Esophageal sensation in premature human neonates: temporal relationships and implications of aerodigestive reflexes and electrocortical arousals

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1Sections of Neonatology, Pediatric Gastroenterology, and Nutrition, The Neonatal and Infant Feeding Disorders Program, Center for Perinatal Research, Department of Pediatrics, The Ohio State University College of Medicine, The Research Institute at Nationwide Children’s Hospital, Columbus; 2The Neonatal and Infant Feeding Disorders Program, The Research Institute at Nationwide Children’s Hospital, Columbus; 3Pediatric Sleep Medicine, Department of Pediatrics, The Ohio State University College of Medicine, Nationwide Children’s Hospital, Columbus; 4Center for Biostatistics, The Ohio State University College of Medicine, Columbus, Ohio; and 5Division of Gastroenterology, Department of Internal Medicine, Medical College of Wisconsin, Milwaukee, Wisconsin

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Jadcherla SR, Parks VN, Peng J, Dzodzomenyo S, Fernandez S, Shaker R, Splaingard M. Esophageal sensation in premature human neonates: temporal relationships and implications of aerodigestive reflexes and electrocortical arousals. Am J Physiol Gastrointest Liver Physiol 302: G134–G144, 2012. First published August 18, 2011; doi:10.1152/ajpgi.00067.2011.—Electrocortical arousal (ECA) to perceived stimuli. Accordingly, arousal difficulties have been proposed as causal mechanisms in the pathogenesis of sudden infant death syndrome or apparent life-threatening events (ALTE) (13, 36). Prevalence of life-threatening events in premature infants is common and such episodes usually warrant further investigations. Sleep studies are often indicated in conditions in which survival mechanisms are at risk. Infants with ALTE undergo evaluation for esophageal and sleep pathology, and the mechanisms for arousals in relation to esophageal motility or gastroesophageal reflux (GER) events are unclear in preterm infants either in health or disease.

An arousal response due to positional changes or various physical or chemical stimuli has been described in infants (10, 27) and represents a protective response or constellation of reflexes (22). The infant arousal response involves subcortical and cortical responses occurring as a sequence of stereotyped behavior and may occur in response to somatic or visceral stimuli or physical or chemical stimuli. However, the mechanisms for arousals in relation to esophageal provocations are unclear. Spontaneous arousals have been described as sequences of varying combination of events ranging from apneas or augmented breaths followed by startle to wakeful states (33). Typically, an infant arousal response involves an augmented breath, coupled with a startle, followed by cortical arousal. Each of these responses has been shown to occur during quiet or non-rapid eye movement sleep stage and active or rapid eye movement sleep in infants (9, 20, 23).

We have previously studied the effects of midesophageal sensory stimuli on esophageal peristaltic reflex and upper esophageal sphincter contractile reflex (UESCR) to divert visceral stimulus away from the aerodigestive tract in premature infants (15, 18). Although spontaneous electrocortical arousals
esophageal neuromotor responses. Therefore, this study was undertaken to investigate the effects of pharyngoesophageal motility sequences and esophageal provocation on the temporal changes in sleep state and respiratory patterns. Our aims were (1) to characterize the spatiotemporal relationship of spontaneous ECAs in relation to pharyngoesophageal motility changes as in a) spontaneous swallows and b) gastroesophageal reflux events, and (2) to define the sensory-motor characteristics of esophageal reflexes in sleeping healthy premature infants during concurrent esophageal manometry and polysomnography (PSG) by testing the hypothesis that esophageal provocation can result in both esophageal reflex responses and ECAs during sleep and that arousals are dependent on the frequency characteristics of esophageal neuromotor responses.

**SUBJECTS AND METHODS**

**Participants**

Entally fed preterm neonates \((N = 16)\) were studied when they were physiologically stable. Gestational age (GA) was determined by maternal history and obstetric data. Postmenstrual age (PMA) was determined by adding GA to chronological age. Subjects were studied when they had recovered from neonatal transition and respiratory distress at birth and were on a stable phase of feeding and growth. All subjects were evaluated by the principal investigator (S. R. Jadcherla) and the attending neonatologist and were deemed healthy at study. None of the subjects had a presumed or proven clinical diagnosis of GER, and none were receiving prokinetics, acid suppressive therapy, or xanthines at the time of study or discharge. None were diagnosed with necrotizing enterocolitis or bronchopulmonary dysplasia during their hospital stay. Subjects with perinatal asphyxia, gastrointestinal abnormalities, birth defects, chromosomal disorders, and neurological abnormalities were excluded.

The study procedures were approved by the ethics committee at the Institutional Research Review Board at the Nationwide Children’s Hospital Research Institute, Columbus, OH. The study protocol conforms to the standards set by the latest revision of the Declaration of Helsinki, and the methods and procedures were approved by Nationwide Children’s Hospital Research Institute’s constituted Institutional Research Review Board as well as in accordance with the Health Insurance Portability and Accountable Act (HIPAA). Written, informed consent and HIPAA authorization were obtained from parents prior to study.

**Manometry**

The use of esophageal manometry methods in neonates was described by us before (15, 16, 18). Briefly, the catheter assembly (Dentsleeve International, Mui Scientific, Mississauga, Ontario, Canada) was connected to the pneumohydraulic micromanometric water perfusion system via the resistors, pressure transducers (TNF-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\(^{-1}·\text{port}^{-1}\) for esophageal ports, 0.01 ml·min\(^{-1}·\text{port}^{-1}\) for the pharyngeal port, and 0.04 ml·min\(^{-1}·\text{port}^{-1}\) for the sleeves. The catheter was passed nasally in the supine lying neonate without the aid of sedation. All studies were done in the same manner, with the transducers at the level of the subject’s esophagus (midaxillary line).

PSG, respiratory inductance plethysmography (Respitrace, Viasys, Conshohocken, PA), heart rate, and pulse oximetry were synchronized and recorded concurrent with manometry. The computer clocks recording PSG (Grass methods, Astro-Med, West Warwick, RI) and manometry (MMS methods) were synchronized to the millisecond before start of the study and verified at the end of the study.

**Manometric Experimental Protocol**

Continuous data acquisition and analysis were performed during manometric study based on waveform characteristics, as defined before (15, 16, 18). During manometric pull-through, the high-pressure zones of the lower and upper esophageal sphincters (LES and UES, respectively) were identified by the presence of a consistent increase in pressure greater than 5.0 mmHg above the baseline for at least 15 s, in addition to the changes in pressure with respiration (15, 16, 18). Neonates were allowed to adapt to catheter placement for ~15 min. Spontaneous swallow-mediated primary peristalsis sequences, spontaneous GER events, and responses to midesophageal infusions were evaluated.

**Manometry Data Analysis**

**Stimulus characteristics.** Abrupt esophageal infusions (air, water, apple juice) were given via the midesophageal infusion port and responses were analyzed. Infusion duration was measured as the duration from the start of infusion to the waveform peak of the infusion signal. Sham stimuli markers were placed during a period of esophageal quiescence when no stimulus was given. Esophageal motility and PSG characteristics were measured up to 10 s after sham stimulus.

**Analysis related to swallowing reflexes.** These characteristics were analyzed as defined before (12, 15, 16, 18), and described briefly as follows: 1) Spontaneous primary peristalsis was defined as a spontaneous pharyngoesophageal sequence that begins in the pharynx and is associated with UES relaxation and propagation of the peristaltic wave front across the proximal, middle, and distal esophagus and accompanied by LES relaxation (12). 2) Esophagodeglutition response (EDR) was defined as a deglutition response to esophageal stimulation that begins with onset of the pharyngeal waveform associated with UES relaxation and propagation of the peristaltic wave front across the proximal, middle, and distal esophagus and accompanied by LES relaxation (17). 3) Secondary peristalsis (SP) occurs in response to midesophageal provocation and is defined as the propagation of waveforms distally from the proximal, middle, and distal esophageal segments in the absence of pharyngeal waveform and UES relaxation. The onset of proximal esophageal upstroke from the stimulus onset defines the response latency for SP (17). 4) Response onset to peristaltic reflex was taken from onset of stimulus to the onset of EDR or SP. 5) Duration of peristaltic response was defined as the period between the onset of peristaltic response originating in pharynx to the offset of distal esophageal contraction waveform. 6) Velocity of primary peristalsis was measured as the product of distance (from pharynx to distal esophagus) over time (onset of pharyngeal waveform to the onset of esophageal contraction in the distal esophagus). 7) Velocity of SP was measured as the product of distance (from proximal esophagus to distal esophagus) over time (onset of proximal esophageal waveform to onset of distal esophageal waveform).

**Analysis related to esophageal body characteristics.** These characteristics are analyzed as defined before (12, 15, 16, 18) and described briefly as follows: 1) Complete propagation was considered as sequential propagation of esophageal waveforms that begin in the proximal esophagus (PE), leading to middle esophagus (ME) and distal esophagus (DE). 2) Incomplete propagation was determined as two or fewer sequential esophageal waveforms that do not reach the distal esophagus. 3) Failed propagation was considered when no esophageal waveforms were noted despite presence of a pharyngeal waveform and UES relaxation. 4) Amplitudes of PE, ME, and DE
were measured as the maximum amount of pressure (mmHg) reached during respective esophageal waveforms. Durations of PE, ME, and DE were measured as the total time taken from the onset of esophageal waveform to the offset of the waveform.

Analysis related to sphincter characteristics. These characteristics are analyzed as defined before (15, 16, 18, 29), and described briefly as follows: 1) Resting UES pressure was measured as an average of five UES pressure measurements at end expiration observed prior to stimulus or spontaneous swallow. 2) Response onset to UESCR is defined as the time taken from the onset of stimulus for an increase in UES pressure of at least 4 mmHg above baseline. 3) Maximum UES contraction pressure was taken at the maximum pressure reached after onset of UES contraction. 4) UESCR duration is measured from the onset of UES contraction, to the peak of UESCR. 5) Resting LES pressure was measured as an average of five LES pressure measurements observed prior to stimulus or spontaneous swallow. All pressures were taken at end expiration and in relation to gastric pressure.

Respiratory arousal characteristics. These characteristics are analyzed on the basis of changes in the respiratory inductance plethysmography recordings. 1) Respiratory change was scored if deglutition apnea or a change in rhythm was present. 2) Onset of respiratory arousal was considered as an abrupt change in respiratory rate and rhythm characterized by increased exhalations and inhalations, sometimes associated with stridor, gape, or cough and wakefulness or startle. 3) Respiratory arousal duration was determined from the onset of respiratory arousal to restoration of respiration normalcy.

GER characteristics. 1) GER was defined manometrically as LES relaxation followed by a change in esophageal common cavity pressure of at least 2 mmHg and associated with retrograde esophageal waveforms (14, 25). 2) Transient lower esophageal sphincter relaxation (TLESR) is the most common mechanism of GER (25) and is a prolonged period of LES relaxation lasting longer than 10 s (26) that occurs in the absence of any preceding pharyngeal or esophageal waveforms. 3) TLESR duration was measured from the onset of prolonged LES relaxation to restoration of LES resting pressure. 4) Onset of GER event to complete clearance was calculated from

<table>
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<th>Sleep (48)</th>
<th>Awake (11)</th>
<th>P Value</th>
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<tr>
<td>Resting UES pressure, mmHg</td>
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<td>Resting LES pressure, mmHg</td>
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<td>17.5 ± 4.3</td>
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<td>PE amplitude, mmHg</td>
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<tr>
<td>ME amplitude, mmHg</td>
<td>65.2 ± 5.6</td>
<td>64.2 ± 10.0</td>
<td>0.9</td>
</tr>
<tr>
<td>ME duration, s</td>
<td>3.9 ± 0.4</td>
<td>3.3 ± 0.6</td>
<td>0.4</td>
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<tr>
<td>DE amplitude, mmHg</td>
<td>64.2 ± 9.0</td>
<td>65.5 ± 15.4</td>
<td>0.9</td>
</tr>
<tr>
<td>DE duration, s</td>
<td>4.9 ± 0.4</td>
<td>5.5 ± 0.8</td>
<td>0.6</td>
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Values are expressed as means ± SE; significance is based on mixed model with repeated measurements. LES, lower esophageal sphincter; PE, proximal esophagus; ME, middle esophagus; DE, distal esophagus.

Fig. 1. Pharyngoesophageal manometry and concurrent polysomnography (PSG). Effect of spontaneous swallow on sleep. Example of swallow with electrocortical arousal. Notice the abrupt upper esophageal sphincter (UES) pressure increase followed by an immediate relaxation. Next, UES gradually increases in unison with changes in respiration. Concurrently, changes in sleep pattern occur. Thereafter, baseline UES pressure is restored as well as normal respiratory patterns.
onset of GER mechanism to complete esophageal peristalsis along with restoration of respiratory normalcy.

Video PSG methods. Infant sleep study was performed and scored by accepted EEG methods. The Grass sleep system (Grass Technologies, Astro-Med) and the TWin PSG Clinical Software were used. A standard infant montage including C3, C4, CZ, O1, O2, A1, A2 gold-plated electrodes were used. EOG electrodes and chin EMG electrodes were applied, in addition to the EKG, thoracic, and abdominal RIP belts. Observations include eyes open, eyes closed, and movements. The studies were scored by the dedicated certified PSG technician (I. Keith) as per the established guidelines for neonates (5, 35a, 34).

PSG data analysis. The PSG analysis was verified and confirmed by sleep board-certified sleep physiologists (M. Splaingard, S. Dzodzomenyo) using an a priori criteria and was classified into different sleep stages and arousals (11). The scorers commented on the sleep stage during a point of interest (e.g., onset of infusion, onset of TLESR, sham events, onset of primary peristalsis, somatic stimuli, etc.); however, the scorers were blinded to the nature of stimulus or the type of the manometric event. Sham events were also included, during which no stimulation was given. Infant sleep stages were separated as per the standard criteria into active and quiet sleep and awake states, respectively, were 65:25:10 proportions (%) of complete vs. incomplete vs. failed propagation of spontaneous solitary primary peristalsis sequences during sleep state and awake state, respectively, were 65:25:10 and 73:9:18 (P = 0.5, χ²).

Differentiating the Effect of Arousals on Basal Esophageal Motor Activity and Respiratory Patterns

Overall, 30 of 59 (50.7%) spontaneous swallows were associated with arousals. Furthermore, frequent changes in sleep state following arousals were observed during swallow-related arousals (26.7 vs. 3.5% in nonarousals, P = 0.05), and similar changes in respiratory patterns were more frequent (76.7 vs. 0% in nonarousals, P = 0.0001) (Fig. 1). After completion of the UES relaxation phase of swallow, the return of the UES pressure to baseline was followed. An increase in UES contractile pressure after the UES relaxation phase of spontaneous primary peristalsis was noted more frequently during arousals (66.7 vs. 6.9% nonarousals, P = 0.002), and the onset of UES pressure increase was delayed during swallows associated with arousals (9.3 ± 1.1 s vs. 0.35 ± 3.5 s in nonarousals, P = 0.04). Completely propagated primary peristaltic sequences were more frequent during swallows with nonarousals compared with swallows with arousal (72.4 vs. 26.7% with arousals, P = 0.0007). Response onset to LES relaxation and onset of swallow to complete esophageal clearance are prolonged with those swallows associated with arousal (Fig. 2).

Relationship Between Electro cortical Arousal and Solitary or Multiple Swallow Sequences

Of 30 swallow-arousal sequences, the median number of pharyngeal swallows during an episode was three per occur-
Characteristics of esophageal motility and arousals were differentiated on the basis of pharyngeal swallow waveform frequency per burst as minimal (1–3) swallows or maximal (>3) swallows per episode. ECA duration (s) during minimal swallows was 7.1 ± 1.8 and during maximal swallows was 11.7 ± 1.8, P = 0.1. The time (s) taken for complete esophageal clearance from onset of swallowing event was significant (P = 0.01) between minimal (33.4 ± 9.6) and maximal swallows (74.5 ± 9.9). Response time (s) from the onset of pharyngeal waveform to the onset of ECAs during minimal and maximal swallows was 11.2 ± 3.7 and 22.5 ± 3.8, respectively (P = 0.03). Frequency of postdeglutition rise in UES pressure over baseline was different, 46.7% among minimal swallows vs. 86.7% for maximal swallows (P = 0.01). UES contractile duration was similar during nonarousals (26.7 ± 10.1) for minimal swallows vs. 55.0 ± 7.9 for maximal swallows (P = 0.1). Frequency of respiratory arousals during minimal and maximal swallows was 53 and 100%, respectively (P = 0.01).

**Effect of GER Events on Changes in Sleep and Respiratory Patterns**

Fifty-one of 69 GER events (73.5%) were associated with arousals (GER arousals), and the esophageal motility, clearance characteristics, as well as sleep state and respiratory changes were examined. Comparisons were made between GER arousals vs. GER with nonarousals (Fig. 3). Onset of GER event to completion of esophageal clearance (restoration of esophageal quiescence after distal esophageal waveform) was lesser during nonarousals (44.1 ± 9.3 s vs. 75.7 ± 6.3 s with arousals, P = 0.01). Response time to the onset of peristalsis facilitating clearance was also lesser during nonarousals (33 ± 8.9 s vs. 61.6 ± 5.9 s with GER arousals, P = 0.01). During GER with nonarousals, UES contractility was noted in 61% (vs. 92.2% in GER arousals, P = 0.01). Changes in sleep state were not witnessed in those GER events associated with nonarousals, in contrast to sleep state modification in 64.5% of GER arousals (P = 0.002). Respiratory arousal changes were noted in 72.2% of GER events with nonarousals vs. 92.2% in GER arousals (P = 0.06). Respiratory arousal lasted for 29.2 ± 8.8 s in GER without arousal vs. 55.6 ± 5.3 s in GER arousals (P = 0.02). Significant positive correlation (ρ = 0.7, P < 0.0001) was noted between spontaneous GER duration and respiratory arousal duration (Fig. 6A).

**Examination of Sleep Stage Changes and Respiratory Patterns After Abrupt Midesophageal Provocations During Sleep**

Abrupt midesophageal infusions (N = 95, 30 air, 65 liquids) and 31 sham infusions were given in sleep defined by PSG. No esophageal responses or arousals were noted with 31 sham stimuli. In contrast, 51 of 95 esophageal stimuli (54%) resulted in arousals (P < 0.0001 vs. sham stimuli) (Fig. 4). The characteristics of stimulus provoking the esophagus were similar during arousals (N = 44) vs. nonarousals (N = 51). Particularly, there was no difference (P = NS) in infusion duration (s) between arousals (2.6 ± 0.3) and nonarousals (2.3 ± 0.3); nor was there variation in flow rate (ml/s) between arousals (0.7 ± 0.1) vs. nonarousals (0.8 ± 0.1). There was no difference between distribution of media (air, water, apple juice) during arousals (14:19:18) and nonarousals (16:11:17). However, when arousals occurred, the response onset to arousal was 13.8 ± 1.5 s, and the total duration of arousals with each stimulus was 11 ± 1.6 s.

Peristaltic reflex response frequency was greater during those infusions that were associated with arousals (96 vs. 70% in nonarousals, P = 0.0001). Similarly, UESCR was more frequently noted during those infusions that were associated with arousals (96 vs. 57% in nonarousals, P = 0.0004). Significant prolonged response onset and duration with peristaltic reflex responses (Fig. 5A) and UES contractile reflex (Fig. 5B) were noted during those infusions associated with arousals (both P < 0.05, Fig. 5, A and B). The frequency of LES relaxation during those infusions associated with arousal was 94.1% (vs. 75% in infusions with nonarousals, P = 0.001). However, the response onset to LES relaxation (8.3 ± 1.5 vs. 4.3 ± 1.4 s) and LES relaxation duration (15.5 ± 5.5 vs. 4.0 ± 6.5 s) were similar between infusions evoking arousals and nonarousals. Frequent changes in sleep state were noted during those infusions that were arousal associated (35.3 vs. 6.8% during nonarousals, P < 0.0001) as well as the changes in respiratory pattern (78.4 vs. none during nonarousals, P < 0.0001). In addition, significant positive correlation (ρ = 0.9, P < 0.0001) was noted between spontaneous infusion response duration and respiratory arousal duration (Fig. 6B).

**DISCUSSION**

The frequencies of feeding periods, swallowing, and GER events as well as the amount of time spent in sleep are all greater during neonatal period than at any other stages of life. Therefore, aerodigestive provocation and concerns of aspiration are frequent considerations in premature infants convalescing in the neonatal nurseries, and these infants therefore undergo constant monitoring for aerodigestive safety and respiratory signs. Arousals from sleep have been considered an important protective response in infants and can occur with or without stimuli (22, 36). The relationship of such arousals with esophageal events or the temporal relationships or the sensory-motor mechanisms for such events has been unclear in human neonates. In the present study, the important findings are that 1) basal esophageal motility characteristics are similar in sleep or awake state; 2) sleep state changes are seen frequently following swallows; 3) swallow-arousal sequences are associated with prolonged UES, LES, and esophageal body motility changes; 4) esophageal provocation during spontaneous GER events in sleep significantly modifies sleep state and respiratory patterns and causes arousals; 5) GER arousals are associated with prolonged esophageal motility and clearance; and 6) adaptive esophageal motility patterns evoked upon induced
provocation are associated with arousals and altered sleep stage changes and respiratory patterns.

Sleep state changes were seen frequently after spontaneous swallows in this study, in that ~51% were associated with arousals. It was interesting to note that completely propagated primary peristaltic sequences were significantly \((P = 0.0007)\) more frequent during swallows with nonarousal compared with swallows with arousal. Thus longer esophageal transit or incomplete propagation of peristaltic reflexes may result in hypervigilant state characterized by ECA. Significant changes \((P = 0.05)\) in sleep state following arousal was evident more frequently, and are also associated with changes \((P = 0.0001)\) in respiratory patterns. Our findings are suggestive of activation of secondary (respiratory) and tertiary (electrocortical) vigilance mechanisms during esophageal transit and may be related to modulation of respiratory-related neurons implicated in swallow-respiratory coordination (21). Spontaneous pharyngeal swallow-associated respiratory pauses were seen in sleep and were also observed by others (8, 19, 24, 36). Sleep-stage associated changes in UES contraction reflex have been described in adults (2) but not are clear in neonates.

Swallow-arousal sequences were associated with prolonged UES, LES, and esophageal body motility changes, in that two-thirds of the swallow-arousal sequences were associated with postdeglutition increase \((P = 0.002)\) in UES contractile pressure, and when that occurred, prolonged response onset \((P = 0.04)\) to UESCR by 27-fold was also evident. Similarly, response onset to LES relaxation \((P = 0.03)\) by 3.7-fold, and onset of pharyngeal peak to complete esophageal clearance \((P = 0.0003)\) was prolonged by 5.7-fold with swallow-arousal sequences. These findings are reflective of activation of vagal and glossopharyngeal afferents and vagal efferents that regulate UESCR, peristaltic reflexes, and LES relaxation reflex. These reflexes are activated during swallows without arousals also; however, during arousal, the response delays to activation of UESCR and increased and prolonged activation of UESCR suggest that UES plays an important role in neonates to facilitate postdeglutition airway protection, whereas esophageal peristalsis and LES relaxation remains prolonged.

During arousals, multiplicity of swallows was associated with significantly prolonged changes in esophageal motility, in that the time \((s)\) taken for complete esophageal clearance from pharyngeal waveform was greater \((P = 0.01)\) by 2.3-fold during multiple swallows. Response time \((s)\) from the onset of pharyngeal waveform to the onset of electrocortical arousals for maximal swallows was also greater \((P = 0.03)\) by twofold. Frequency of postdeglutition rise in UES pressure over baseline was also greater \((P = 0.01)\) with maximal swallows, as well as the frequency \((P = 0.01)\) of respiratory arousals. These findings suggest that with solitary or fewer than three swallows per sequence, when completely propagated, the need for arousal is lesser. When peristalsis is not efficient in clearance, then multiple swallows result in prolonged esophageal motor activity and postdeglutition increase in UESCR as well as ECAs, thus activating multiple levels of airway protection mechanisms.

Esophageal provocation during spontaneous GER events in sleep significantly modifies sleep state and respiratory patterns and causes arousals, and GER arousals are associated with prolonged esophageal motility and clearance. Overall, 73.5% of GER events were associated with arousals (GER arousals), and when arousals did not happen neonates slept well without significant changes in sleep state. Conversely, respiratory arousals were seen regardless of ECAs during GER events. However, respiratory arousal lasted nearly twofold greater \((P = 0.02)\) during ECAs compared with nonarousals. Interestingly, the esophageal motility and clearance characteristics were significantly longer during GER arousals. Specifically, the response onset to peristaltic reflexes was longer by nearly twofold and the onset of GER event to restoration of esophageal quiescence following peristaltic clearance was also longer by nearly twofold. Although increase in UESCR was evident regardless of ECAs, it was more frequent by 1.5-fold during GER arousals (compared to nonarousals).

Examination of sleep stage changes and respiratory patterns after induced abrupt midesophageal provocations during sleep (Figs. 4 and 5) reveal that sensory stimulus provided to esophageal lumen activates afferents to the brain stem and cortex, as evidenced by the presence of respiratory arousals, sleep state changes, ECAs, and adaptive esophageal reflexes. Although the physical (air and liquids) and chemical (water and apple juice) characteristics of stimulus during induced abrupt midesophageal provocations were similar, overall, only 54% of the stimuli resulted in arousals, None of the sham stimuli resulted in arousals. Significantly, during the infusion-induced arousals, the peristaltic reflex response frequency was greater \((P = 0.0001)\), UESCR was more frequent \((P = 0.0004)\), and prolonged response onset and prolonged response duration with both, peristaltic reflex responses \((P < 0.05, \text{Fig. } 5A)\) and UESCR responses \((P < 0.05, \text{Fig. } 5B)\) were noted, as well as the increased frequency of LES relaxation reflex \((P = 0.001)\). Thus, in sleep, esophageal afferent stimulation can activate dormant supratentorial pathways and cortical activation, and restoration of esophageal quiescence may restore sleep rhythm.

Arousal and increased ventilatory response characterized by changes in respiratory rate and rhythm have been seen in hypoxia and hypercarbia in both animal and human adult and infant studies (8, 30, 31, 36, 37). In our study, separately as well as in parallel, upon spontaneous and induced esophageal stimulation, arousal responses have been noted in both quiet and active sleep states. However, the response onset to arousal and relationship of ECA with respiratory changes varied. Interestingly, when ECA occurred, respiratory change was greater, implying that ascending brain stem afferents and thalamocortical pathways were not inhibited, thus activating ECAs, and that brain stem efferents activated respiratory and/or ventilatory changes. That respiratory arousal changes lasted until esophageal clearance and peristaltic activity per-
sisted suggests that sustained aerodigestive stimulus is perceived to warrant respiratory vigilant state and that upon restoration of esophageal quiescence a normalcy of breathing pattern ensued. The implications of these findings in sleeping premature neonates and high-risk ICU infants with chronic lung disease, who are at great risk of life-threatening events and sudden infant death syndrome, are that aerodigestive provocative stimulus can activate defense responses at multiple levels: 1) Activation of local enteric reflexes evokes SP reflex that may have facilitated clearance without the need to stimulate supraesophageal afferents and thus preserved sleep. 2) Activation of local enteric afferents facilitates secondary peristaltic reflex, brief esophagodeglutition response (1–3 swallows), UES relaxation, and transient respiratory changes, thus facilitating esophageal clearance and airway protection, and during this process ECAs also occurred. The fact that ECAs occurred briefly and much earlier than the completion of esophageal reflexes suggests that the arousal is indeed a hypervigilant defensive response needed to coordinate swallowing, respiratory changes, and restoration of esophageal quiescence. Additionally, this direct observation during induced abrupt esophageal provocation also supports that premature infants can perceive esophageal stimuli in sleep. 3) Activation of local enteric and supraesophageal afferents that resulted in disrupted sleep pattern, ECA, increase in UESCR activity, respiratory change, prolonged esophageal waveform activity and multiple swallowing patterns. This defensive phenomenon ends when esophageal clearance is facilitated, and respiratory rhythm is restored. 4) Duration of esophageal provocation is a major factor that is proportional to the duration of respiratory change, regardless of the origin of provocation, where it is of gastric origin as in GER or persistence of esophageal provocation as in delays in esophageal peristaltic clearance. ECA during such anterograde or retrograde esophageal transit may serve to respond to acute threats to survival during sleep, and

Fig. 5. A: comparison of esophageal peristaltic activity that was associated with arousal. When stimulus was given, onset of peristaltic response was prolonged during arousals vs. nonarousals ($P = 0.0007$) as well as the response duration during arousals vs. nonarousals ($P < 0.0001$). B: comparison of UES contractile acuity. Frequency of UES contractility was noted more frequently during arousals vs. nonarousals (96 vs. 57%, $P < 0.0001$). UES contractility response time is delayed in arousals compared with nonarousals. UES contractility, after occurrence, is prolonged in arousal vs. nonarousals.

Fig. 6. A: relationship of spontaneous GER duration vs. respiratory arousal (RA) duration. B: relationship of induced abrupt esophageal provocation vs. respiratory arousal duration.
multiple afferent-efferent systems are integrated by virtue of brain stem viscerosomatic convergence phenomena (28, 35) resulting in aerodigestive defensive reflexes that maintain aerodigestive safety.

In summary, prolonged ECAs are associated with delayed clearance, heightened UESCR, and acute respiratory and sleep state changes. Swallow and GER events without arousals were associated with faster esophageal clearance and infrequent UESCR state. When UESCR occurs, the response time and duration are shorter. The study data also support that visceral sensitivity can be perceived by premature neonates during antegrade esophageal transit as in swallowing airway protection and is associated with activation of the cortical and subcortical arousal mechanisms. Similarly, retrograde esophageal transit during GER events is also associated with differential activation of the nature of arousal mechanisms. Perceived threat is more apparent with GER events and retrograde transit, resulting in heightened excitation and prolonged activation of cortical pathways. This is evidenced by activation and recruitment of multiple sequence patterning of visceral and somatic motor reflexes prior to restoration of normalcy. Presence of arousals and basal esophageal peristalsis may represent the existence of mechanisms in sleep to facilitate diversion of perceived stimulus to safe peristalsis. Infusion-related ECAs were associated with delayed onset and prolonged duration of the esophageal reflexes participating in peristalsis and airway protection. Premature infants can perceive visceral sensitivity resulting in 1) arousals, 2) respiratory changes, and 3) esophageal reflexes. In sleep, esophageal afferent stimulation can activate dormant supratentorial pathways and cortical activation; restoration of esophageal quiescence may restore sleep rhythm.

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DISCLOSURES

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