UNDERSTANDING THE DEVELOPMENT OF INFANT FEEDING: A SPECTRAL ANALYSIS APPROACH

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Feeding problems in preterm neonates stem from complications of early delivery. Attainment of independent feeding is a prerequisite for Neonatal Intensive Care Unit (NICU) discharges. Some quantitative studies of infant feeding involve excessive amounts of time for data processing. Multivariate spectral analysis was used to minimize time for investigation of variability in these rhythms. Auto and Cross-spectral parameters of the rhythms were correlated with Gestational Age (GA), Postmenstrual Age (PMA), Birthweight (BW), Days of Life (DOL), and Time Since First Nipple feeding (TSFN). Auto-spectral analysis showed 25.55% increase in Bandwidth of suck (bw-su) for a 2-week increase in GA (DOL fixed) and 8.99% increase in bw-su for a 10-day increase in DOL (GA fixed). Cross-spectral analysis showed a decrease of 0.158Hz of Bandwidth of Suck-Swallow (bw-SS) for a 2-week increase in GA for GA later than 28 weeks. For GA earlier than 28 weeks, peak coherence decreased by 0.774 for a 2-week increase in GA (PMA fixed) and decreased by 0.126 for a 2-week increase in PMA (GA fixed). The method describes the progression of feeding rhythms through correlations with clinical indexes, thus providing clinicians with an understanding of the development of infant feeding and helps predict long-term developmental outcomes.

Keywords: preterm infants, rhythmic suckle and swallow, neurological maturation, developmental outcomes, spectral analysis.

Pooja Vijaygopal
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UNDERSTANDING THE DEVELOPMENT OF INFANT FEEDING:
A SPECTRAL ANALYSIS APPROACH

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UNDERSTANDING THE DEVELOPMENT OF INFANT FEEDING:
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THESIS

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Biomedical Engineering in the Graduate School at the University of Kentucky

By
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2009

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DEDICATION

I would like to dedicate this work to my parents for I would not have been where I am today without their efforts. I would also like to dedicate it to Ren, whose unrelenting and unconditional friendship I will cherish forever.
ACKNOWLEDGEMENTS

The following thesis, while an individual work, benefited from the insights and direction of several people. I would like to express my deepest gratitude to my mentors, Dr. Abhijit Patwardhan and Dr. Eric W. Reynolds. I would like to thank them for believing in my abilities and for being patient with my shortcomings. This achievement would not have been possible without their support and guidance at various stages of this project and throughout my time here at University of Kentucky.

I would also like to thank Deb Grider, who was truly a pleasure to work with on this project.

In addition, I would like to thank my family and friends. I thank my parents and sister for their support against all odds, from the time I stepped out of home to fulfill my dreams of a foreign education. I would also like to thank Ren and Eesha for their unstinting support and prayers.

Finally, I wish to thank all my friends in Lexington and in the Center for Biomedical Engineering who have made my life worthwhile in the past three years.

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GLOSSARY OF TERMS

1. GA: Gestational Age
2. PMA: Postmenstrual age
3. DOL: Day-of-Life
4. TSFN: Time since first nipple feed
5. BW: Birthweight
6. bw-su: Bandwidth of suck
7. 50p-su: 50% power of suck
8. pk-freq-su: Peak frequency of suck
9. bw-sw: Bandwidth of swallow
10. 50p-sw: 50% power of swallow
11. pk-freq-sw: Peak frequency of swallow
12. bw-SS: Bandwidth of suck-swallow relationship
13. 50p-SS: 50% power of suck-swallow relationship
14. pk-freq-SS: Peak frequency of suck-swallow relationship
15. pk-pha-SS: Peak phase of suck-swallow relationship
16. max-phase: Maximum phase in bandwidth of suck-swallow relationship
17. min-phase: Minimum phase in bandwidth of suck-swallow relationship
18. norm-phase-SS: Normalized phase of suck-swallow relationship
19. pk-coh-SS: Peak coherence of suck-swallow relationship
20. avg-coh-SS: Average coherence in bandwidth of suck-swallow relationship
CHAPTER ONE: INTRODUCTION

Preterm births are defined as those occurring prior to 37 weeks of gestation. Births occurring before 32 weeks are classified as very preterm. While most of the morbidity associated with preterm birth occurs among the very preterm infants, even late preterm delivery (32-37 weeks) is associated with increased risk of poor outcomes. It is in this group of infants that most disorders associated with prematurity are observed. This group also accounts for a majority of neonatal deaths[1]. Preterm birth rates have increased over the last decade. The March of Dimes census showed a substantial increase of 29% in the rate of preterm births in the state of Kentucky between 1996 and 2006 [2]. In 2006, 15.1% of live births babies were born prematurely in the state of Kentucky[2].

Infants born preterm can have severe physiological problems that stem from their immature body systems and low birthweight as well as other associated illnesses due to prematurity. Some of the common disorders in these infants are Bronchopulmonary dysplasia (BPD) or chronic lung disease characterized by the abnormal development of lung tissue and Intraventricular Hemorrhage (IVH) characterized by bleeding in the brain’s ventricular system. Another disorder common in preterm infants is inefficient feeding. Wang et al in 2004 found that as much as 27% of late preterm infants (infants born between 32 and 37 weeks of gestation) had feeding problems versus only 5% of term infants[3]. Feeding issues are even more common in the very preterm group. Since attainment of efficient feeding is imperative to independent survival, these
feeding problems often lead to delayed discharge from the hospital and increased cost [3, 4].

With increased survival of these preterm infants, there is an increase in feeding problems. Consequently, there is a need to expedite the understanding of these issues. Understanding feeding and its associated problems may predict the developmental outcomes leading to an overall decrease in the duration of stay of these patients in the Neonatal Intensive Care Unit (NICU).

Currently there are very few reliable methods to investigate infant feeding. Most researchers have used qualitative investigations to describe the morphologies of the rhythms, as well as feeding behaviors of the infants [3, 4]. Some others have shown stability of these feeding rhythms using quantitative analyses, with stress on temporal relationships and statistical correlations between these physiological events [5, 6]. These quantitative analyses involve manual computations that are labor intensive and time-consuming requiring many hours of data processing per study. The analysis method used in the present study was based on multivariate spectral analysis that allows us to understand the underlying frequencies in these rhythms and the correlations between the three rhythms of suck, swallow and respiration.
CHAPTER TWO: BACKGROUND

Healthy term infants usually demonstrate efficient feeding skills at birth. However, feeding difficulties or disorders are common among preterm infants. Feeding is the most complex activity an infant must accomplish and is crucial to the independent survival of the newborn. It requires an efficient coordination of the three rhythms of feeding i.e. suckle, swallow and respiration[7, 8]. Inefficient coordination of these rhythms may lead to insufficient ventilation during the feed and improper nutrition and growth as a long term consequence [5].

It has been shown that the near-term fetus can swallow 450 ml/day[9]. In a study by Humphrey et al, the fetus was shown to swallow amniotic fluid at 12 weeks Postmenstrual age (PMA) [10]. Newborn infant feeding skills are known to be almost entirely reflexive and under the control of the brain stem[11]. Rhythm regulators situated in the brain stem control the regulation of feeding rhythms. These are nucleus solitarius, nucleus ambiguous and nucleus hypoglossus, and the nucleus trigeminalis [7, 12-14]. Feeding is an important milestone in neural development. Feeding problems in the early stages have been correlated with a number of disorders especially neurological deficits such as cerebral palsy[15]. This suggests that efficient coordination of the rhythms may indicate central nervous system maturity. Thus, the study of preterm infant feeding provides an insight into the neurological development and maturation of the infant.

There are various therapies available for treatment of feeding disorders if identified early in the infants. Speech therapists develop programs with feeding
strategies once such problems have been identified. Previous studies have included qualitative investigations, which focus on morphologies of the rhythms as well as feeding behaviors; for example, Lau et al have described and differentiated the components of suck and developed a unique ‘hyoid drum’ for non-invasive measurements of swallows. They showed that the suck was constituted by a suction component (the negative intraoral pressure) and an expression component (the positive pressure on the nipple to squeeze the nipple between the upper palate and the tongue for milk flow)[16]. They have also proposed the different stages of sucking by an infant to aid clinicians in understanding the initiation and development of oral feeding in an infant with feeding difficulty[17]. These stages were classified as a progression of the suction and expression components with an increase in amplitude of suction with time and duration of the suck bursts. Gewolb et al have approached these issues through quantitative analyses, with stress on temporal relationships and statistical correlations between the physiological events [5, 6, 18]. They have described the ontogeny of these rhythms and the progression of the suck-swallow dyad leading to achievement of an efficient coordination[5]. Coefficient of variation (COV) for pairs of rhythms (suck-suck, swallow-swallow, and suck-swallow) were calculated and used as a measure of successful coordination and stability in these rhythms. They demonstrate that the development of these rhythms is a function of PMA implying that the basis of successful feeding lies in the innate neurological maturity of the infants.
In 2002, Qureshi et al studied the changes in the suckle rhythm during the first month of life of the infants. The group also introduced another method using X-Y plots to check for stability of the rhythms[6]. Koenig, Davies and Thach in 1990 have elegantly shown the coordination of these rhythms through temporal measures of the onset of each of the rhythms. They have clearly delineated the relationships between suck-swallow, suck-breath and swallow-breath and illustrated the interplay of respiration in the coordination of the rhythms [19, 20].

Gewolb et al, in their quantitative analyses approach, define 'runs' as an aggregation or occurrence of \( \geq 3 \) events with inter-event intervals of \( \leq 2 \) seconds. Sucks and swallows in runs were analyzed and their peak-to-peak intervals were computed. Further, the COV was calculated for the suck-suck and swallow-swallow interval. The method required an investigator to manually inspect each rhythmic waveform, and then to \textit{subjectively} identify a significant deflection, and \textit{manually} input the data into a spreadsheet. A labor-intensive method such as this can require several days of data processing for just one feeding study. Thus, new methods that would automate this computation are desired.

The present project was designed to overcome these inadequacies with digital signal processing techniques. Spectral analysis provides a novel approach to these investigations. It enables us to describe the fundamental frequencies associated with the feeding rhythms. Auto-spectral estimates give us an idea of variability in the individual rhythms. Cross-spectral analysis help in describing cross correlations between these rhythms and their interactions through phase relationships. The results of these analyses are presented in two parts. First,
auto-spectral analysis of suck and swallow rhythms was performed and the correlations of the spectral measures with demographic and maturational data from the infants were investigated. Then, an extension of the procedure was adopted using cross-spectral and coherence analyses to investigate the relationships between the spectral parameters and the maturational indices.
CHAPTER THREE: METHOD AND ANALYSES

As part of an ongoing study of infant feeding, we collected data from a group of twenty-five low risk preterm infants. Low risk was defined as having no IVH, and not likely to develop BPD, and other congenital anomalies.

All the study participants were selected from the Neonatal Intensive Care Unit at the Kentucky Children’s Hospital. The study protocol complied with all HIPAA and IRB regulations. Informed parental consent was obtained for all infant participants. The infants were prepared approximately half hour before their regular time of feeding. An illustration of the set up is shown in Figures 1. Two acoustic devices, a microphone and an accelerometer were fixed to either side of the neck of the infant with double-sided tape. Outputs from these devices were routed into the data acquisition device through a mixing board (Yamaha MG10/2, Yamaha Corp, Indonesia). The acoustic devices were used for measurements of swallow sounds as a part of data collection for another project that is focused on cervical auscultation. ECG signals were acquired with a cardio-respiratory monitor (SpaceLabs Model 90623A, SpaceLabs Inc., Redmond WA). Oxygen saturation was measured with a standard Pulse Oximeter (Masimo Radical, Masimo Corporation, Irvine, CA). A 5F nasopharyngeal catheter was connected through a pressure transducer (Transpac IV Neonatal/Pediatric Pressure Monitoring Kit, Hospira Inc., Lake Forrest IL) to a nipple and was cut such that the catheter tip was flush with the tip of the nipple. This was used to measure suckle pressure. A second catheter was placed such
that the tip was in the infant’s pharynx via the nostrils and connected to a pressure transducer to measure swallow pressure. Nasal airflow was measured with a small thermistor bead (Omega 44030, Omega Engineering Inc., Stamford, CT) in a custom assembly placed at the opening of the nares. Respiratory effort was measured with a thoracic chest band (Pneumotrace II, Model 1132, UFI, Morro Bay, CA).

With the above stated eight channels of recording in place, a 1-minute session of non-nutritive suck (data from which are not used in this project) was performed followed by a session of nutritive feeding for 15 minutes. The infants were studied once a week from the onset of feeding at their normal feeding time until discharge. They were given the amount of breast milk or infant formula that was prescribed by the clinical caregivers. The entire session with all biometric and cervical auscultation data was collected and displayed as a linear graph using Windaq Data Acquisition System and Waveform Browser (DATAQ Instruments, Akron OH.). A sample recording in Windaq Acquisition System is shown in Figure 3, which displays about 75 seconds of recorded data. The first two channels are the accelerometer and microphone data respectively. ECG is displayed as the third channel. Sucks and swallows are seen as deflections in the nipple and pharyngeal recordings on the fourth and fifth channel respectively. Respiratory data measured by the nasal thermistor and the thoracic band are also noted as deflections for outward and inward movements of airflow and the chest motion shown in channels 6 and 7. Pulse Oximeter recording for the baby’s oxygen saturation is shown in channel 8. Only the suck and swallow recordings
from channel 4 and 5 were used for the following analyses. Figure 2 shows the setup during the course of a feeding study.
Figure 1: Schematic of the instrumentation setup.
Figure 2: Photograph of the instrumentation during a feeding episode.
Figure 3: Sample data recording on Windaq Acquisition Instruments.
SECTION A: AUTO SPECTRAL ANALYSIS

Fifty-one recordings from twenty-one low-risk preterm infants were used for this analysis. The demographic data for these infants is shown in Table 1.

Table 1: Demographics of subject population for Auto spectral analysis.

<table>
<thead>
<tr>
<th></th>
<th>Mean</th>
<th>Std. Dev.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Birth weight (BWT) grams</td>
<td>1030</td>
<td>342</td>
</tr>
<tr>
<td>Gestational Age (GA) weeks</td>
<td>28.85</td>
<td>2</td>
</tr>
<tr>
<td>Post-Menstrual Age (PMA) weeks</td>
<td>35.28</td>
<td>2.28</td>
</tr>
<tr>
<td>Day of Life (DOL) days</td>
<td>49</td>
<td>20</td>
</tr>
<tr>
<td>Time Since First Nipple (TSFN) days</td>
<td>18</td>
<td>11</td>
</tr>
</tbody>
</table>

These demographic measures were used as independent variables in our statistical analyses. Gestational Age (GA) is defined as the total number of weeks of pregnancy completed at the time of delivery. Postmenstrual age (PMA) is defined as the total number of weeks since the mother’s last menstrual period. Day-of-Life (DOL) refers to the number of days since the baby’s birth at the time of the study. Time since first nipple feed (TSFN) refers to the number of days since the baby started nipple feeding and is a measure of learning or practice. Birthweight (BW) was the baby’s weight at the time of delivery. Figure 3 shows
aggregation of suck and swallow with apnea in between periods of no feeding activity (denoted by a flat line). These aggregations of sucks and swallows are referred to as ‘runs’ and are defined as suck or swallow deflections occurring in groups of no less than 3 events separated by not more than 2 seconds. For our auto-spectral analysis, we intended to look at the periods of active feeding and thus these runs constitute our period of interest. The suck and swallow data channels were acquired at 500 samples per second using Windaq Pro+ Acquisition device and recorded in the Windaq Waveform Browser. The data was then exported from the Windaq Waveform Browser into MATLAB v7.5 (The Mathworks Inc. Natick, MA). A program was written to identify the runs and excise the periods of inactivity between them. The concurrent period of swallows were also extracted. These runs were then spliced together to form a continuous waveform of suckle activity. Similarly, the corresponding segments of swallows that were extracted were also spliced together. Figure 4 illustrates this process. Section A represents the suck and swallow channels from Figure 3. Section B shows the period of runs with the inactivity period excised. Section C shows the spliced suck waveform with the corresponding swallow segments.
Figure 4: Process of data splicing for Auto-spectral analysis.
Auto-spectra were estimated for these waveforms of suck and swallow in MATLAB using built in functions. The calibrated data from Windaq was imported into MATLAB. The data were divided into 10-second segments and the mean of each segment was subtracted from the segment (i.e. zero meaning). The MATLAB function computed the spectra using the Welch’s method of estimation with no overlap. A Hanning window of 10-second duration (5000 points) was used for windowing the data prior to spectral estimation. The length of the FFT used was 8192 (power of 2 greater than 5000), thus providing a frequency resolution of 0.061Hz. The following spectral parameters, frequency ($pk\ freq$), amplitude, bandwidth containing 50% power ($bw-su$), and 50% power ($50p-su$) were computed. An illustration of these computations is shown in Figure 5. These parameters were correlated with the demographic factors such as Gestational Age (GA) Postmenstrual Age (PMA), Birth Weight (BWT), Days of Life (DOL), and Time Since First Nipple feeding (TSFN).
Figure 5: Auto spectrum of suck data obtained during a trial from an infant with measured parameters.
PROC MIXED in Version 9.1 of SAS (SAS Institute Cary, NC) was used to fit linear mixed models with random effects capturing the correlations among repeated measurements on the same babies. Natural logarithm transformed data were used in order to mitigate the effects of outlying spectral values before fitting the statistical model. Stepwise-backward elimination was performed on the dependent spectral variable against every individual independent demographic variable. Stepwise-backward elimination was performed by fitting a model of the dependent variable against all the independent variables. The t-statistic or the F-statistic was computed and the corresponding p-value for each of the independent variables was calculated. Since the significance threshold of $\alpha=0.05$ was used, the p-values of each independent variable was compared for significance. The variable with the highest non-significant p-value was eliminated and the process was repeated. This backward elimination was carried out until all remaining independent variables, if any, had p-values less than 0.05. These independent variables, if any, were deemed significant predictors of the dependent variable. The first set of results was obtained for the cohort as a whole. The next set of results was obtained based on stratifications by gestational age in order to identify changes in spectral parameters.
SECTION B: CROSS-SPECTRAL AND COHERENCE ANALYSES

These analyses are an extension of the analysis discussed in Section A. In this analysis, cross-spectra and coherence were computed on the complete nutritive recording of the infants using a program in MATLAB. The calibrated data from Windaq was imported into MATLAB. The data were divided into 10-second segments and the mean of each segment was subtracted from the segment (i.e. zero meaning). The MATLAB function estimated the cross spectra of these data using the Welch’s method of estimation with no overlap. A Hanning window of 10-second duration (5000 points) was used for windowing the data prior to spectral estimation. The frequency resolution was calculated as mentioned earlier to be 0.061Hz (i.e. sampling frequency / N_{FFT}). This analysis was performed on the low risk infants for suck-swallow (SS), swallow-respiration (SR) and suck-respiration (SkR) interactions. Data were collected from 23 infants over 69 recordings. The demographics for this group of infants are shown in Table 2 below.
Table 2: Demographics of subject population for Cross-spectral and Coherence analysis.

<table>
<thead>
<tr>
<th></th>
<th>Mean</th>
<th>Std Dev</th>
</tr>
</thead>
<tbody>
<tr>
<td>Birthweight (BW) gms</td>
<td>1060</td>
<td>329</td>
</tr>
<tr>
<td>Gestational Age (GA) weeks</td>
<td>28.85</td>
<td>2</td>
</tr>
<tr>
<td>Post-Menstrual Age (PMA) weeks</td>
<td>35.14</td>
<td>2.28</td>
</tr>
<tr>
<td>Days of Life (DOL) days</td>
<td>47</td>
<td>20</td>
</tr>
<tr>
<td>Time Since First Nipple (TSFN) days</td>
<td>17</td>
<td>12</td>
</tr>
</tbody>
</table>

Calculations for spectral parameters such as peak freq \((pk -freq)\), bandwidth containing 50% power \((bw)\), 50% power \((50p)\), peak phase \((pk-pha)\), normalized phase \((norm-pha)\) and peak coherence \((pk-coh)\) and mean coherence in the bandwidth \((avg-coh)\) were noted for the all the three paired interactions. The first three parameters were measured similarly from the magnitude spectrum as mentioned in previous section. From the phase spectrum, we measure the phase value corresponding to the peak of the magnitude spectrum and call it peak phase. Phase measurements in the bandwidth were also studied. Since phase values maybe positive or negative, averaging phase in the bandwidth can lead to errors in computation. We use
normalized phase as a measure of phase variation in the bandwidth instead. Normalized phase (norm phase) is defined as the percentage change of phase in the bandwidth. It is computed as

\[ \text{Norm phase} = \frac{\text{maximum phase in bw} - \text{minimum phase in bw}}{\text{Peak phase}} \times 100\% \]

High values of normalized phase indicate a high variation in phase within the bandwidth. Coherence provides a non-dimensional measure of correlation between the two time series as a function of frequency. Figure 6 below illustrates the measurements of the spectral parameters discussed above.
Figure 6: Cross-spectrum and coherence between suck and swallow rhythms obtained from an infant with measured parameters.
Coherence is given by the following formula

\[ \chi^2 = \frac{|P_{xy}(f)|^2}{P_x(f) \cdot P_y(f)} \]

Where \( P_{xy}(f) \) is the cross-spectrum of the two time series

\( P_x(f) \) is the auto-spectrum of the first time series

\( P_y(f) \) is the auto-spectrum of the second time series [21]

A large value of coherence indicates correlation between the two time series at the corresponding frequency \((f)\). A high value of coherence may occur in two cases. First, if \( P_{xy}(f) \) is truly large, coherence will be high. Next, if \( P_{xy}(f) \) is not a large value and the individual auto spectra \( P_x(f) \) and \( P_y(f) \) are very low, the reciprocal would cause a high coherence. To evaluate true coherence, we use 95% confidence intervals on zero coherency. This test is based on the assumption that both time series are uncorrelated and hence their theoretical coherence is zero[21]. Upon calculating the degrees of freedom for the time series, theoretical coherence maybe computed using the Fisher or F-distribution. If the coherence estimated between the two time series at the peak is greater than this theoretical coherence, then it signifies true coherence and therefore true cross correlation [21]. The equation for the above test is given by

\[ \frac{(\nu - 2) \chi_{est}^2(f)}{2 (1 - \chi_{est}^2(f))} \geq F_{2, \nu-2, \alpha} \]

Where \( \nu \) is the degrees of freedom for the Hanning window, \( \chi_{est}^2(f) \) is the squared coherency computed at frequency \( f \), \( F_{2, \nu-2, \alpha} \) is the F- value for the given \( \nu \) and \( \alpha=0.05 \)[21].
Twenty-two infants over fifty-one recordings (about 70% of the total recordings) passed the test for zero coherency and showed true coherence. With swallow-breath and suck-breath interactions, about fifteen percent of the total recordings showed true coherencies. As such, these were not considered large enough for further statistical analysis.

Similar statistical analysis as mentioned earlier using linear mixed modeling with random effects (PROC MIXED) was performed on this data as well. In this case, the spectral parameters were not logarithmically transformed. Logarithms of negative phase values yield complex values. The results of the mixed modeling are established for real-valued dependent variables. As such, such a transformation will not be able to provide a method for interpretation. Stepwise-backward elimination as mentioned in the earlier section was performed similarly on the dependent variables against every individual independent spectral variable. The results of the statistical analysis were obtained at a significance threshold of $\alpha=0.05$. The first set of results was obtained for the cohort as a whole. We then employed stratification by gestational age to help identify corresponding changes in spectral parameters.
SECTION A: AUTO-SPECTRAL ANALYSIS

Initially we performed statistical analysis for the cohort as a whole. The first statistical model was fit for the dependent variable natural logarithm of bandwidth-suck (ln (bw-su)) and the independent variables GA, PMA, BW, DOL and TSFN. After stepwise backward elimination, GA and DOL were the only significant predictors of bw-su (GA: slope = 0.1138 p = 0.0072; DOL: slope = 0.008, p = 0.0367). The second model was fit for ln (50p-su) with the same independent variables. DOL was a significant predictor (slope = 0.007 p-value = 0.0028).

The process was repeated for the spectral parameters for swallow. Models were fit between the ln (bw-sw), ln (50-p), ln (pk freq-sw) and the same independent variables. DOL was negatively associated with the ln (bw-sw) (slope = -0.007, p = 0.0461) and TSFN were significant in predicting the ln (bw-sw) (slope = 0.017, p = 0.01).

Based on the premise that older infants at higher GA are expected to be more neurologically developed, we performed the similar statistics with GA stratification. Table 3 shows the statistical results obtained. We found that for this cohort, the median GA was 28 weeks and we used this arbitrarily as the point of stratification for this analysis. Most of the results showed significant changes in Bandwidth, 50% power, and peak frequency for babies born before 28 weeks. For babies born after 28 weeks there was only one significant result.
Table 3: Results of Auto-spectral analysis for GA before 28 weeks.

<table>
<thead>
<tr>
<th>In(x)</th>
<th>BW</th>
<th>PMA</th>
<th>DOL</th>
<th>TSFN</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>bw-su</strong></td>
<td>NS</td>
<td>m = 0.2126</td>
<td>p = 0.0163</td>
<td>NS</td>
</tr>
<tr>
<td>50p-su</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>pk freq-su</td>
<td>NS</td>
<td>m = -0.351</td>
<td>p = 0.0121</td>
<td>m = 0.075</td>
</tr>
<tr>
<td><strong>bw-sw</strong></td>
<td>m = -0.001</td>
<td>p = 0.0343</td>
<td>m = 0.181</td>
<td>p = 0.04</td>
</tr>
<tr>
<td>50p-sw</td>
<td>NS</td>
<td>NS</td>
<td>m = -0.035</td>
<td>p = 0.0092</td>
</tr>
<tr>
<td>pk freq-sw</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

m: slope, p: p-value

*The only significant association for babies born after 28 weeks is for 50p–su with PMA (slope = 0.07399, p = 0.0044)*

With GA stratification in the statistical model, we fit a model between the dependent variable (bw-su) and the same independent variables. After stepwise backward elimination for GA less than 28 weeks, PMA was found to be a significant predictor of the bw-su and was associated positively (slope = 0.212, p = 0.0163). We fit a second model between 50p-su and all the independent variable. For infants with GA greater than 28 weeks, PMA showed a positive association with 50p-su (slope=0.073 p = 0.0044). The third model was fit between the pk freq-su and the independent variables. For GA less than 28
weeks, there were three significant predictors PMA, DOL and TSFN. PMA and DOL showed negative associations i.e. decrease in the dependent variable with increase in independent variable (PMA: slope = -0.351, p = 0.0121; DOL: slope = -0.017 p=0.0243). TSFN showed a positive association i.e. increase in the dependent variable with increase in the independent variable (slope=0.075, p= 0.0002).

The model fit for the dependent variable bw-sw showed significant associations with the predictors PMA, DOL, BW. There was a positive association between PMA and bw-sw (slope = 0.181, p = 0.04) for GA less than 28 weeks. DOL showed negative association with bw-sw (slope= -0.02125 p =0.02). BW showed negative association with bw-sw as well (slope= -0.00101 p = 0.0343). The last model was fit for the dependent variable 50p-sw and the independent variables for GA less than 28 weeks. DOL was the only significant predictor and had a negative association with 50p-sw. (slope= -0.03528 p = 0.0092).

Natural logarithm transformations of the dependent variables cause the slopes of the statistical computations to be very small. To reveal their true significance, we converted them back to their original scale. For the first statistical test using the whole group, if we increase GA by 2 weeks while holding DOL fixed, the typical increase in the bw-su is an estimated 25.55%. If we increase DOL by 10 days while holding GA fixed, the typical increase in the bw-su is an estimated 8.99%. If we increase DOL by 10 days, the typical increase in the 50p-su is an estimated 7.55%. If we increase DOL by 10 days while holding
TSFN fixed, the typical decrease in bw-sw is an estimated 7.31%. If we increase TSFN by 10 days while holding DOL fixed, the typical increase in the bw-sw is an estimated 19.17%.

For Gestational Age less than 28 weeks, a 1-week increase in PMA showed a corresponding 23.68% change in bw-su. There was no change in bw-su with respect to any maturational index for GA greater than 28 weeks. However, for infants in this group, the 50p-su showed significant a change with increase in PMA. A 1-week increase in PMA showed a corresponding 7.67% increase in 50p-su. For GA less than 28 weeks, PMA, DOL and TSFN correlate with the peak frequency of suck. A 1-week increase in PMA, keeping the other two parameters constant, shows a corresponding decrease of 29.6% in the frequency of suck. An increase in 10 days of DOL, keeping the PMA and TSFN constant shows a corresponding decrease of 16.33%. An increase in 10 days of TSFN for the same PMA and DOL shows an increase in the peak frequency by a factor of 113.5%.

The results also showed significance for parameters measured from the swallow rhythm. For Gestational age less than 28 weeks, bandwidth of swallow showed significant changes with respect to changes in PMA, DOL, and BWT. A 1-week increase in PMA, keeping the other two parameters of DOL and BWT the same, showed a corresponding increase of 19.84% in the bandwidth of swallow. With BWT and PMA constant, an increase in 10 days of DOL showed a corresponding decrease of 19.14% in the bandwidth of swallow. Keeping PMA and DOL constant, an increase of 50 grams in BWT gives a corresponding
decrease of 4.92% in the bandwidth. For Gestational age less than 28 weeks, 50% power of swallow shows a dependence on the DOL. A 10-day increase in DOL shows a corresponding negative change of 29.72% in the 50% power of swallow. All other variables of the spectral analysis showed no relationships with the maturational indexes with the application of the GA stratification.
SECTION B: CROSS-SPECTRAL ANALYSIS

Similar statistical analysis was performed for the cross-spectral analysis. We used PROC MIXED with random effects in SAS. Logarithmic transformation was not used in this analysis since some values of phase are negative and hence the transformations would introduce difficulty in both computation and interpretation. Thus, all parameters were used as absolute values. Table 4 below shows the results obtained upon considering the cohort as a whole.

Table 4: Results of Cross-spectral & Coherence analysis for the whole cohort.

<table>
<thead>
<tr>
<th></th>
<th>BW</th>
<th>GA</th>
<th>PMA</th>
<th>DOL</th>
<th>TSFN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bw-SS</td>
<td>NS</td>
<td>NS</td>
<td>m=0.03133, p=0.0099</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Pk-pha-SS</td>
<td>m=0.1853, p=0.0377</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

m: slope, p: p-value

The first model was fit between the dependent variable bandwidth (bw-SS) with all the independent variables. The bw-SS was found to be dependent significantly with PMA (slope=0.031, p=0.0099). Another model was fit between peak phase (pk-phase-SS) and the independent variables. A positive association
was found between pk-phase-SS and birthweight (BW) (slope=0.185, p=0.0377).

The process was then repeated with stratification using the median GA (28 weeks) of the group. We found significant results for both GA greater than and GA less than 28 weeks. For GA later than 28 weeks, bw-SS had a significant negative association with GA (slope=-0.079, p=0.0454). In addition, 50-p-SS was found to have a small but significant association with TSFN (slope=0.006, p=0.019). The results are shown in Table 5.

Table 5: Results of Cross-spectral & Coherence analysis for GA after 28 weeks.

<table>
<thead>
<tr>
<th></th>
<th>BW</th>
<th>GA</th>
<th>PMA</th>
<th>DOL</th>
<th>TSFN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bw-SS</td>
<td>NS</td>
<td>m=-0.079, p=0.0454</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>50p-SS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>m=0.006, p=0.019</td>
</tr>
</tbody>
</table>

m: slope, p: p-value

For GA earlier than 28 weeks, 50p-SS was found to have two significant predictors in GA (slope=-0.518, p=<0.0001) and DOL (slope=-0.008, p=0.0036). Pk-freq-SS showed a significant dependence on BW (slope=0.0007, p=0.0004). Normalized phase (norm phase-SS) was found to have a significant positive association with GA (slope=100.11, p=0.0002). The peak coherence was also found to have a negative association with both GA (slope=-0.387, p=0.0005)
and PMA (slope=-0.063, p= 0.016) and the average coherence showed negative association with both GA (slope=-0.487, p=<0.0001) and DOL (slope=-0.008, p=0.0076). The results are shown in Table 6.

Table 6: Results of Cross-spectral & Coherence analysis for GA before 28 weeks

<table>
<thead>
<tr>
<th></th>
<th>BW</th>
<th>GA</th>
<th>PMA</th>
<th>DOL</th>
<th>TSFN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bw-SS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>50p –SS</td>
<td>NS</td>
<td>m=-0.518, p=&lt;0.0001</td>
<td>NS</td>
<td>m=-0.008, p=0.0036</td>
<td>NS</td>
</tr>
<tr>
<td>Pk-freq-SS</td>
<td>m=0.0007, p=0.0004</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Pk-pha-SS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Norm-pha-SS</td>
<td>NS</td>
<td>m=100.11, p=0.0002</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Pk-coh-SS</td>
<td>NS</td>
<td>m=-0.387, p=0.0005</td>
<td>m=-0.063, p=0.016</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Avg-coh-SS</td>
<td>NS</td>
<td>m=-0.487, p=&lt;0.0001</td>
<td>NS</td>
<td>m=-0.008, p=0.0076</td>
<td>NS</td>
</tr>
</tbody>
</table>

m: slope, p: p-value
Without a logarithmic transformation of the dependent variable, the interpretations of regression coefficient estimates are cast in terms of absolute changes. Thus for the results with the whole cohort, we have a 2-week increase in PMA causing an increase of 0.0626 Hz in the bw-SS and a 10-g increase in BW correlates with an increase of 1.85 degrees in the peak phase-SS.

For GA less than 28 weeks, there is a decrease of $0.51V^2Hz^{-1}$ in 50p-SS for a 1-week increase in GA keeping DOL fixed. With GA fixed, the 50p-SS also decreases by $0.081V^2Hz^{-1}$ for a 10-day increase in DOL. Peak-freq-SS showed an increase of 0.078Hz for a 100g increase in BW. Normalized phase showed an increase of by a factor of 100% for a 1-week increase in GA. The peak coherence showed a decrease of 0.387 for increase in 1 week of GA keeping PMA fixed and a decrease of 0.063 for a 1-week increase in PMA keeping GA fixed. The average coherence also showed a decrease with both GA and DOL. Keeping DOL fixed, for a 1-week increase in GA, the average coherence decreased by 0.48 and keeping GA fixed, the average coherence decreased by a very small factor of 0.08 for a 10-day increase in DOL.
CHAPTER FIVE: DISCUSSION

Achievement of effective feeding requires the coordination of the rhythms of suckle, swallow and respiration. This is considered the most complex skill in the life of a newborn infant. To achieve independent survival, an infant must be proficient in coordinating these rhythms. Feeding difficulties are prevalent amongst preterm infants [3, 4, 22, 23]. Since the regulators of the feeding rhythms are located in the brainstem, the study of the development of feeding may provide insight into function of the brainstem. Consequently, poor feeding is often thought to be one of the first signs of neurological damage[24]. There is immense pressure on health care providers to limit costs and discharge patients earlier [25]. Clinicians are trying to understand infant feeding patterns so that they can design better interventions to improve outcomes, decrease length-of-stay in the hospital for preterm infants and possibly predict long-term developmental outcomes.

Gewolb et al, have investigated the ontogeny of feeding rhythms with linear graphic representations through a quantitative approach. They have also studied how various disease states of preterm infants affect their development in cross-sectional studies [5, 6, 18, 26-28]. The approach for their studies involved labor-intensive techniques. The analyses used by them detected peaks in the suck and swallow data. The method required scrolling through the entire data channel of interest for peaks of the events (i.e. sucks and swallows). With this study designed to record 15 minutes of a single feeding episode, and two
channels of interest, this process of manual peak detection would be very cumbersome. In addition to this difficulty, 25 infants were enrolled in this Low risk category with 175 infants to be enrolled in the entire project. Thus with multiple studies, on multiple subjects, the previous method would be inefficient. Our aim in this project was to introduce multivariate spectral analysis as an effective tool to understand these rhythms and their interactions. Spectral analysis is a technique that is used to study variability in the waveforms. The auto-spectral measures used in the analysis were peak frequency ($pk$-$freq$), area under the curve containing 50% power ($50p$) and bandwidth containing the 50% power ($bw$). The x-coordinate (frequency axis) corresponding to the peak depicts the most dominant frequency contained in the signal and is defined as the peak frequency ($pk$-$freq$). It is a measure of the rate of occurrence of the events (i.e. sucks and swallows). The computation of area under the curve containing 50% power is an extension of the concept of a spectral edge frequency. Spectral edge frequency is a measure used in signal processing to indicate the amount of power (usually in %) contained in the spectrum below a certain frequency ‘$\lambda$' [29]. In our case, we modified it to define the middle 50% of the spectrum obtained from the power spectral density of the suck or swallow signal. The frequency band or bandwidth ($bw$) containing this 50% power was used as a measure of variability. The choice of 50% was arbitrary. An increased bandwidth shows increased variability and vice versa.

Taking the cohort as whole with the auto-spectral analysis, GA and DOL were positively associated with $bw$-$su$, indicating that older babies had more
variability in their suckle rhythm. On stratifying the data by GA less than or equal to 28 weeks, only PMA was positively associated with bw-su. This seems intuitive, as GA+ (DOL/7) = PMA. However, the positive association between bw-su and PMA indicates that there is increased variability in the suck rhythm with increasing age of the infant. This is in contrast to the previous works that have shown decreasing variability in the suckle feeding rhythm with advancing age.

The results for bw-sw bring in further complexity to our interpretations. In this case, TSFN and DOL have opposing effects on the bw-sw. DOL is an index of the maturation and increase in DOL shows lesser variability. On the other hand, TSFN, which is used as an index of the learning, shows an increase in variability. GA stratification showed that bw-sw could be predicted by changes in PMA, DOL and BWT. There is increasing rhythm variability with increase in PMA but a decrease in variability with increases in DOL and BWT. Infants born at a higher birthweight have lesser variability or increased stability in their swallow rhythm.

For GA less than 28 weeks, we had significant changes in peak frequency measures of suck. Three factors, PMA, DOL and TSFN play a combined role in predicting the behavior of peak frequency. Increases in PMA and DOL show decreases in peak frequency. The suck frequency increases with increasing TSFN. This is a significant observation because contrasting changes with these three predictors shows that the maturational indices of PMA and DOL control the suck rate by decreasing it whereas, the learning index of TSFN apparently increases it.
The next step was to investigate the cross-spectra and coherence between the rhythms for a more comprehensive idea of the phase relationships between the rhythms. The results of the cross-spectral and coherence analysis when the cohort was considered as a whole also showed that the bandwidth of the suck-swallow relationship \((bw-SS)\) varied positively with PMA, indicating that there is increased variation with increase in age. An interesting point to note is that when the cohort was stratified with GA, for GA greater than 28 weeks, \(bw-SS\) showed decreased variability with increase in GA. This clearly shows that the suck-swallow interaction achieves stability with increasing age.

Peak-phase \((pk-pha-SS)\) showed positive correlation with birthweight \((BW)\) for the whole group of infants. For a 10-gram increase in BW, we find that the phase-shift between the two rhythms of suck and swallow increases by 1.85 degrees. In other words, the lag between the suck and swallow rhythms increases with birthweight.

For GA earlier than 28 weeks, 50p-SS showed a decrease with increasing GA and DOL showing that the suction amplitude maybe significantly lower at early GA. The pk-freq-SS also showed a small but significant increase with corresponding increases in BW. The normalized phase showed a very high increase with increased GA earlier than 28 weeks suggesting a large variation of phase in the bandwidth. There was no significant relationship between them for GA later than 28 weeks.

The peak coherence of suck swallow \((pk-coh-SS)\) was shown to decrease with GA and PMA for GA earlier than 28 weeks. The average coherence also
decreased with GA and DOL. This may be due to the fact that for an earlier GA, the rhythms of suck and swallow are less rhythmic and is consistent with previous findings[5].

We have presented a novel method of analyses for the study of infant feeding. In our effort to improve the data analysis process, we investigated the differences between the maturational components of infant feeding and contributions to the development of feeding behaviors from learning. PMA and GA are measures of maturation. BW is usually proportional to GA. TSFN and DOL are both measures of the baby’s opportunities to practice or learn to feed. The results of the auto-spectral analysis shows a few differences when compared to the cross-spectral analysis. The most significant difference was in the bandwidth measurements. The difference could be because the auto-spectra were analyzed for runs and the cross-spectra were not. The bandwidth results of the auto-spectral analysis are in contrast with those in literature, whereas the cross-spectral result for bandwidth showed consistency with those in literature. The results of this study show that the maturational and the learning theories jointly contribute to the development of infant feeding. Neither of the theories may be solely responsible for the development of these skills. Our method uses multivariate spectral analysis as a quantitative approach to measure the characteristics of the rhythms. The method describes the progression of feeding rhythms through correlations with clinical indexes, thus providing clinicians with an understanding of the development of infant feeding, to help decrease length-of-stay and possibly predict long-term developmental outcomes. Many studies of
infant feeding have used maturational indexes such as GA, PMA, and BW as comparable indices. Using indexes such as DOL and TSFN, gives us a slightly wider perspective with regard to the indexes of learning. Most of our results show that more than one index is a predictor of the measured spectral parameter.

We have thus provided a basis for future studies to consider both aspects of maturation and learning. Since we have investigated only Low-risk infants in this study, we may use the results as a standard to compare with those from infants with other pathological conditions.
APPENDIX

Figure A1: Example of Auto Spectrum of suckle data obtained during a trial in a infant (GA>28 weeks).

Figure A2: Example of Auto spectrum of swallow data obtained during a trial in an infant (GA> 28 weeks).
Figure A3: Comparison of Auto spectra of Suck between one infant with GA > 28 weeks and one infant with GA < 28 weeks.

Figure A4: Comparison of Auto spectra of Swallow between one infant with GA > 28 weeks and one infant with GA < 28 weeks.
Figure A5: Example of Cross Spectrum of Suck-swallow relationship from data obtained during a trial in an infant (GA>28 weeks).
Figure A6: Example of Cross Spectrum of Swallow-Respiration relationship from data obtained during a trial in an infant (GA>28 weeks).
Figure A7: Example of Cross Spectrum of Suck-Respiration relationship from data obtained during a trial in an infant (GA>28 weeks).
Figure A8: Comparison of Cross spectra of Suck-Swallow relationship between one infant with GA > 28 weeks and one infant with GA < 28 weeks.
**Auto spectral results: Whole cohort**

![Graph](image)

**Bw-Su vs GA (DOL fixed)**

Figure A9: Bandwidth of suck varying with GA (DOL fixed).

All figures are shown with the regression line (in pink) drawn with the respective slope values as mentioned in the text.

![Graph](image)

**Bw-Su vs DOL (GA fixed)**

Figure A10: Bandwidth of suck varying with DOL (GA fixed).
Figure A11: 50% power of suck varying with DOL.

Figure A12: Bandwidth of swallow varying with DOL (TSFN fixed).
Figure A13: Bandwidth of swallow varying with TSFN (DOL fixed).
Stratification using GA: GA earlier than 28 weeks

Figure A14: Bandwidth of suck varying with PMA.

Figure A15: Peak frequency of suck varying with PMA (DOL, TSFN fixed).
Figure A16: Peak frequency of suck varying with DOL (PMA, TSFN fixed).

Figure A17: Peak frequency of suck varying with TSFN (PMA, DOL fixed).
Figure A18: Bandwidth of swallow varying with BW (DOL, PMA fixed).

Figure A19: Bandwidth of swallow varying with DOL (BW, PMA fixed).
Figure A20: Bandwidth of swallow varying with DOL (BW, PMA fixed).

Figure A21: 50% power of swallow varying with DOL.
Stratification with GA: GA later than 28 weeks

Figure A22: 50% power of suck varying with PMA.
**Cross spectral results: Whole cohort**

**Figure A23:** Bandwidth of suck-swallow varying with PMA.

**Figure A24:** Peak phase of suck-swallow varying with BW.
Stratification using GA: GA later than 28 weeks

Figure A25: Bandwidth of suck-swallow varying with GA.

Figure A26: 50% power of suck-swallow varying with TSFN.
Stratification with GA: GA earlier than 28 weeks

Figure A27: 50% power of suck-swallow varying with GA (DOL fixed).

Figure A28: 50% power of suck-swallow varying with DOL (GA fixed).
Figure A29: Peak frequency of suck-swallow varying with BW.

Figure A30: Normalized phase of suck-swallow varying with GA.
Figure A31: Peak Coherence of suck-swallow varying with GA (PMA fixed).

Figure A32: Peak Coherence of suck-swallow varying with PMA (GA fixed).
Figure A33: Average coherence of suck-swallow varying with GA (DOL fixed).

Figure A34: Average Coherence of suck-swallow varying with DOL (GA fixed).
REFERENCES:


VITA

Pooja Vijaygopal was born in Bombay (now Mumbai), India on 2nd October 1983. She received the degree for Bachelor of Engineering (Medical Electronics) in Bangalore from M.S. Ramaiah Institute of Technology, affiliated to Visvesvaraya Technological University in July 2006. She joined the Biomedical Engineering program at the University of Kentucky in August 2006.