Esophageal reflexes modulate frontoparietal response in neonates: Novel application of concurrent NIRS and provocative esophageal manometry


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Submitted 21 October 2013; accepted in final form 24 April 2014

Jadcherla SR, Pakiraih JF, Hasenstab KA, Dar I, Gao X, Bates DG, Kashou NH. Esophageal reflexes modulate frontoparietal response in neonates: Novel application of concurrent NIRS and provocative esophageal manometry. Am J Physiol Gastrointest Liver Physiol 307: G41–G49, 2014. First published May 1, 2014; doi:10.1152/ajpgi.00350.2013.—Central and peripheral neural regulation of swallowing and aerodigestive reflexes is unclear in human neonates. Functional near infrared spectroscopy (NIRS) is a noninvasive method to measure changes in oxyhemoglobin (HbO) and deoxyhemoglobin (HbD). Pharyngoesophageal manometry permits evaluation of aerodigestive reflexes. Modalities were combined to investigate feasibility and to test neonatal frontoparietal cortical changes during pharyngoesophageal (visceral) stimulation and/or swallowing. Ten neonates (45.6 ± 3.0 wk postmenstrual age, 4.1 ± 0.5 kg) underwent novel pharyngoesophageal manometry concurrent with NIRS. To examine esophagus-brain interactions, we analyzed cortical hemodynamic response (HDR) latency and durations during aerodigestive provocation and esophageal reflexes. Data are presented as means ± SE or percent. HDR rates were 8.84 times more likely with basal spontaneous deglutition compared with sham stimuli (P = 0.004). Of 182 visceral stimuli, 95% were analyzable for esophageal responses, 38% for HDR, and 36% for both. Of analyzable HDR (n = 70): 1) HbO concentration (μmol/l) baseline 1.5 ± 0.7 vs. 3.7 ± 0.7 poststimulus was significant (P = 0.02), 2) HbD concentration (μmol/l) between baseline 0.1 ± 0.4 vs. poststimulus −0.5 ± 0.4 was not significant (P = 0.73), and 3) hemispheric lateralization was 21% left only, 29% right only, and 50% bilateral. During concurrent esophageal and NIRS responses (n = 66): 1) peristaltic reflexes were present in 74% and HDR in 61% and 2) HDR was 4.75 times more likely with deglutition reflex vs. secondary peristaltic reflex (P = 0.016). Concurrent NIRS with visceral stimulation is feasible in neonates, and frontoparietal cortical activation is recognized. Deglutition contrasting with secondary peristalsis is related to cortical activation, thus implicating higher hierarchical aerodigestive protective functional neural networks.

esophageal motility; aerodigestive reflexes; visceral sensation; swallowing; near infrared spectroscopy

THE MECHANISMS OF CORTICAL activation during aerodigestive provocation and swallowing are not well understood in human neonates. However, a large body of evidence from studies in human adults provides at least two levels of control systems that reside in brain stem and cerebral cortex (28, 33). This knowledge has emanated from the use of concurrent functional magnetic resonance imaging (fMRI) methods and aerodigestive stimulation methods (5, 6, 17). Performance of such studies in human adults requires subject cooperation, ability to perform tasks, and occasionally sedation. Furthermore, the aerodigestive testing methods are required to be magnet-safe for simultaneous evaluation. Unfortunately, these concurrent methodologies are not feasible in human neonates because of the aerodigestive safety monitoring issues and body positioning concerns in a closed setting. Therefore, crib side methods incorporating approaches to map neural connections involving aerodigestive reflexes are critical to advance the scientific field and perform repeated observations to understand developmental physiology of the aerodigestive apparatus.

Recently, functional near infrared spectroscopy (NIRS) systems have been used to measure cortical hemodynamic physiological signals correlating with the underlying neural activity (13, 40, 47). Analogous to pulse oximetry, this permits measurement of cerebral oxygenation and quantification of cerebral hemodynamics based on the sensitivity to optical properties of absorption and scattering. Several studies have demonstrated that near infrared light propagates through the intact skull to reach the brain (13, 40, 47), which can be applied to neonatal care (48). Therefore, by positioning infrared light sources and detectors on the scalp, NIRS can be used to monitor brain activity and location. Through the application of modified Beer-Lambert Laws, changes in oxyhemoglobin (HbO) and deoxyhemoglobin (HbD) for distinct wavelengths that correlate to HbO and HbD can be calculated (8, 38, 39). Ideally, neuronal activation results in increased cerebral metabolic rate (oxygen consumption) resulting in an HbO decrease and HbD increase and 2) increased cerebral blood flow [hemodynamic response (HDR)] by 2–10 times compared with the cerebral metabolic rate, resulting in HbO increase and HbD decrease (38). Consequently, NIRS is a noninvasive and cost-efficient method with the capability to detect changes in brain activity on multiple fields that may allow further clarification of aerodigestive physiology and development of new approaches toward assessment of cortical activation.

Recently validated methods in human neonates (20, 24, 26, 27, 45) allowed definition of the vagus nerve-mediated upper esophageal sphincter (UES) contractile reflex, esophageal peristaltic reflexes, and lower esophageal sphincter (LES) relaxation response-evoked esophageal provocation. Physiological effects of esophagus-airway interactions have also been evaluated by quantifying the effects of esophageal sensory stimulation on glottal reflexes and effects of esophageal sensory...
stimulation on electrophysiological characteristics of sleep (20, 27) in human neonates.

By combining NIRS and esophageal manometry methodology, corticoesophageal interactions can be evaluated. To our knowledge, the current study is the first study to define the effects of visceral stimulation on frontoparietal activation by analyzing the changes in the concentrations of HbO and HbD and to establish the feasibility of using esophageal manometry provocation methods concurrent with NIRS. Our working hypothesis was that there will be significant changes in the concentrations of HbO and HbD levels in the brain associated with specific aerodigestive reflexes evoked upon pharyngeal and esophageal stimulation with air and liquids.

MATERIALS AND METHODS

Subjects

Ten human infants (5 male, 25–41 wk gestation) underwent esophageal manometry concurrent with NIRS. Studies were performed in a neonatal intensive care unit (NICU) setting at Nationwide Children’s Hospital. Subjects were studied after recovery from neonatal health issues and were on a stable phase of feeding and growth. In general, in our nursery, oral feedings are initiated and continued as per infant’s cues and clinical physiological stability. The NICU at Nationwide Children’s Hospital is an all referral one. Subjects were evaluated by the attending neonatologist and were deemed stable at study. Subjects with birth defects and genetic syndromes were excluded. The study procedures were approved by the ethics committee at the Institutional Review Board at the Nationwide Children’s Hospital Research Institute (Columbus, OH). The study protocol conforms to the guidelines of the Institutional Review Board policy and the health insurance portability and accountably acts. Written informed consent was obtained from the parents before study.

Manometry Methods

Pharyngeal and esophageal provocations were administered during esophageal quiescence, and recordings were made using esophageal manometry methods in neonates, as previously published (21, 22). Briefly, the catheter assembly (Dentsleeve, Mui Scientific, Ontario, Canada) was connected to the pneumatic-hydraulic micromanometric water perfusion system via resistors, pressure transducers (TFN-R disposable pressure transducers), and amplifiers (Solar modules, Solar 2; MMS Medical Instruments, Dover, NH). The esophageal manometry catheter assembly with dual sleeves and four side ports recording from pharynx and proximal, middle, and distal esophageal loci and a terminal gastric recording port was used. The water perfusion rate was 0.02 ml·min⁻¹-port⁻¹ for esophageal ports, 0.01 ml·min⁻¹-port⁻¹ for the pharyngeal port, and 0.04 ml·min⁻¹-port⁻¹ for the sleeves. The catheter was calibrated at the midaxillary line before placement.

NIRS Methods

NIRScout system (NIRx Medical Technologies) was used to analyze changes in HbO and HbD concentrations (41). With input from our neuroradiologist (Bates) and established literature on frontoparietal cortex-related surface markings (19, 46), a purpose-built noninvasive NIRS EEG cap (NIRx Medical Technologies), designed to accurately position sources and detectors, was placed on the cranium to measure changes in the frontoparietal cortex. The cap contained four infrared light sources and eight detectors bilaterally to characterize frontoparietal cortical activity on left and right hemispheres with 1.5 cm between the optodes (Fig. 1). Infrared light sources used two wavelengths of 760 and 850 nm. The sampling rate for the NIRScout system depends on the number of sources and detectors used. For the purpose of this study, four sources and eight detectors were used for a sampling rate of 10.42 Hz.

Near-infrared analysis, visualization, and imaging software (version 1.0; NIRx Medical Technologies) was used for NIRS data analysis. After data acquisition, a postprocessing band pass filter (0.017–0.25 Hz) was applied to remove systemic oscillations such as heart rate and low-frequency drift. Additionally, to quantify the data, the filtered NIRS recordings were analyzed using MATLAB software. Experimental and analytical protocols included analysis of spontaneous basal deglutition and adaptive reflexes to visceral stimulation and its effects on frontoparietal cortical HDR activity under quiet testing states.

Manometric Experimental Protocol

In the unstimulated supine lying neonate, the manometry catheter was passed nasally with the transducers at the level of the subject’s esophagus. A pull-through technique was performed to identify the UES and LES for proper catheter placement and then well secured. Infants were allowed to adapt to catheter placement before the experimental protocol was carried out. Continuous data acquisition and analysis were performed during manometric study based on waveform characteristics, as previously defined (21, 22, 26). Manometric responses evoked upon abrupt infusions of midesophageal stimulation (air and liquids), pharyngeal stimulation (water), or sham (random sham event marker placement during study) stimulation were observed. Midesophageal infusions simulate gastroesophageal reflux, pharyngeal infusions simulate an oral bolus, and sham stimulation allows for the evaluation of background esophageal activity. Through visceral (esophageal or pharyngeal) stimulation or sham stimulation, respective sensorimotor-induced responses can be evaluated (15, 24).

Visceral-Cortical Activation Experimental Protocol

All studies were done in a closely monitored quiet setting. Although ambient room temperatures vary between rooms and day to day throughout the study, the room temperature was kept constant. Also, throughout the study, the lights were dimmed to the lowest setting to keep light intensity constant. The NIRS cap was placed on the neonate’s head carefully by the neonatologist based on the sutures and fontanel as guidelines. The sources and detectors were placed on the right and left frontoparietal regions of the brain. Elastic straps were used to secure the cap on the subject’s head comfortably. Periodic checks were made to verify the snugly placed optode positions. After allowing the infant to adapt to the manometry catheter placement and NIRS cap placement, baseline esophageal motility and NIRS activity were concurrently recorded, and both modalities were time-synchronized. Infant safety, movement, and behaviors were continuously recorded.

The neonatologist administered abrupt visceral provocations and sham stimuli during a phase of esophageal quiescence and quiet state as per our pharyngeal and esophageal infusion protocol as previously defined (20, 27). Pharyngo-esophageal events, spontaneous swallowing, coughing, aerodigestive reflexes, movement artifacts, touching, and inadvertent stimuli were also recorded to enhance validation of our focused visceral-cortical evaluation. Cardio-respiratory events were also monitored to document subject safety. Continuous data acquisition for manometry and NIRS signals were processed, and pharyngo-esophageal biomechanical events were specifically documented. The timing of esophageal stimuli could not be given during NIRS quiescence because NIRS quiescence could not be predicted because of limitations in NIRS technology in displaying continuous waveforms for onsite immediate interpretation. Thus, for the purpose of the current study, the timing of visceral and sham stimuli was a random event for NIRS changes. Therefore, baseline HbO/HbD changes between baseline and upon stimulation were analyzed to
observe the effect of visceral stimuli vs. background NIRS activity (sham).

Data Analysis

Esophageal manometry characteristics and analysis. Esophageal reflexes, as described by us before (16, 21–26), were classified as pharyngeal reflexive swallow (PRS), pharyngo-UES-contractile reflex (PUCR), secondary peristalsis (SP), esophago-deglutition responses (EDR), or UES contractile reflex. For the sake of simplicity with our pilot observations, these characteristics were then grouped into presence of esophageal response or none when any of these were absent. Also, PRS and EDR were grouped as deglutition reflex, and PUCR, SP, and UES contractile reflex were grouped as secondary peristaltic reflex. Esophageal response latency was defined as the time period from the onset of infusion to the onset of the esophageal response. Manometric data examiners (Hasenstab and Jadcherla) were blinded to the NIRS data, and NIRS examiners (Pakiraih, Dar, and Kashou) were blinded to the manometric data. Statistical analysis and correlation was performed by an independent person (XG) who was unaware of manometry and NIRS waveform signals.

NIRS characteristics and analysis. HbO and HbD concentration changes, presented as micromoles per liter (μmol/l), were examined with respect to the near infrared wavelengths being emitted so that functional changes of distinct portions of the frontoparietal cortex could be analyzed for trends. NIRS baseline concentration (μmol/l) was defined as the average concentration value before visceral stimulation. HDR was defined as an increased HbO concentration and a decrease in HbD concentration in response to visceral stimulation (Fig. 2). Cortical HDR latency (s) was defined as the duration between visceral stimulation onset to HDR onset. HDR duration (s) was defined as the duration between the HDR onset and offset. HbO percent increase was defined as the percent change from the baseline concentration to the highest HbO concentration of the HDR duration for a given stimulus. HbD percent decrease was defined as the percent change from the baseline concentration to the lowest HbD concentration of the HDR duration for a given stimulus.

Statistical Analysis

Experimental and analytical protocol included analysis of spontaneous and provocative swallows and its effects on frontoparietal cortical HDR activity under quiet testing states. The changes in HbO and HbD from the baseline to the response were analyzed. Data are presented as means ± SE or as percent. Discrete variables were compared between the situations when HDR was present and HDR was absent using Chi square test or Fisher Exact Test, as appropriate. For demographic characteristics, where data were normally distributed, parametric tests were applied for comparisons between measurements at study and measurements at birth, whereas nonparametric tests (Wilcoxon rank-sum test) were used for data that were not normally distributed. To examine the effects of infusion volume and infusion media on the esophageal response relationship, a generalized estimating equation (GEE) approach was used to test for differences in volume relationships. Linear mixed-effect models were applied for the comparisons of the changes in HbO and HbD from the baseline to the response. In these models, GEE and linear mixed model, a random effect was included to account for correlation among the subjects. Log transformation was performed to achieve normality for the statistical analysis (mixed model). Mean ± SE values are reported, unless stated otherwise. Because data are not normally distributed, values are shown as median (interquartile
Demographic characteristics are stated in Table 1. Changes in growth were noted: gestational age vs. postmenstrual age (PMA) at evaluation \((P = 0.013)\), birth weight vs. weight at evaluation \((P = 0.006)\), and head circumference at birth vs. at evaluation \((P = 0.11)\). The PMA at first oral feed ranged from 37.4 to 42.1 wk.

Effect of Visceral and Sham Stimulation on Adaptive Esophageal and NIRS Response Characteristics

Data from a total of 182 visceral stimuli were analyzed across the study population. Manometry responses to midesophageal infusions (air and liquids) or pharyngeal infusions (water) were evaluated concurrent with NIRS analysis. The average synchronized NIRS recording time concurrent with manometry recording was 54 min (25–60 min). NIRS recording time was lesser in those cases of excessive motion or crying. Out of 182 visceral stimulations, 173 events (95%) were analyzable for esophageal manometry characteristics; specifically there were 134 (77%) peristaltic reflexes, and 39 stimuli (23%) had no esophageal responses. During 182 visceral stimulations, NIRS signals were analyzable only during 70 visceral stimulation events (38%). However, only 66 of the 70 events were analyzable for both NIRS and manometry concurrent events.

Characteristics of NIRS HDRs to 70 visceral stimuli are described (Table 2; Figs. 2 and 3). Comparison of HbO concentration between baseline vs. poststimulus values was 1.5 ± 0.7 and 3.7 ± 0.7 \(\mu\)mol/l, respectively \((P = 0.02)\). HbD concentration between baseline vs. poststimulus values was

Table 1. Demographic characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Study Population ( (n = 10) )</th>
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<tbody>
<tr>
<td>Gestational age at birth, wk</td>
<td>34.9 ± 2.0</td>
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<tr>
<td>Weight at birth, kg</td>
<td>2.4 ± 0.4</td>
</tr>
<tr>
<td>Head circumference at birth, cm*</td>
<td>32.1 ± 1.7</td>
</tr>
<tr>
<td>Postmenstrual age at evaluation, wk†</td>
<td>45.1 ± 3.0</td>
</tr>
<tr>
<td>Weight at evaluation, kg</td>
<td>3.9 ± 0.4</td>
</tr>
<tr>
<td>Head circumference at evaluation, cm</td>
<td>35.0 ± 1.0</td>
</tr>
</tbody>
</table>

Values are shown as means ± SE; \( n \), no. of subjects. *\( P = 0.01 \) for comparison with gestational age at birth.

Table 2. Effect of visceral stimuli on hemodynamic characteristics that were analyzable for NIRS responses

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Mean ± SE</th>
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<tbody>
<tr>
<td>Stimulus duration, s</td>
<td>3.2 ± 0.4</td>
</tr>
<tr>
<td>Cortical response latency, s</td>
<td>3.8 ± 0.5</td>
</tr>
<tr>
<td>HbO increase, %</td>
<td>555.4 ± 107.8</td>
</tr>
<tr>
<td>Hb decrease, %</td>
<td>271.8 ± 71.4</td>
</tr>
<tr>
<td>Cortical response duration, s</td>
<td>29.2 ± 1.7</td>
</tr>
</tbody>
</table>

NIRS, near infrared spectroscopy; HbO, oxyhemoglobin; HbD, deoxyhemoglobin. \( n = 70 \) Subjects.
0.1 ± 0.4 and −0.5 ± 0.4 μmol/l, respectively ($P = 0.73$). NIRS hemispheric lateralization responses, i.e., left only-right only-bilateral, were 21:29:50%, respectively. Additionally, left and right hemispheric cortical response latency ($P = 0.92$) and duration ($P = 0.97$) were similar.

In addition, comparisons were made between the responses during 66 esophageal stimuli vs. 22 sham stimuli. The esophageal peristaltic response rate was 49 out of 66 visceral stimuli (74%), with a response latency of 2.8 ± 0.3 s. The esophageal peristaltic response rate with sham stimuli was zero (0%). The NIRS response rate to visceral stimulation was 40 out of 66 (61%), whereas, with sham stimulus, NIRS responses were noted only five times (23%; vs. visceral stimulation, $P = 0.002$). Additionally, the subjects receiving visceral stimulation were 5.23 times more likely to have a HDR present compared with patients with sham stimulation (Table 3). To explore and determine if SP has less impact on central cortical activation, we analyzed the effect of deglutition reflex vs. SP reflex on NIRS changes. The association of peristaltic reflexes with NIRS changes is shown (Table 4). The presence of an HDR was 4.75 times more likely with a deglutition response compared with SP ($P = 0.016$).

We tested the hypothesis that the visceral stimulus volume or media (air or liquids) modifies cortical HDR latency and duration. Regardless of the stimulus volumes, the HDR latency time ($P = 0.09$) and HDR duration ($P = 0.29$) were similar. However, as the stimulus volume increases, the magnitude of the HbO also increases ($P = 0.045$). Regardless of media type, the cortical latency time ($P = 0.16$) and HDR duration ($P = 0.86$) were similar.

### Effect of Basal Spontaneous Deglutition on NIRS Responses

Overall, 18 basal spontaneous deglutition events were analyzable for NIRS characteristics. Of these, the cortical HDR rate was seen in 13 out of 18 (72%). Compared with sham

Table 3. Effect of stimuli on HDR that were analyzable for both NIRS and esophageal responses

<table>
<thead>
<tr>
<th>Stimulation</th>
<th>HDR Present</th>
<th>HDR Absent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Visceral ($n = 66$)</td>
<td>40 (60.6)</td>
<td>26 (39.4)</td>
</tr>
<tr>
<td>Sham ($n = 22$)</td>
<td>5 (22.7)</td>
<td>17 (77.3)</td>
</tr>
</tbody>
</table>

Values are shown as n(%), where $n$ is the no. of subjects. HDR, hemodynamic response. $P$ value = 0.002 for comparison between visceral and sham stimulation.

Table 4. Effect of adaptive peristaltic reflexes to visceral stimuli on HDR that were analyzable for both NIRS and esophageal responses

<table>
<thead>
<tr>
<th>Esophageal Response</th>
<th>HDR Present</th>
<th>HDR Absent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deglutition reflex ($n = 23$)</td>
<td>19 (82.6)</td>
<td>4 (17.4)</td>
</tr>
<tr>
<td>Secondary peristaltic reflex ($n = 26$)</td>
<td>13 (50.0)</td>
<td>13 (50.0)</td>
</tr>
<tr>
<td>No esophageal activity ($n = 17$)</td>
<td>8 (47.1)</td>
<td>9 (52.9)</td>
</tr>
</tbody>
</table>

Values are shown as n(%), where $n$ is the no. of subjects. $P$ value <0.0001 for comparison of adaptive esophageal responses.
stimulation, basal spontaneous deglutition was 8.84 times more likely to have a cortical HDR rate (odds ratio: 8.84; confidence interval 2.11–37.11; \( P < 0.001 \)). Basal spontaneous deglutition and adaptive deglutition have different effects on frontoparietal NIRS signals as evidenced by the contrasting recruitment frequency of NIRS changes between these deglutition events (Table 5). However, we found similarities in central cortical activation. Furthermore, the cortical response duration and magnitude were also similar with basal spontaneous deglutition vs. stimulus-induced adaptive deglutition response (\( P = 0.41 \) and 0.64, respectively).

**DISCUSSION**

In this mechanistic study, we observed the effects of visceral stimulation on frontoparietal responses in human neonates using a novel provocative esophageal manometry concurrent with NIRS. This is the first study to define cortical activation using these modalities in neonates at the crib side. The cardinal findings were: 1) concurrent provocative esophageal manometry and NIRS in neonates is feasible and safe; 2) activation of the frontoparietal region is 3.6-fold more frequent with deglutition response compared with sham stimuli; 3) extraction of oxygen from HbO was evident in the frontoparietal region with deglutition, and significant brisk and sustained changes in HbO and HbD were recognized to implicate a neurophysiological response; 4) lateralization of cortical response was not evident, thus suggesting bilateral representation for swallowing; and 5) over a twofold increase is evident in HbO with deglutition, suggesting an increase in neuronal response and blood flow, whereas extraction rates are similar.

Swallowing involves a complex sequential coordination of carefully orchestrated aerodigestive muscular contractions that facilitates the bolus transport and maintains airway safety. Brain and brain stem interactions modulate a primary response to enhance safe transport of the oral bolus and a secondary response that involves cortical vigilance. This process involves afferent and efferent fibers from cranial nerves V, VII, IX, X, and XII. The nuclei reside in pons and medulla oblongata (7, 36). Cortical perception and vigilance during human adult swallowing studies revealed the key neurosensory areas to include but are not limited to: thalamus, cerebellum, basal ganglia, pyramidal tracts, frontal operculum, and the insula (18). Similar information, however, is not known from human neonates, who have formidable neurosensory and nociceptive stimulations to their aerodigestive tracts during their recovery from neonatal illness. Unfortunately, neuroimaging studies in neonates require that infants should be calm and motionless. Such situations are difficult to achieve without giving sedation; thus, this scenario modifies the testing conditions for defining cortical activation. Testing for functional brain mapping for prolonged periods is also not feasible in situations where instructions cannot be followed, such as in neonates. Therefore, we adopted the alternative technology of NIRS at the crib side in sedated neonates to characterize and quantify the changes in HbO and HbD in the regions of interest, such as frontoparietal and insular areas that house the majority of swallowing networks. In the current study, we have shown the feasibility of accurately defining stimulus-induced swallowing and presence or absence of NIRS response. Such studies will pave the way for mapping sensorimotor circuits involved with swallowing and feeding. Because the human neonatal brain is a rapidly progressing developmental system, longitudinal studies using our approaches will be beneficial to investigate maturational changes in health and disease, as well as provide avenues to map functional and dysfunctional sensorimotor neural circuits related to aerodigestive reflexes.

Various phases of swallowing in relation to cortical activation have been studied (2, 6, 11, 28) in human adults. Specifically, changes in the metabolic activities were evident in the somatosensory motor cortex, anterior cingulate gyrus, insular cortex, frontal operculum, and superior temporal gyrus. Additionally, differences were also noted with regards to volitional aspects of intent and preparation for swallowing (anterior cingulate gyrus and insular cortex mediated) vs. reflexive swallowing, which is brain stem mediated (32, 34). While we cannot control volitional swallowing in neonates, activation of the somatosensory cortex and insular cortex occurred as was evidenced by frontoparietal cortical activation during spontaneous swallowing and adaptive deglutition responses. Some of these findings are similar to adult human studies during fMRI when volitional swallowing occurs with sequential changes in cortical activation, as the adult human is able to define perception, take commands, and execute motor functions. In neonates, adaptive deglutition responses are also associated with cortical activation. This adaptive deglutition phenomenon is not seen in adults during esophageal provocation (35). Cortical response changes have been determined in adults using concurrent EEG, fMRI, and fNIRS (3, 4, 6, 31). We have also previously determined cortical response changes in infants using polysomnography (27). Similar to these approaches, we conclude that the physiological cortical response is actually the change in fNIRS, and pharyngeal stimulation-induced cortical response changes are manifest by changes in fNIRS.

As known from adult studies (3, 4, 6, 31), deglutition or central swallowing mechanisms require a larger sensorimotor field and involve oromotor and pharyngo-esophageal muscular participation; thereby, activation of the regions of interest supports that afferent pathways were activated from esophagus to brain stem nuclei, and then to thalamus, basal ganglia, and frontoparietal-insular areas. From our neonate studies, the presence of well-coordinated swallowing motor response supports the activation of ascending and descending motor pathways, thus regulating well-orchestrated swallowing behavior. Whether these neonatal mechanisms involve multisynaptic pathways is not clear.

Swallowing and midesophageal provocation is likely activating the somatosensory cortex and corresponding motor areas in the lateral precentral cortices of the neonate. Thus, these sensorimotor events may be manifestations of esophageal sensitivity-induced fNIRS changes. Exaggeration of esophageal sensitivities can occur in the context of gastrointestinal reflux symptoms. Further studies are needed to clarify potential

<table>
<thead>
<tr>
<th>Deglutition Type</th>
<th>HDR Present</th>
<th>HDR Absent</th>
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<tbody>
<tr>
<td>Basal spontaneous deglutition (n = 18)</td>
<td>13 (72.2)</td>
<td>5 (27.8)</td>
</tr>
<tr>
<td>Adaptive deglutition reflex (n = 23)</td>
<td>19 (82.6)</td>
<td>4 (17.4)</td>
</tr>
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</table>

Values are shown as n(%), where n is the no. of subjects. \( P \) value = 0.47 for comparison of vagally mediated deglutition.

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**Table 5. Effect of vagally mediated deglutition on HDR that were analyzable for both NIRS and esophageal responses**
clinical mechanisms as the basis for symptoms. Our study shows that there is activation in the somatosensory cortex and the insular cortex during spontaneous swallowing. This corresponds to adult studies, which also show that there is activation in the insular and somatosensory cortex during volitional swallowing. On the other hand, reflexive swallowing has activations in the brain stem, as evidenced by vagal nerve-mediated pharyngeal reflexive swallowing and PUCR. Study of reflexive swallowing associated with other complex somatosensory behaviors and characteristics of cortical activation remain to be explored in further studies, regardless of the age spectrum.

The neonatal brain is in a period of rapid growth and development, and neonates spend a considerable amount of time in sleep, feeding, and breathing. Like other motor and cognitive skills, development of swallowing and feeding reflexes continues to advance and mature during infancy and toddler periods. At what stage of human development lateralization of specific functional connectivity occurs is not known.

One-half of the NIRS responses were bilateral and the rest unilateral. Predominance in lateralization of cortical response was not evident. We infer that activation of swallowing reflexes may have bilateral cortical representation for swallowing. This observation is in agreement with other adult human studies using fMRI (6, 28, 32, 33). On the other hand, there was also no difference between signals from the left or right hemisphere overall. The development and distribution of ascending or descending neural pathways on both sides in relation to glossopharyngeal and vagal nuclei centered in medulla in human premature infants are not known. Furthermore, the functions of these pathways in relation to swallowing reflexes and cortical response changes are not known. All we can say from our study is that both sides and bilateral representation of cortical response changes are evident.

The insular cortex is located in the region that is being analyzed as well as the part of the motor homunculus that controls the mouth, tongue, and esophagus. Lesions located at the insula have been shown to cause dysphagia (9); thus, HbO activation in this region reaffirms previous studies and shows that the NIRS system can analyze regions associated with swallowing. These results show that NIRS can be used to complement other instrumentation to help determine the correlation between brain activity and feeding disorders.

Other researchers have used NIRS in delineating visual and voice recognition in neonates to determine the areas of brain activation and interpretation of such stimulations (14, 30, 37, 42, 43). Yet others have used NIRS to help characterize tissue oxygen saturation of neonates with congenital heart defects and neurological disorders (10, 12, 44). Our current study is the first to systematically examine the mapping of circuits related to swallowing and visceral stimulation in human neonates.

Clearly our current approaches have advantages: 1) concurrent NIRS permits prolonged sensorimotor examination of different tasks in multiple experimental paradigms; 2) these approaches can be performed at crib side without the need to transport infants or the need for sedation; 3) direct observation of behaviors is possible, leading to accurate documentation of time-related motor patterns that have a central basis; and 4) there is no need for radiation exposure or placement in magnets for fMRI. Thus, safety concerns for concurrent studies such as ours are negligible.

Our approaches have shortcomings as well that need further refinement in future studies, and these include: 1) development of robust methods to evaluate indeterminate responses and motion artifacts. In the current study, this was alleviated by using a good band pass filter to remove the sharp increases in HDRs; 2) minimizing patient motion in future research protocols by using the practice of swaddling to soothe the infant while allowing maximum comfort with minimal restraint; 3) hair obstruction can lead to noisy signals since it obstructs the light path (1, 43) but can be minimized by displacing the hair out of the way when setting up the sources and detectors on the infant; and 4) increasing the signal-to-noise ratio to minimize a mirror effect on the NIRS signal by adjusting the cap design. The cap was chosen based on the neonatal head circumference, which varies between subjects. Smaller caps than the patient’s head circumference can lead to a snug fit, but we did not use that approach because it may apply pressure on the infant’s head. Less reliable contact between the cap and scalp results in a high gain between the source and detector pair during calibration. The gain and the weak signals received by the detector reduce the signal-to-noise ratio of the source-detector pair and cause a mirroring effect between the HbO and HbD. Thus, these signals were considered not analyzable, but steps were taken to mitigate this problem.

This preliminary feasibility study was designed to investigate the physiology of brain-foregut interactions, specifically characterizing changes in HbO at different loci. The experimental paradigms used have the potential to differentiate cortical activation during specific esophageal reflexes and functions (deglutition, gastroesophageal reflux, esophageal peristalsis, UES reflexes, and LES reflexes). Future studies could help elucidate and clarify the pathophysiology of dysphagia and gastroesophageal reflux disease in infants. This methodology is far from being used as a new diagnostic method, but is rather a proof of concept.

Our approaches have several implications for future clinical and translational research from the current study. 1) Better understanding of cortical activation during swallowing and esophageal reflexes is critical for understanding feeding aversion and dysphagia syndromes. We are far from the development of objective evidence-based therapies in this group of infants. 2) The human infant brain is in a state of rapid neuroplasticity and growth, and evaluation of neurosensorv pathways during recovery from illness is possible in longitudinal studies. Therefore, both longitudinal and sequential changes in swallowing architectural neural networks can be characterized using the methods adopted by us. Such approaches can enhance our understanding of the central basis of dysphagia symptoms or of their modification. 3) Finally, we are not in a position to use these concurrent methods for routine clinical diagnostic testing, since further refinement in data analytical methods, signal-noise filtration methods, and development of indications for clinical testing are needed.

In conclusion, this is the first study to define cortical activation using NIRS and esophageal manometry modalities in neonates at the crib side. Concurrent provocative esophageal manometry and NIRS in neonates is feasible, safe, and practical. Activation of the frontoparietal region is 3.6-fold more frequent with deglutition response. Extraction of oxygen from HbO was evident in the frontoparietal region with deglutition. Significant brisk and sustained changes in HbO and HbD are...
recognized, suggesting a neurophysiological response. Later-
alization of cortical response was not evident, thus suggesting
bilateral representation for swallowing.

ACKNOWLEDGMENTS
We are grateful to Rebecca Moore for nursing observations with this study.

GRANTS
This study is supported in part by National Institute of Diabetes and
Dietetic and Kidney Diseases Grant RO1-DK-068158 (S. R. Jadcherla).

DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS
I.D., X.G., and N.H.K. interpreted results of experiments; S.R.J., J.F.P.,
J.F.P., K.A.H., I.D., X.G., and N.H.K. performed experiments; S.R.J., J.F.P.,
K.A.H., I.D., X.G., and N.H.K. analyzed data; S.R.J., J.F.P., K.A.H., I.D.,
X.G., D.G.B., and N.H.K. edited and revised manuscript; S.R.J., J.F.P.,
K.A.H., I.D., X.G., D.G.B., and N.H.K. approved final version of manuscript;
J.F.P. and K.A.H. prepared figures.

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