) had mild injury, and 3 (12%) had moderate to severe brain injury. Characteristics of the study population are summarized in Table 1.

Systemic Cardiovascular Responses

Summary of heart rate and blood pressure changes over time are presented in Table 1. With stimulating interventions (DC, PP), the Intact ANS Group showed an expected increase in HR, while conversely the Impaired ANS Group decreased HR in response to these stimuli (Figure 1A/D). The Intact ANS Group showed an expected mild decrease in HR 2–3 minutes after vagal stimuli with PE, while the Impaired ANS Group showed no response at this timepoint with a later increase in HR at 4–5 minutes (Figure 1B). Responses were similar between groups for ET manipulation, except for a period at 2 minutes after stimulus where the Impaired ANS group had a slight increase in HR that was not observed in the Intact ANS group (Figure 1C). Similarly, infants in the Intact ANS Group demonstrated an overall increase in BP between 1–3 minutes after DC (Figure 1E) and 3 minutes after PP (Figure 1G). The Impaired ANS Group showed a decrease in BP after PE (Figure 1F). There were no significant differences in BP response after ET between the two groups.

Cerebral Hemodynamic Responses

Post-event changes in cerebral blood flow (HbD) and cerebral blood volume (HbT) are also summarized in Table 2. Overall, the Intact ANS Group had wider variability in HbD and HbT compared to the Impaired ANS Group (Figure 1H/I). HbT (Figure 1H), and to a lesser extent HbD (Figure 1I), increased 1 minute after PP in the Impaired ANS group, whereas these measures remained stable in the Intact ANS group. Differences in hemodynamic responses were not significant between groups after DC, PE and ET.

Autonomic Dysfunction Index and Severity of Brain Injury

Higher ADI was significantly associated with the poor outcome assessed by death or degree of brain injury by MRI (Figure 2). This relationship remained significant even after adjusting for the encephalopathy grade at presentation ($p = 0.041$).

Discussion

This study finds that HIE newborns with depressed HRV, indicative of autonomic dysfunction, have different physiologic responses to routine interventions that occur during neonatal critical care. While previous research has shown that HRV metrics during hypothermia can be useful predictors of mortality, neurodevelopmental outcomes, EEG grade severity, and MRI evidence of brain injury in HIE affected infants,^{23–27} this is the first study to demonstrate a temporal link between real-time assessment of autonomic function by HRV metrics and an infant's immediate physiologic response to stimuli during NICU care. Whether these differing responses are simply a manifestation of cerebral injury in HIE, have a protective effect, or contribute to the pathogenesis of evolving brain injury cannot be fully elucidated by this study. However, the association between the cumulative duration of autonomic dysfunction (assessed by ADI) and increasing severity of brain injury in this population, support the notion that these aberrant responses may be potentially injurious and contribute to secondary pathways leading to irreversible brain injury. Thus, using HRV metrics to identify when infants are most vulnerable to interventions may provide opportunities for individualized neuroprotective strategies during intensive care.

The design of this study was informed by prior investigations of systemic and cerebral circulatory regulation in preterm infants.⁴⁴ Limperopoulous et al⁴⁴ examined 82 preterm infants and found that they exhibited dramatic circulatory changes in response to routine NICU care events. The magnitude of these circulatory responses was lowest among the most immature and critically ill infants in the study population. Although the impact of these hemodynamic changes on brain development in premature infants is complex and poorly understood, it is reasonable to hypothesize that these circulatory fluctuations may contribute to the risk of cerebral injury, particularly during states of cerebral pressure-passivity. While autonomic regulation in term infants may be more robust than in preterm infants, our prior work has demonstrated that this physiology is altered after hypoxia-ischemia in the term infant.⁴⁵ The current study extends this earlier work by establishing that detection of impaired ANS function relates to distinct profiles of NICU care tolerance.

We observed a predictable systemic circulatory response following stimulating events such as diaper changes or painful procedures during periods of intact ANS function, with increased HR and BP, likely through activation of the sympathetic nervous system. However, when these care events took place during periods of ANS dysfunction, we observed only minimal changes in BP and a paradoxical decrease in HR. This decrease in HR may represent an aberrant response to stressful stimuli in ANS Impaired infants, potentially decreasing cardiac output with risk for extending ischemic injury. Conversely, the ANS Intact Group demonstrated a mild decrease in HR (and overall stable to increased BP) in response to pupillary exam and ETT manipulation. The response to these interventions, known to trigger parasympathetic outflow,⁴⁶ suggest intact baroreflex function in the ANS

Intact Group which can serve as a protective mechanism to maintain cardiac output and end-organ perfusion in response to changes in heart rate. In contrast, the ANS Impaired Group had only minimal change in HR and BP in response to pupil exams and ETT manipulation.

Our study did not find statistically significant differences in the cerebral hemodynamic responses to NICU care events. This may be attributable to the wide fluctuations of the HbD and HbT signals, particularly among the ANS Intact Group, which makes it difficult to detect small differences between the two groups. Overall, the Intact Group had greater variability in HbD and HbT signals compared to the Impaired Group. This observation may reflect the normal physiology of cerebral vasoreactivity and autoregulation. Prior studies have suggested that cerebral autoregulation is a dynamic process and that variability in NIRS parameters may be a marker of an intact cerebral autoregulatory system.^{47–51}

There are several limitations of this study. The ideal time scale for identifying changes in physiologic variables in response to care events is unknown. We evaluated changes in HR, BP, HbD and HbT over a 5-minute period following the start of each care event. However, if response latency and duration extended beyond this observation period, we may have underestimated the magnitude of differences in physiological responses. Likewise, assessing whether responses differ during other key time periods of treatment such as rewarming or normothermia requires further study. While several methods of HRV interrogation have been reported,⁵² we relied on our previously described approaches in the frequency and time domains to quantify HRV. We stratified infants into a category of general ANS impairment if *any or all* of the analyzed HRV metrics fell below previously defined thresholds as this was thought to be a sensitive approach to defining autonomic impairment. Larger studies are needed to develop more robust predictive models to assess the optimal combination of HRV metrics. Additionally other methods to assess ANS integrity such as coupling between HR and BP (reflecting baroreflex function⁵³) or between BP and NIRS (reflecting cerebral autoregulation⁴⁵) or assessment of peripheral vasomotor tone may also help stratify at-risk patients. The current study was also limited by its retrospective design. Given the reliance on clinically indicated video EEG for identification of care events, we did not have a control group of non-HIE babies for comparison. The clinical events that were observed occurred during routine NICU care, rather than evaluation of an evoked response paradigm with a preset stimulus. Variability in provider technique for these care events could have obscured some of the findings. For example, while ET and PE procedures were more protocolized and standardly performed, more variability in degree of positional changes was observed with DC. Similarly, PP encompassed a variety of care events of different durations and intensities including heelsticks and insertion of intravenous or intra-arterial lines. Additionally, some eligible care events may have been missed due to an obscured camera view. Another limitation of this study was the small sample size. Although a large number of events were available for analysis, we did not consider intra-subject correlation, and the physiologic data of certain subjects may have been over-represented. While we excluded epochs with electrographic seizures, the limited sample size precluded adjustment for other potential confounders such as medications or other clinical variables that could affect BP, HR or NIRS measures.

Our results show that HIE infants with depressed HRV have aberrant physiologic responses to routine caregiving events in the NICU. These findings may serve as an impetus for future research to better understand the role of autonomic dysfunction and systemic and cerebral circulatory responses in the multifactorial pathogenesis of brain injury in HIE. From a clinical perspective, our data supports the use of HRV metrics as a bedside tool for monitoring ANS integrity in real-time in order to guide neuroprotective care. In this study, we also find that the cumulative duration of autonomic impairment, described by an Autonomic Dysfunction Index, is significantly correlated with severity of brain injury in HIE infants. Collectively, these data support the inclusion of heart rate variability metrics in our current predictive armamentarium for infants with HIE.

List of Abbreviations

HIE	hypoxic-ischemic encephalopathy
TH	therapeutic hypothermia
ICU	intensive-care unit
EEG	electroencephalogram
MRI	magnetic resonance imaging

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1.

Twelve second segments of physiological data are shown when responses were most significantly different by ANS group. Changes in heart rate (HR) (a) 1 minute after diaper change, (b) 2 minutes after pupillary exam, (c) 2 minutes after endotracheal tube manipulation and (d) 4 minutes after painful procedures. Blood pressure (BP) changes are shown at (e) 1 minute after diaper change (f) 4 minutes after pupillary exam, and (g) 3 minutes after painful procedure. NIRS data at 2 minutes after painful procedures for (h) hemoglobin difference [HbD; cerebral blood flow] and (i) total hemoglobin [HbT; cerebral blood volume]. Data represent mean change with + or - 1 standard deviation for the Intact ANS Group (blue) compared to the Impaired ANS Group (red).

2.
Autonomic dysfunction index (ADI) by ANS group.

Table 1:**Characteristics of Study Population (n= 25)**

Gestational age, wk (mean $\pm$ SD)	38.1 $\pm$ 1.9
Birth weight, kg (mean $\pm$ SD)	3.22 $\pm$ 0.71
Race/Ethnicity	
Caucasian, <i>n</i> (%)	11 (44)
Black, <i>n</i> (%)	11 (44)
Asian, <i>n</i> (%)	1 (4)
Other/unknown, <i>n</i> (%)	2 (8)
Gender, <i>n</i> males (%)	16 (64)
Initial pH, median (range)	6.93 (6.55–7.21)
Apgars, median (range)	
1 minute	1 (0–5)
5 minute	4 (0–7)
10 minute	6 (0–8)
Encephalopathy grade, <i>n</i> (%)	
Moderate	20 (80)
Severe	5 (20)
EEG seizures, <i>n</i> (%)	7 (28)
Received vasoactives, <i>n</i> (%)	16 (64)
Dopamine	16 (64)
Dopamine and epinephrine	5 (20)
Age monitoring initiated, hrs, median (range)	13.8 (6.93–23.9)
Duration of study, hrs, median (range)	6.58 (2.91–8)
Ventilator support (at time of study), <i>n</i> (%)	16 (64)
Died, <i>n</i> (%)	3 (12)

Summary of Physiological Response by Group

Table 2.

Stimulus	Heart Rate			Blood Pressure			NIRS HbD			NIRS HbT		
	Intact	Impaired	Accuracy ^a	Intact	Impaired	Accuracy ^a	Intact	Impaired	Accuracy ^a	Intact	Impaired	Accuracy ^a
Diaper Change												
1 minute	↑	↓	0.56 *			0.28			0.37			0.40
2 minute	↑	↓	1.00 *	↑	↔	0.64 *			0.26			0.33
3 minute	↑	↓	0.77 *	↑	↔	0.55 *			0.29			0.34
4 minute	↑	↓	0.60 *	↑	↔	0.53 *			0.34			0.38
5 minute	↑	↓	0.62 *			0.50			0.42			0.20
Pupillary Exam												
1 minute			0.44			0.25			0.22			0.25
2 minute			0.32	↔	↓	0.66 *			0.44			0.44
3 minute	↓	↔	0.73 *			0.15			0.25			0.10
4 minute	↓	↔	0.75 *	↔	↓	0.79 *			0.22			0.17
5 minute	↔	↑	0.56 *	↔	↓	0.75 *			0.22			0.25
ET Manipulation												
1 minute			0.19			0.17			0.12			0.12
2 minute			0.29			0.43			0.17			0.17
3 minute	↔	↑	0.64 *			0.47			0.19			0.17
4 minute			0.34			0.39			0.24			0.23
5 minute			0.22			0.50			0.20			0.20
Painful Procedure												
1 minute			0.34			0.27			0.00			0.00
2 minute	↑	↓	0.55 *	↔	↑	0.14	↔	↑	0.57 *	↔	↑	0.54 *
3 minute	↑	↓	0.59 *			0.18			0.44			0.47
4 minute			0.34	↑	↔	0.55 *			0.24			0.35

Stimulus	Heart Rate			Blood Pressure			NIRS HbD			NIRS HbT		
	Intact	Impaired	Accuracy ^a	Intact	Impaired	Accuracy ^a	Intact	Impaired	Accuracy ^a	Intact	Impaired	Accuracy ^a
5 minute	↑	↓	0.63 *			0.39			0.33			0.00

Arrows represent a given parameter's direction of change from baseline after intervention.

^aNearest mean classification accuracy

* Denotes differential response by group, significance is defined by nearest mean classification accuracy >0.5