Significance of the ability to differentiate emotional prosodies for the early diagnosis and prognostic prediction of mild hypoxic-ischemic encephalopathy in neonates

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Abstract
Background: Perinatal brain injury affects around 300,000 neonates in China each year, early diagnosis and active intervention are also crucial for timely treatment and better prognoses. As hearing is the earliest as well as the most sensitive sense to develop in neonates, we propose that the ability to differentiate among different emotional prosodies may differ between neonates with and without brain injuries.

Methods: We enrolled full-term neonates admitted to the neonatology department of Peking University First Hospital from January 2016 to December 2016, conducted functional near-infrared spectroscopy (fNIRS) monitoring within 24 hr of admission, and analyzed changes in oxyhemoglobin (ΔHbO2) and deoxyhemoglobin (ΔHb) to study the ability of neonates to differentiate among emotional prosodies. The neonates were followed up to 36 months for neurological outcome evaluation.

Results and conclusions: We found that neonates showed the early ability to differentiate among emotional prosodies, responding most sensitively to positive emotions, and this ability may have been impaired following brain injury.

Keywords
brain injury, diagnosis, emotional prosody, neonates, prognosis

1 | INTRODUCTION

The most common residual sequela and neurological disability in full-term infants is hypoxic–ischemic brain injury, which has an incidence of up to 3‰–4‰ (Lee et al., 2013), of which, mild hypoxic ischemic encephalopathy (HIE) accounts for more than 50% (Conway et al., 2018). Recent studies have found that among children with mild brain injury who were previously considered to have normal prognosis, some showed poor prognosis during long-term follow-up, manifesting as delayed language development, slower gross and fine motor development, cognitive dysfunction, and others (Murray et al., 2016). Although mild HIE is generally not an indication for therapeutic hypothermia, it has been shown...
to improve the prognosis of patients with HIE, reducing the incidence of sequelae from 37%–40% to 29%–31% (Gagné-Loranger et al., 2016). This implies that early diagnosis and active intervention are also needed for infants with brain injury, even those with mild brain injury, to improve their prognosis.

Magnetic resonance imaging (MRI) and amplitude-integrated electroencephalography (aEEG) are used to evaluate mild brain injuries in clinical practice, but they have limitations. Although brain structural injuries could be found in clinically diagnosed mild HIE using MRI at 3–7 days of life, its diagnostic efficacy within the 24-hr postnatal period, especially within the 6-hr time window for therapeutic hypothermia, remain unclear (Chen et al., 2019). aEEG is currently the most commonly used method for the bedside detection of brain function in neonates, which can be employed for the timely detection of moderate to severe brain injury within 6 hr postpartum. However, neonates with mild brain injury may show normal aEEG at the early stages, even in cases presenting with neurological sequelae at subsequent follow-up (Oliveira et al., 2018). Therefore, it is necessary to find more modalities for early diagnosis to guide hypothermia treatment and improve prognosis. Compared to neonates with moderate to severe encepha-lathy, the poor prognosis of neonates with mild brain injury is more likely to manifest as cognitive impairment (Decety & Howard, 2013), previous studies have shown that neonates with HIE have impaired ability to differentiate among emotional prosodies, which constitutes a crucial component of cognitive function (Sun et al., 2019). Therefore, the rationale of the present study was to examine whether it is possible to use changes in the ability to differentiate among emotions for the early diagnosis of mild brain injury in neonates.

FNIRS is a non-invasive bedside measure to evaluate brain function of different cortical areas. It reflects cerebral blood flow by measuring changes in ΔHbO₂ and ΔHb (Ferrari & Quaresima, 2012; Scholkmann et al., 2014). Studies have shown that changes in cerebral blood flow can be observed in neonates when stimulated by different emotional prosodies (Zhang et al., 2017, 2019), by comparing the response between healthy neonates and HIE neonates using event-related potentials (ERP), studies have found an impaired ability to differentiate emotional prosodies in neonates with brain injuries (Sun et al., 2019), but ERP lacks spatial resolution for determining the brain region that is impaired in function.

The aim of this study was to apply fNIRS to detect the ability of neonates with mild HIE to differentiate among emotional prosodies through the ΔHbO₂ and ΔHb of different brain regions in response to different vocal emotional stimuli, thereby addressing the following questions: (a) Is the ability of neonates with mild HIE to differentiate among emotional prosodies affected? (b) If so, which cortical areas are affected? (c) What is the predictive value of early neonatal differentiation of emotional prosodies for the prognosis of neonates with mild brain injury?

2 | MATERIALS AND METHODS

2.1 | Participants

The participants enrolled in this study were full-term neonates admitted to the neonatology department of Peking University First Hospital from January 2016 to December 2016. Neonates diagnosed with mild HIE according to the Sarnat Grading Scale and EEG grading (Montaldo et al., 2015) were assigned to the study group, and neonates without brain injury were assigned to the control group. All neonates had normal otosacoustic emission (OAE) results and fNIRS examinations were conducted within 24 hr of life, and were followed up until 36 months old. Exclusion criteria were (a) inability to complete the fNIRS examination due to crying, (b) presence of diseases that may affect fNIRS detection, including brain injuries or developmental abnormalities other than mild HIE, congenital heart disease, and severe oxygen-dependent pneumonia, and (c) those whose artifact could not be removed. Based on these criteria, a total of 57 neonates were enrolled in this study, of whom 37 had mild HIE and 20 did not.

2.2 | Stimuli and procedure

Four types of vocal emotional stimuli were used in this study, including of happy (positive), fearful, angry (negative), and neutral speech. The vocal materials were retrieved from a database of Chinese vocal emotional stimuli (Liu & Pell, 2012). The neutral, happy, fearful, and angry vocal stimuli presented to each neonate were randomly sampled from a set of emotional vocal materials in the database, each emotional prosody would appear 10 times in total, but the order and specific content of the material were randomly generated by the preset program, and delivered through a loudspeaker (HiFier-010A, Shenzhen, China). The sound intensity level was 55–60 dB, and the background environmental noise was approximately 30 dB. Each unit of vocal emotional stimuli is known as a block, and the same type of emotional prosody was played continuously for 15 s in each block. During the experiment, vocal stimuli were alternated with silence (~15 s each), i.e., there was an interval of 15 s between each block. The four blocks (i.e., four types of emotional prosodies) were presented in a random order. The total duration of the experiment was approximately 20 min.
2.3  fNIRS recording and data generation

fNIRS was performed using a near-infrared optical brain functional imaging system (NIRScoat 1624 system, NIRx Medical Technologies, LLC. Los Angeles, USA). The light sources in this system are dual-wavelength NIR sources emitting light at a constant intensity at 780 and 850 nm. Light attenuation is detected to calculate the concentration changes in HbO2 and Hb in brain tissues and to perform imaging.

The layout of eight sources and eight detectors was arranged according to the EEG international 10–20 system of electrode placement. The distance between each source and detector was ≤ 3 cm (Benavides-Varela et al., 2011). The detectors and sources formed a total of 20 recording channels, with 10 in each cerebral hemisphere (see Figure 1 and Table 1) (Zhang et al., 2017).

Before starting the procedure, the neonates wore a custom-designed neonatal test cap, which ensured optical contact between the probe of the source and the scalp. The test was carried out during the 30 min after the infant had been fed. Optical and acoustic stimulations were avoided during the testing process.

The ΔHbO2 and ΔHb recorded through fNIRS after the presentation of different vocal emotional stimuli were analyzed using the nirsLAB v2017.6 software developed by NIRx Medical Technologies, LLC (Los Angeles, USA).

The procedures for data generation were as follows: (a) The raw data detected using the fNIRS imaging system were imported into the software. (b) The four types of vocal emotional stimuli were labeled and set markers. (c) Data from 20 s before the start of the experiment to 20 s after all sounds were presented were selected for analysis. (d) Artifact removal, discretization, and filtering were performed manually.
a low cutoff frequency of 0.01 Hz and high cutoff frequency of 0.2 Hz were selected as the filtering parameters. (e) Based on the modified Beer-Lambert law, the optical data for two wavelengths measured during each stimulus was transformed into oxy-Hb and deoxy-Hb signals, respectively. In every patient, the ΔHb and ΔHbO2 in response to each prosody in every channel was averaged.

2.4 | Neurodevelopment assessment

Neurodevelopment assessment was performed using the Ages and Stages Questionnaire (ASQ) for all enrolled neonates at 36 months postpartum. It covers five areas of child development, including the communication, gross motor, fine motor, problem solving, and personal-social domains (Committee on children with disabilities, 2001; Council on children with disabilities et al., 2006). Each area consists of six items, and each item represents a developmental milestone for that age group. One of three options must be selected for each item, where “yes” indicates that the child can perform the item and is assigned 10 points, “sometimes” is assigned 5 points, and “not yet” is assigned 0 points. The sum of the six items constitutes the score for each area, based on which, the area can be divided into normal development, risk of suspected developmental delay (borderline), and diagnosis of developmental delay.

2.5 | Statistical analysis

All data were processed and analyzed using SPSS 22.0 (SPSS Inc., Chicago, IL). Repeat measures ANOVA was conducted for ΔHbO2 and ΔHb in the 20 channels in response to the 4 emotional prosodies in the control group and HIE group. The statistical results in individual channels were corrected for multiple comparisons across channels by the false discovery rate (FDR) procedure implemented in Matlab (v2015b, the Mathworks, Inc., Natick, MA), a corrected $q < 0.05$ is considered statistically significant (Storey & Tibshirani, 2003).

| Channel | Corresponding source and detector | Brain region | $p$ |
|---------|-----------------------------------|--------------|-----|
| 1       | T8-P8                             | Middle and superior temporal gyri (R) | .872 |
| 2       | T8-F8                             | Middle temporal gyrus (R) | .152 |
| 3       | T8-C4                             | Supramarginal gyrus (R) | .118 |
| 4       | F4-F8                             | Dorsolateral prefrontal cortex and pars triangularis (R) | .122 |
| 5       | F4-C4                             | Frontal eye fields, pre-motor and supplementary motor cortices, dorsolateral prefrontal cortex (R) | .611 |
| 6       | F4-FC2                            | Frontal eye fields (R) | .387 |
| 7       | P4-P8                             | Angular gyrus (R) | .472 |
| 8       | P4-C4                             | Supramarginal gyrus (R) | .538 |
| 9       | CP2-C4                            | Primary somatosensory cortex (R) | .418 |
| 10      | CP2-FC2                           | Pre-motor and supplementary motor cortices (R) | .151 |
| 11      | T7-P7                             | Middle and superior temporal gyri (L) | .277 |
| 12      | T7-F7                             | Middle temporal gyrus (L) | .614 |
| 13      | T7-C3                             | Supramarginal gyrus (L) | .422 |
| 14      | F3-F7                             | Dorsolateral prefrontal cortex and pars triangularis (L) | .294 |
| 15      | F3-C3                             | Frontal eye fields, pre-motor and supplementary motor cortices, dorsolateral prefrontal cortex (L) | .303 |
| 16      | F3-FC1                            | Frontal eye fields (L) | .252 |
| 17      | P3-P7                             | Angular gyrus (L) | .777 |
| 18      | P3-C3                             | Supramarginal gyrus (L) | .619 |
| 19      | CP1-C3                            | Primary somatosensory cortex (L) | .319 |
| 20      | CP1-FC1                           | Pre-motor and supplementary motor cortices (L) | .151 |

Note: L, left; R, right.
In the channels that had statistically significant results, independent-samples T test was performed to determine the kind of emotional prosody that elicited a different response, \( p < .05 \) is considered statistically significant. Finally, Pearson correlation was performed to study the association between the channel that showed a difference in ability to recognize an emotional prosody and the patients ASQ. \( p < .05 \) is considered statistically significant, and \( r > 0 \) is considered a positive correlation.

3 | RESULTS

3.1 | Clinical status

The demographic characteristics of the mild HIE group and the no brain-injury (control) group are summarized in Table 2.

The main diseases diagnosed in the control group were mild neonatal pneumonia (i.e., normal blood gas analysis and percutaneous oxygen saturation, not requiring oxygen therapy; 11 cases), neonatal jaundice (7 cases), and high-risk neonates born to mothers with connective tissue disease who showed no organ damage after evaluation (2 cases). None of the patients showed neurological symptoms, and no abnormalities were found in complete cranial ultrasonography and aEEG during hospitalization.

3.2 | fNIRS

RMA analysis showed that among the 20 channels, channel 6 (covering the right middle frontal gyrus area) showed a difference in \( \Delta HbO_2 \) but not \( \Delta Hb \) when comparing the two groups (\( q < 0.05 \)), indicating a certain difference in ability to recognize emotional prosodies. The other channels showed no difference in \( \Delta HbO_2 \) or \( \Delta Hb \) in any channels (Table 3).

We then tried to determine which emotion in particular was recognized differently between the 2 groups in channel 6, and we found the \( \Delta HbO_2 \) in response to the angry prosody was different in the control group and the mild HIE group (\( p = .014 \)). As seen in Figure 2, healthy neonates were capable of differentiating among the four emotional prosodies, showed a higher \( \Delta HbO_2 \) when listening to the angry and happy prosodies than the neutral prosody (angry vs. neutral: \( t = 3.307, p = .002 \), happy vs. neutral: \( t = 2.699, p = .01 \)) In the mild HIE group (Figure 3), however, the four emotions evoked similar \( \Delta HbO_2 \) changes, indicating a possible impairment in the right middle frontal gyrus area to recognize the angry vocal emotion or differentiate between different vocal emotions. \( \Delta Hb \) in response to any emotional prosody in either group was not statistically significant.

| TABLE 2 | Demographics of the study and control groups |
|---------|---------------------------------------------|
|         | Study group | Control group |
| No. of cases | 37 | 20 |
| Male | 21 | 10 |
| Female | 16 | 10 |
| Gestational age (weeks) | 39.0 ± 1.3 weeks | 38.8 ± 1.3 weeks |
| Birth weight | 3,190 ± 448 g | 3,077 ± 448 g |
| Age at testing | 3.9 ± 2.8 days | 4.3 ± 2.2 days |
| Hb (g/L) | 176.8 ± 20.5 | 160.1 ± 26.7 |
| BP (mmHg) | 73.6 ± 7.14 | 76.3 ± 9.54 |
| Note: None of the patients required respiratory support and maintained a SpO2 above 95%. |
3.3 Neurological prognosis

Of all 57 neonates enrolled in our study, 49 (20 in the control group and 29 in the HIE group) were followed up until 36 months. For the control group, all 20 neonates had normal ASQ scores in all skills, while for the HIE group, 5 patients had borderline abnormal scores in their fine motor skills, but none showed developmental delay in any skills. The results showed that the scores of the control group for the five areas of communication, gross motor, fine motor, problem-solving, and personal-social were 58.0 ± 2.5, 58.1 ± 2.4, 33.4 ± 5.6, 48.8 ± 4, and 50.5 ± 4.3, respectively, which indicated normal development. As for the study group, the scores for the five areas were 59.0 ± 2.3, 58.6 ± 2.1, 30.2 ± 4.3, 47.8 ± 4.6, and 49.5 ± 3.2, respectively. Among which, the score for fine motor skills was borderline abnormal and was significantly lower than that of the control group.

As channels 6 showed significant changes in ΔHbO₂ after the presentation of angry vocal stimuli, these indicators were used to perform correlation analysis with neonatal prognosis. The correlation coefficient of ΔHbO₂ at channel 6 with prognosis was 0.569, \( p = .002 \), indicating that the changes ΔHbO₂ in the right middle frontal gyrus area in response to the angry voice may be positively correlated with the ASQ score at 36 months of age.

4 CONCLUSIONS

In this study, we found that neonates showed the early ability to differentiate among emotional prosodies, responding most sensitively to positive emotions, and this ability may have been impaired following mild brain injury.
Recent studies have found that neonates with mild brain injury may still have neurological sequelae (Kariholu et al., 2018; Lally et al., 2018; Scholkmann et al., 2014). However, in contrast to severe brain injury, current clinical, imaging, and brain functional detection techniques can only play a limited role in the early diagnosis of mild brain injury in neonates, their
optimal diagnostic efficacy being at 3–7 days of life, which is past the 6 hr time window for therapeutic hypothermia (Lally et al., 2018; Walsh & Inder, 2018). Although therapeutic hypothermia is not usually indicated in mild HIE, we still hope by finding a more sensitive parameter for earlier diagnosis, preferably within 6 hr of life, it could provide us with the possibility for a timelier intervention. Given that the prognosis of patients with mild brain injury is more likely to involve cognitive impairment, we applied fNIRS to detect one of the cognitive functions, namely, the ability to differentiate among emotional prosodies, to help achieve early diagnosis and early intervention of mild brain injury along with other diagnostic tools such as the ultrasound and aEEG, thereby improving the patient's prognosis. When comparing the results of healthy and mild HIE neonates, we found a difference in their ability to recognize emotional prosodies in the right middle frontal gyrus area, especially when listening to the angry prosody. This implies that mild brain injury can affect the ability of neonates to differentiate among emotional prosodies, which is consistent with the findings of Sun et al. (Sun et al., 2019).

In our study, all neonates went through fNIRS examination within 24 hr of life, for neonates at high risk of brain injury, even those without typical clinical manifestations, we may still be able to use fNIRS to test their ability to differentiate among emotional prosodies to achieve early discovery, and hence, early treatment of mild brain injury.

Despite the significant differences in ΔHbO2, the differences in ΔHb were not statistically significant. ΔHbO2 reflects oxygen uptake by brain tissues, whereas ΔHb reflects oxygen utilization by brain tissues. Thus, our result may indicate that following the presentation of different emotional prosodies, the brain tissues of neonates may actively increase the uptake of oxygen to ensure ample oxygen supply to the brain regions responsible for emotional differentiation. With HIE, the ability to uptake oxygen and increase blood flow may be impaired. Nevertheless, if the body does not have systemic issues, such as ischemia or anemia, then, even with brain injuries, additional oxygen consumption for the differentiation among emotional prosodies should not be required. Other studies have reported that due to the signal properties of HbO2 and Hb, HbO2 has a larger signal to noise ratio, is more sensitive to changes in cerebral blood flow, and therefore, is more likely to produce statistically significant results (Sato et al., 2012). In conclusion, further research is needed to uncover the specific mechanisms involved.

In conclusion, this study found that neonates with mild HIE have an impaired ability to recognize the angry vocal emotion in the right middle frontal gyrus area compared with healthy neonates. Therefore, fNIRS can be clinically used to detect ΔHbO2 to judge the ability of neonates to differentiate among emotional prosodies, which can serve as a means to assist the clinical diagnosis of mild HIE. Whether the early ability to differentiate among emotional prosodies is correlated with the long-term prognosis of cognitive function remains to be verified by longer term and larger scale follow-up data.

CONFLICT OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ETHICS APPROVAL STATEMENT

The research was conducted ethically in accordance with the World Medical Association Declaration of Helsinki and was approved by the ethics committee of Peking University First Hospital. Written informed consent was acquired from the patients’ parents.

PATIENT CONSENT STATEMENT

Written informed consent was acquired from the patients’ parents.

AUTHOR CONTRIBUTIONS

Lili Liu and Yuehang Geng conducted the experiments and wrote the manuscript with the help of Yun Cui, Yanxia Zhou, Guoyu Sun, Cheng Peng, Rui Zhang, Yuanpei Ma, Yanan
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