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Opioid Effects on Premature Infant Brain Function Measured by Limited Channel EEG

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Abstract

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Background and Aim: Neonatal opioid use for pain management and therapeutic sedation may contribute to untoward patterns of brain function, or change rhythmic cycles that form the basis for sleep structure. This study aimed to: 1) describe differences in pattern characteristics of limited channel EEG, and 2) evaluate changes in brain wave signal variability with quantification of cyclicity by spectral density distribution before and after standard morphine administration.

Method: This within-subject naturalistic experiment acquired EEG data by limited channel amplitude-integrated (aEEG) in the neonatal intensive care unit (NICU). Participants were recruited in two phases: 1) medically stable (n=16, 28-32 weeks postmenstrual age (PMA)) and 2) critically ill receiving morphine for pain or sedation (n=9, 26-39 weeks PMA). The first phase established feasibility, the second employed aEEG to describe brain function before and after opioid administration. Amplitude-integrated EEG signal was quantified by measures of bandwidth voltage, lower border voltage, and variance of each with two simple quantitative

measures of peak counts 10 μ V above baseline, and proportion of time below 10 μ V. Further analysis presented frequency changes across time to describe morphine effect on EEG spectral measures in younger infants who received morphine in the NICU.

Results: Less mature infants had higher peak counts and more time at low voltage within epoch: median peak counts 3.625 (SD1.740), median proportion less than 10 μ V 22% (SD 0.200).

Except for spectral edge frequency (SEF50), morphine effect did not emerge until focused analysis included mini-bands (2Hz) of spectral power. Moreover, the log transformed “mini-band” of 14-16 Hz showed a significant difference in mean rank ($n=9$, $\chi^2 = 5.444$, $df=1$, $p<.020$) between 15 minutes prior and 60 minutes after the dose. Younger infants demonstrated high power in lower frequency (delta) bands. Among older infants, shifts within the spectrum after morphine administration were observed from high frequency bands toward lower frequencies.

Conclusion: Description of brain function patterns is quantifiable. Although morphine effects may be masked by the heavy presence of power in the delta range early in gestation, exploration of spectral densities offers a framework for understanding shifts of spectral frequencies in premature infant brain function.

Table of Contents

LIST OF FIGURES	iii
LIST OF TABLES	iv
Chapter 1: Overview of Dissertation Structure and Content	1
Organization of the Dissertation Content	1
Neonatal Brain Function Patterns	2
Chapter 2: Quantification of Neonatal Limited Channel EEG Patterns	5
Methods	12
Participants.	12
Procedure.	12
Instrument.	12
Analysis.	13
Results	15
Measures of continuity and discontinuity.	15
Measures of cycling.	16
Effect of maturation.	17
Discussion	18
Chapter 3: Spectral Analysis of Neonatal Brain Function: Effect of Opioids	28
Methods	31
Participants.	31
Study design and measures.	32
Method of analysis.	35
Statistics.	37
Results	38
Discussion	40
Chapter 4: Under the Spectrum: Searching for Cyclic Effects of Opioid on Premature Infant Brain Function	50
Brain function and development of sleep.	50
Opioids and brain function.	54
Problem.	56
Purpose.	56
Methods	56
Design.	56
Setting and sample.	57
Instruments and measures.	58
Procedures.	58
Data analysis.	59
Statistics.	63

Results.....	63
Sample characteristics.	63
Data reduction due to artifact (impedance).	64
Morphine characteristics.....	64
Individual participants.	65
Effect of age.....	66
Discussion.....	67
Chapter 5: Summary and Conclusions.....	76
References.....	80

LIST OF FIGURES

Figure Number	Page
2.1a. Example of a Discontinuous Pattern	23
2.1b. Example of a Continuous Pattern	23
2.2. Effect of postmenstrual age (PMA) on Cerebral Function Monitoring (CFM) Signal Characteristics	25
2.3. Log Bandwidth (individual median) by Postmenstrual Age (PMA).....	26
3.1. Example of Neonatal EEG Signal	47
3.2. Example of Participant Box Plot for Visual Analysis (SEF 90).....	48
3.3. Effect of Maturation on SEF 50 (Hz).....	49
4.1. Spectral Power Shifts by Morphine Time for a Premature Infant (PMA 26.67 weeks).....	72
4.2. Spectral Power Shifts by Morphine Time for a Term Infant (PMA 39.71 weeks).....	73

LIST OF TABLES

Table Number		Page
2.1	Conceptual Framework for Limited Channel EEG Interpretation.....	24
2.2	Descriptive Summaries of Continuity with Comparison by low PMA (<30 weeks).....	27
3.1	Demographic Characteristics of Participating Infants	46
4.1	Differences in Power Spectral Mini-Bands Across Morphine Time Points	71
4.2	Effect of Morphine and Age on Variability of EEG Spectral Density.....	74
4.3	Effect of Morphine at Two Time Points and Age on Variability of EEG Spectral Density.....	75

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DEDICATION

To Philip Thorngate and Bob Burr

Kindred spirits who led me to know that to find the forest one must examine the trees.

Chapter 1: Overview of Dissertation Structure and Content

This dissertation presents the methods and data from a two-phase naturalistic study of brain function in premature infants conducted in the Neonatal Intensive Care Unit (NICU). The description and exploration of opioid effects on brain function were central to this hypothesis generating work. These data are presented as three separate papers outlining varied aspects of the study. Each paper is written individually for publication with respective tables and figures inclusive for each; references are consolidated for the entire document.

Organization of the Dissertation Content

The first individual paper makes up Chapter 2 and serves to introduce the conceptual model and operationalize brain function measurement concepts for translation to future research uses. The entire study sample (medically stable premature infants and critically ill mechanically ventilated infants receiving morphine) from both phases are combined to explore quantification of cerebral function monitoring (CFM) signal with respect to development and maturation of brain function. The genesis for the analysis presented as the first paper came from earlier pilot work to establish feasibility of amplitude-integrated electroencephalogram (aEEG) in the NICU. Within the research team, we were unable to establish reliability of interpretation of aEEG signal data when applying a previously published scoring tool (Burdjalov, Baumgart, & Spitzer, 2003). The tool lacked precision and operational definitions for key characteristics of premature infant brain function. The preliminary data set (phase 1) was combined with morphine study participants (phase 2) to develop and test simple analytic approaches for quantifying brain function before exploration of the opioid effects.

The next two papers address the effects of morphine on brain function. Chapter 3 consists of a paper outlining the data preparation and signal processing steps required to conduct spectral analysis to evaluate the effect of morphine administration on spectral power, as summarized by spectral edge frequencies and slope of the full spectrum. Nine participants are included from phase 2 of the study. The final paper is presented in Chapter 4 focusing again on the nine infants recruited for the morphine effect phase of the study and taking the analysis deeper into spectral inquiry through the comparison of changes in spectral bands across time to search for morphine effects. The dissertation concludes with an overall summary of the findings, measurement and analytic discovery, and possible avenues for future work.

Neonatal Brain Function Patterns

Brain function develops in a complex non-linear fashion over time as the infant proceeds through gestation and begins to experience the world outside the womb. Characteristics of electrical signals as measured by electroencephalogram (EEG) have long been described in qualitative terms by neurophysiologists, clinicians and researchers. The literature has included premature infant brain function for many decades (Parmelee, Akiyama, Stern, & Harris, 1969). The conceptual foundation for this work is a model of brain function patterning including the common features of discontinuity, continuity and cyclic patterns between the two.

Early in gestation, the dominant pattern of brain function is discontinuity, typified by wide changes in signal amplitude and intermittent high voltage bursts interspersed with quiescent intervals of very low amplitude, appearing as an interrupted signal. Discontinuity is strongly evident until at least 30 weeks gestation when the signal becomes more synchronized, and recedes in prominence, as continuity emerges to become the primary pattern (Vanhatalo & Kaila, 2006). Continuity of brain wave signal is the result of steady oscillation of cortical neuronal

firing with increasing intensity as the infant brain matures and the interconnected neuronal network is built (Amzica & Steriade, 2000). Continuous EEG signal presents as a more consistent lower amplitude (as compared to the erratic high amplitude bursts present in discontinuity) and appears denser on aEEG trended graphs relative to high frequency waves, resulting in a narrow bandwidth or amplitude excursion. Continuity emerges over time with initial presence often detectable by 29 weeks gestation. By 40 weeks or term gestation, continuity patterns develop into an alternating cyclic process of high frequency low amplitude signal with approximately 20 minute periods reflecting more discontinuity.

The primary neonatal EEG patterns of discontinuity and continuity are each essential in their own functional development. However, the interactive process of cycling between the two is even more critical because it forms the basis of sleep and wake states upon which the infant will develop normal sleep architecture. The promotion of early sleep patterns and optimal brain function patterns that will become sleep states in early life is of great import to neonatal care. Sleep states are associated with critical processes of brain growth and neural plasticity, including synaptic formations and remodeling that will become cognitive pathways for the life of the infant (Mirmiran & Ariagno, 2003; Peirano, Algarin, & Uauy, 2003). Neonatal nurses have the challenging responsibility to deliver life saving care while trying to avoid interruption of sleep thus promoting optimal neurodevelopment in the setting of intensive care.

This small sample descriptive study made use of a non-invasive clinical device, limited channel EEG to acquire long recordings of raw EEG signal. Exploration included analytic techniques for both quantification of discontinuity, continuity and cycling; and determining the effect of opioids given for pain management and sedation in the NICU. Although the number of participants was small, the very large data set presents a unique opportunity to investigate the

depth and variation of EEG signal, a virtual window into the brain of the vulnerable infant. The development of tools to enhance understanding and quantify premature infant brain function offers key contributions to optimizing the care and outcome of developing humans. In particular, describing the effect of opioids on brain function raises further research questions which may lead to better approaches to pain and sedation management early in life.

Chapter 2: Quantification of Neonatal Limited Channel EEG Patterns

Limited channel electroencephalogram (EEG) is an established and clinically valuable tool for the assessment of neonatal brain function. While this simplified type of EEG captures global background brain function in contrast to the detail available with conventional EEG, it affords the benefits of applying fewer electrodes and obtaining continuous recordings in the neonatal care setting. Limited channel EEG is a general term that encompasses various models of clinical monitors designed to collect less than the many channels and montages of a full conventional EEG.

Amplitude-integrated EEG recordings are time compressed processed EEG signal from one or two channels without eye movement, EKG, or respiratory data that is usually collected in polysomnography/conventional bedside EEG. Once acquired from a single or double limited channel device, EEG signal is processed by algorithms to integrate amplitude changes into a time compressed trend. Thus an amplitude-integrated EEG signal (aEEG) is derived from the original EEG signal by processing through an algorithm to digitally amplify, smooth, rectify and compress raw EEG onto a piece-wise logarithmic display (Hellstrom-Westas, De Vries, & Rosen, 2008). The process serves to attenuate electrocortical activity between 2 and 15 Hz, signal is then rectified to remove negative EEG inflections in the data, smoothed to reduce possible signal noise and heavily compressed. The display uses a combined (piece-wise) logarithmic scale to show detail in the lower values below 10 μ V on a linear scale plus a log scale to compress the higher amplitude values which may be greater than 100 μ V. Output comprises a graphically depicted trend that is usually categorized by visual analysis of the envelope of amplitude signal data. The signal envelope is commonly described by the lower and upper

border, representing two primary visual patterns depicting brain function (continuity and discontinuity), also the alternating pattern between them (cycling), and the presence of electrical discharges such as seizures (Burdjalov, et al., 2003; Hellstrom-Westas, Rosen, de Vries, & Greisen, 2006; Hellstrom-Westas, Rosen, & Svenningsen, 1995).

Data compression into trended display allows the user to visualize patterns that change over time giving an overview of brain function that can be monitored for hours or days showing changes in response to handling, to medications, and gradual recovery after injury. Cycles of continuity and discontinuity are the primary changes in visual pattern that generally require several hours of recording to appreciate. Long recordings are not usually available with conventional EEG where the many channels provide a snapshot of detailed brain function information often approximately 60-90 minutes in length.

There is growing support for the use of limited channel EEG in research; however comprehensive interpretation is limited in reliability by the current lack of simple approaches for quantifying and summarizing the data. Elucidation of meaning currently involves qualitative interpretation of visual patterns of compressed signal output. Alternatively, some researchers use the raw EEG waves that are not amplitude integrated or compressed, from the limited channel, then conduct transformations to analyze complex signal analysis in the frequency domain. Assessment of premature infant brain function is further complicated by the well-described changes in EEG and brain function patterns that evolve over the weeks prior to term gestation (Olischar et al., 2004; Sisman, Campbell, & Brion, 2005; Soubasi et al., 2009; Zhang et al., 2011).

A specific model of aEEG is the cerebral function monitor (CFM), first developed in the 1970's to assess brain function deficits after serious cardiovascular insult in adults (Maynard,

Prior, & Scott, 1969; Prior, Maynard, & Scott, 1970; Prior et al., 1971). In general, data are recorded in a series of downloadable signals including the aEEG signal, raw EEG, electrode impedance and coordinates for the graphic display of aEEG from that device. Together CFM and aEEG monitoring in the NICU are primarily used in the assessment of cerebral function among full-term infants who have undergone hypoxic events with resulting brain ischemia. More recently, brain function monitoring has been extended to assessment of premature infants to determine gestational age or effect of maturation on brain function with strong reliability (Klebermass et al., 2006; Sisman, et al., 2005; Soubasi, et al., 2009). Visual pattern analyses of limited channel EEG, especially the presence of cycling between continuous and discontinuous patterns are demonstrating increasing sensitivity and specificity for the prediction of neurologic outcomes after brain injury such as intraventricular hemorrhage and periventricular tissue damage. (Bowen, Paradisis, & Shah, 2010; Chalak, Sikes, Mason, & Kaiser, 2011; Klebermass et al., 2011).

The purpose of this study was to explore simple operational measures for quantifying continuity and discontinuity, measured by aEEG, as components of premature infant brain function. In particular, the aim was to quantify variations in the lower border and bandwidth (difference of upper and lower borders) of the limited channel aEEG signal and begin to describe cyclic changes between states evidenced by patterns of continuity and discontinuity. Expanded definitions and analytic techniques will serve to strengthen the application of existing scoring systems for use in naturalistic research settings and clinical practice.

Limited channel EEG captures the two major background brain function patterns of newborns, continuity and discontinuity and the cycling that represents a transition from one to the other. Brain function is the compilation of oscillating electrocortical signal generated by

collective neuronal firing. This weak physiologic electric signal is transmitted across the skull, amplified and captured as measurable changes in voltage. The magnitude of change in voltage is referred to as amplitude. Continuity is the term for the pattern of brain function that is depicted by uninterrupted EEG signal activity, with steady amplitude in a range consistent with gestational development (in the absence of injury, illness, or certain medications). Immaturity and medications such as sedatives and anticonvulsants reduce continuity of signal. In contrast, discontinuity, the other major brain function pattern consists of varied amplitudes patterned as bursts of high amplitude EEG signal interspersed with quiescent intervals lasting for varied time periods again associated with developmental maturation. In gestationally younger infants, longer and more frequent quiescent, near zero amplitude pauses between bursts, result in an interrupted, less dense pattern and a chaotic EEG signal with a wide differentiation between low and high amplitude. *See Figure 2.1a-b. Example of Discontinuous and Continuous Patterns.* Bursts of amplitude and interrupted signal are hallmarks of premature brain function and have been described for decades (Parmelee, et al., 1969).

An emerging model to understand neonatal EEG promotes the idea that healthy brain function is built upon two distinct, yet concurrent processes of neuronal signaling. The first is an intermittent series of neuronal firing described as spontaneous activity transients. These transients are evident early in gestation, and are detectable as bursts of voltage by EEG. The signal appears erratic in frequency, possibly due to unsynchronized autonomous firings in each hemisphere prior to the development of callosal communication networks. The apparently spontaneous nature of the burst-like transients results in an interrupted or discontinuous signal when captured by EEG. The model then depicts a second simultaneous process of ongoing cortical activity with a more regular oscillating frequency. In early gestation, the oscillations are

of low electrical intensity and nearly undetectable. As the brain grows through structural, neuro-chemical, and inter-neuronal network development, the ongoing cortical waves robustly increase in voltage and become more prominent as a continuous signal (Vanhatalo & Kaila, 2006).

With normal maturation, each of the processes develops to reflect a primarily discontinuous pattern early in life with functional maturation to more continuous signal as the infant approaches term gestation. The dual aspects of this model are aligned with the findings of basic science determining that the background EEG signal occurs as the result of neuronal firing in an oscillatory network between the thalamus and the cortex (Amzica & Steriade, 2000; Steriade, Amzica, & Contreras, 1994). As the infant matures, the brain function becomes more organized and begins to consistently cycle between periods of continuity and discontinuity which will ultimately reflect developing sleep states. According to Vanhatalo and Kaila (2006) in their the model presented above, continuity takes over as the primary mode with maturation, thus differing amounts of cortical activity will eventually drive differentiation between sleep states. Superimposed mixed frequencies of the original spontaneous activity transients also grow in complexity with maturation.

At term gestation, brain function is represented as the two noticeably distinct patterns of continuity and discontinuity which then relate to the newborn behavioral states of sleep. *See Table 1 Conceptual Framework for Limited Channel EEG Interpretation.* Continuity features include a dense, narrow band of aEEG signal with amplitude generally above $5\mu\text{V}$ and below $25\mu\text{V}$ reflecting periods where the infant is either awake or in a state known as active sleep. It is not possible to determine wake from active sleep with limited channel EEG alone. Mature segments of discontinuity present on aEEG as widened bands with both higher maximum ($>10\mu\text{V}$) and lower border amplitude ($<5\mu\text{V}$) and less density, representing the intermittent

quiescent intervals between signal bursts of higher amplitude electrocortical activity. This discontinuous pattern is usually associated with the slow EEG waves that occur during quiet sleep in the newborn brain. Although related, both sleep states and brain function patterns are immature before the infant reaches term gestation.

Premature infant brain function is dominated by discontinuity, with rare sections of continuity visible on aEEG tracing. The lower border of the aEEG recording provides information about emerging cyclic changes in pattern, and is often described in the literature as relating to a return of function after injury or evolving maturation in infants born early. Even slight variance in the lower border indicates emerging transition from purely discontinuous as seen before 28-30 weeks postmenstrual age (PMA) to windows of briefly continuous signal, where the brain function has increasing organization and regularity.

A flat lower border, featuring low amplitude ($<3 \mu\text{V}$) without variance, is often associated with injury to the brain in mature infants. Sources of injury including hypoxia-ischemia, intraventricular hemorrhage, infection and trauma, commonly result in low voltage of the signal (Hellstrom-Westas, et al., 2008; Hellstrom-Westas, et al., 2006). These effects are seen on aEEG as a reduced and flattened lower border and may be a feature of collective patterns referred to as disorganized or dysmature brain function if prolonged beyond expected developmental milestones (Hayakawa, Okumura, Kato, Kuno, & Watanabe, 1997; Watanabe, Hayakawa, & Okumura, 1999).

The first signs of clear cycling between discontinuity and continuity emerge as an otherwise healthy infant reaches 29 weeks PMA (Sisman, et al., 2005). Continuity, discontinuity and what is assumed to be sleep-wake cycling between the two patterns form the basis of interpretation of limited channel EEG data. Several classification systems were

developed to assist clinicians with pattern recognition (al Naqeeb, Edwards, Cowan, & Azzopardi, 1999; Burdjalov, et al., 2003; Hellstrom-Westas, et al., 2006) for various populations of term and premature infants with and without cerebral injury. All of the classification systems to date rely upon some level of qualitative judgment to determine cycles of continuity and discontinuity, or complex computation of intervals between bursts of amplitude. Visual interpretation of graphic output is imprecise and made difficult because the higher amplitude values are presented on a logarithmic scale due to the wide ranges of bandwidth of aEEG signal patterns.

As stated in the introduction above, our experience with implementation of one of the popular scoring tools developed for demonstrating maturational changes of aEEG was unsuccessful due to incomplete operational definitions and the necessity to make qualitative judgments to identify and categorize emerging patterns of continuity with resultant cyclicality within each recording. With this study, we intended to develop quantitative approaches to enhance the interpretation of limited channel EEG signal and expand the utility of brain function monitoring as a useful measure for future studies involving premature infants. Although group comparison was not among the study aims, the maturational trajectory of premature infant brain function heavily overlies the patterns and measurements under this inquiry, and the convenience sample reveals a mixed group of early premature and term gestation infants. To that end, we chose to more closely examine data organized by PMA and a few of our analyses were conducted to highlight infants less than 30 weeks of age who are not expected to have fully developed patterns of continuity in the signal or mature cycling between discontinuous and continuous brain function.

Methods

Participants.

The descriptive design made use of data from a feasibility study previously conducted in the Neonatal Intensive Care Unit (NICU) to establish a plan for analysis and quantification of limited channel brain function patterns and from a secondary study of opioid effect on brain function (opioid outcomes are not reported in this paper). Infants were recruited from two Level III NICU's in the Pacific Northwest. Institutional review board approval was obtained from each site, and parents gave informed consent for participation of their infants. Infants were excluded if they had known congenital or acquired conditions of neurologic involvement (grade III or greater intraventricular hemorrhage, periventricular leukomalacia, seizures), were receiving high frequency ventilation or if their parents were not English speaking.

Procedure.

A single limited channel EEG recording was obtained from each infant while in the NICU without disruption of normal care activities. We used standardized placement of three hydrogel electrodes in the P3-P4 location by 10-20 international system (Klem, Luders, Jasper, & Elger, 1999) after a standardized protocol for skin preparation described earlier (Foreman, Thorngate, Burr, & Thomas, 2011). The parietal location is recommended for limited channel EEG placement to obtain a general signal and to avoid muscle artifact from the face and forehead (Hellstrom-Westas, et al., 2008). Recordings ranged from 3-12 hours with most occurring between handling for caregiving or feeding times.

Instrument.

All recordings were made using the Olympic CFM 6000 (Natus Medical, San Carlos, CA). Amplitude-integrated EEG signals, as recorded by this device and other limited channel EEG devices, have established prognostic sensitivity and specificity in both term and premature

infants after brain injury (Bowen, et al., 2010; Chalak, et al., 2011; Hellstrom-Westas, 2006; Hellstrom-Westas, Inghammar, Isaksson, Rosen, & Stjernqvist, 2001; Olischar, Klebermass, Waldhoer, Pollak, & Weninger, 2007; Spitzmiller, Phillips, Meinzen-Derr, & Hoath, 2007) and validity is accepted after comparison with conventional EEG in varied populations. EEG signal is sampled 100 times per second. The concurrent raw EEG signal and continuous impedance monitoring enhance artifact identification and signal fidelity in real time.

Analysis.

After visual review of the graphic output, data were downloaded to disk and prepared prior to statistical analysis. Analysis was conducted on the signal output devised by the manufacturer's algorithm for CFM signal. CFM was filtered and amplitude integrated between 2-15 Hz and has other properties specific to the device. We reduced the CFM signal by a time-sampling strategy to four samples per second to achieve efficiency of analysis and blocked the resulting signal into 60 second epochs across each recording.

We designed an empirical program to quantify discontinuity as evidenced by number of peaks in the signal. First, we estimated baseline amplitude by computing a running median over ten seconds of data and we then counted peaks in the amplitude greater than 10 μV above baseline. We also created a calculated variable to measure the proportion of time with amplitude less than 10 μV in each 1 minute block. Peak counts and time measured in percent below 10 μV together provide a preliminary quantification of discontinuity, reflecting the bursts (peaks) and quiescent periods of low amplitude. We chose 10 μV as a simple strategy for peak count and estimation of low amplitude intervals between peaks, based on previous studies (Bowen, et al., 2010; Hahn, Monyer, & Tharp, 1989; Selton, Andre, & Hascoet, 2000; Vecchierini, Andre, & d'Allest, 2007). Amount of time with the signal at low amplitude influences the overall

amplitude at the lower border as a function of CFM signal. Preliminary testing of these data demonstrated that proportion less than $10\mu\text{V}$ showed the strongest relation to minimum values of the lower border as compared to 5, 15, and $20\mu\text{V}$ thresholds.

Continuity was objectified by the mean and standard deviation of three characteristics; (1) the maximum and (2) minimum CFM values and (3) a calculated bandwidth value represented by the difference between the two for each epoch. Extensive within-subject analyses were conducted to evaluate each of the above variables individually and in combinations to explore quantitative techniques and to generate infant summary measures. Group level analysis was then performed by creating within-subject aggregates compiled for comparison across the sample. In general, for within-subject analysis median values for study variables were chosen to represent each minute of data due to the widely skewed distribution of amplitude and the peak/burst oriented nature of the signal. Group means across subjects are then reported for individual median values of CFM minimum, bandwidth and standard deviation of CFM signal derived from each subject's recording.

After visually analyzing the data through plots by variable across the time series, correlations were conducted between the two calculated variables (peak counts and proportion less than $10\mu\text{V}$) with the quantitative measures of lower border and bandwidth. Postmenstrual age was controlled when exploring variable relations by correlation. A subgroup of infants less than 30 weeks PMA was analyzed in order to establish quantification of discontinuity in early gestations. The subgroup analysis also served to quantify pattern differentiation representative of early signs of evolving continuity. Signal processing was conducted in MatLab R2011a (The MathWorks, Natick, MA), we then exported files to SPSS ver.15 (SPSS, Chicago, IL) for further visual exploration and statistical comparisons.

Results

Twenty five infants were recruited into the study. Twenty four infants (nine male) who had a mean PMA of 33.11 weeks (SD 3.49), with birth weights ranging from 0.54 to 3.49 kg were included in the analysis. Data from one infant were not analyzed due to high impedance for greater than 90% of the recording despite repositioning of electrodes. Two infant participants were recruited from a surgical center and were within 48 hours of surgical intervention for gastroschisis at time of recording. These infants were extubated and receiving standard pain management; post anesthetic physiologic effects were not evident. The remainder of the participants had a primary diagnosis of prematurity with respiratory distress.

Measures of continuity and discontinuity.

Bandwidth.

When evaluating bandwidth, less mature infants had wider spread and similar variation in their individual recordings. Full time series data were reduced to a single median value per minute recorded then statistically summarized. Group mean bandwidth for all participants was 52.98 μV (SD 27.62). For the less than 30 week PMA infants, median bandwidths ranged from 67.51-101.74 μV , group mean 92.56 μV (SD 14.27), $n=5$.

Proportion less than 10 μV and peak counts.

As previously defined, peak counts and proportion less than 10 μV were utilized to quantify discontinuity in the sample. Across the sample, median peak count in 60 second epochs averaged 3.63 (SD 1.74), while the median proportion less than 10 μV was 22% (SD 0.20). Less mature infants had higher peak counts and more time at low voltage. Among the less mature infants of lower gestation (below 30 weeks PMA, $n=5$), median peak count in 60 second epochs ranged from 0-6, mean 4.6 (SD 1.14), while the median proportion less than 10 μV was 51% (SD

0.17). Percentages of low voltage within the epoch are associated with discontinuity and expected to decrease with maturation.

Lower border.

Quantification of continuity included assessment of lower border voltage and emerging changes in voltage across the recording. Less mature infants had a lower mean voltage for the lower border with limited variation. The aggregated group mean of lower border individual medians was $6.20\mu\text{V}$ (SD 2.13). The lower border amplitude for the less than 30 week PMA infants ($n=5$) ranged from 2.3-4.16 μV , group mean 3.22 μV (SD 0.68).

Measures of cycling.

Bandwidth variance.

As narrowing bandwidth is a sign of increasing continuity, the variance across recordings should reflect emerging cycles between continuous and discontinuous periods of brain function. Our data showed only a slight decrease in variance for the younger infants. For the entire sample ($n=24$) the group mean of individual standard deviation for bandwidth was 24.23 μV representing fluctuations within each recording. The variance of the individual bandwidth for the infants with PMA less than 30 weeks ($n=5$) was 23.02 μV (SD 2.80).

Lower Border Variance.

The CFM lower border offers the earliest visual clues to cycling, in the form of gentle undulations along the lowest edge. As the periods of continuity lengthen, lower border variance increases. Our data shows a smaller lower border variance for the younger infants. The group mean lower border standard deviation was $3.96\mu\text{V}$. In contrast, among the younger infants, the group mean standard deviation of the lower border was $1.65\mu\text{V}$ (SD 0.42).

Effect of maturation.

After plotting the individual medians and group aggregated values for each variable, the effect of maturation was apparent upon visual analysis. Within and across subject descriptive analyses revealed expected variations by increasing PMA including increasing voltage at the lower border and narrowing bandwidth highlighted after 30 weeks PMA. *See Figure 2.2. Effect of PMA on CFM Signal Characteristics.* When data were log transformed to achieve a more normal distribution, bandwidth showed a strong negative correlation with increasing PMA ($r = -0.88$, $p < .0001$) by Pearson two-tailed correlation. Partial correlation to control for PMA retained a moderately strong relation ($r = .69$, $p < .0001$) between peak count and a measure of variance across the recording, log standard deviation aggregated by median for each infant. *See Figure 2.3. Log Bandwidth (individual median) by Postmenstrual Age (PMA).* Moreover, the proportion of 1 minute epoch below $10\mu\text{V}$ showed a strong negative correlation with the log of the lower border median ($r = -0.906$, $p < .0001$) after controlling for PMA. As the percent of time with low voltage increases, the lower border decreases, reflecting discontinuity of brain function even after removing the effect of age. Higher mean peak counts and proportion below $10\mu\text{V}$ were evident in younger infants. *See Table 2.3 Descriptive Summaries of Continuity with Comparison by Low PMA (<30 weeks).*

Other analyses.

No differences were noted on the basis of sex, or any other demographic characteristics not associated with maturation. The sample did not equally represent sex, only 37.5% of the sample was male.

Discussion

This study adds to the growing body of literature describing methods to systematically quantify the brain function of premature infants for exploration of varied aspects of neonatal care outcomes (Klebermass, et al., 2011; Kuint, Turgeman, Torjman, & Maayan-Metzger, 2007; Wikström, Ley, Hansen-Pupp, Rosén, & Hellström-Westas, 2008; Wong, 2008). While feasibility of limited channel EEG is now established among early born infants of low PMA, interpretation has continued to rely upon either complex spectral analysis, proprietary software to calculate interburst intervals or expert visual pattern analysis. For pattern analysis of premature infant brain function, researchers are required to include qualitative judgments about presence of cycling between discontinuous and slightly less discontinuous states that represent emerging brain function organization as continuity. A clear need for quantitative interpretation has continued despite increased interest in the study of brain function by EEG and aEEG (Vanhatalo & Kaila, 2006).

We attempted to apply the numerical criteria outlined by Burdjalov and colleagues (2003) during preliminary evaluation of data from our feasibility study of aEEG in premature infants. In that effort, we were unable to obtain scoring reliability between members of our research team. Visual assessment of CFM bandwidth on the piecewise logarithmic scale was a major barrier. Additionally, we found the subtle qualitative descriptions of cycling between continuity and discontinuity and gradual changes from fully discontinuous to some continuity difficult to consistently assign, and concur with the authors that there are inherently subjective aspects to the proposed scoring system. Similar to the findings reported by the Burdjalov (2003) group, our aggregated data reflect the effect of increasing PMA on each of the common CFM characteristics including: lower border, bandwidth, and presence of cycling between. We found bandwidth (log

transformed) to be the strongest reflection of maturation, with further delineation between weeks of gestation.

Our initial approach to introduce a simple process for quantifying discontinuity includes the application of the calculated variables: count of peaks $>10 \mu\text{V}$ above baseline and proportion of time (in one minute block) with amplitude $<10 \mu\text{V}$. These variables are conceptually consistent with the work of the Australian group (Bowen, et al., 2010) who in their study of premature infants conducted quantitative analysis of EEG (from aEEG) for the purpose of predicting outcome after intraventricular hemorrhage or other neurologic injury, used a cut off value of $<80\%$ at $10\mu\text{V}$ over a two hour epoch. Our data reflected the range of developmental progression within our broad sample (26-40 weeks PMA), which led to examination of findings for the small subgroup ($n=5$) of infants with PMA <30 weeks in our sample. As expected when averaged across these young infants, peak counts were higher and proportion below $10\mu\text{V}$ was higher than the means of each for the entire group of mixed gestational ages. Conversely, high peak counts if detected without elevated percent of time below $10 \mu\text{V}$ may signify increasing continuity.

It may be possible to apply these quantifiable methods to the scoring proposed by the Burdjalov (2003) tool, which includes 4 indicators: continuity of the recording, presence of cycling, lower border amplitude, and estimation of bandwidth plus lower border amplitude. The first two of the tool indicators are strictly qualitative, continuity and cycling, requiring expertise or judgments to apply the scoring criteria. Our methods may have value in reducing the reliance on qualitative decisions, in particular for the continuity indicator, although future work is warranted to develop cut points for peak counts and proportion below $10 \mu\text{V}$ to differentiate “somewhat continuous” from either “discontinuous” or “continuous” and to better quantify the

emerging process of cycling between. Outside of scoring maturational changes, peak counts and assessment of low voltage proportions may offer simple quantitative approaches to measuring the effect of interventions conducted in the NICU. Neurologic effects of medications and physiologic therapeutics on vulnerable infants raise many research questions that could be informed by measuring brain function. Interventions such as skin to skin holding, developmentally supported positioning, or gentle parent touch are well suited to enhance brain growth and normal development, brain function patterns would be excellent non-invasive short term outcomes for clinical research. In practice, nurses may be better prepared to plan caregiving to reduce interruption of sleep stages; with brain function monitoring that is quantifiable. CFM data interpretation in the context of optimizing premature infant caregiving will be made more accessible with straightforward techniques to define and gauge both continuity and discontinuity of brain function.

We have been interested in very young premature infants <30 weeks PMA, who present limited channel signals that are the most difficult to differentiate quantitatively—due to absence of continuity and clear cycling. This led us to look for opportunities to quantify subtle differences in discontinuity, within subject. We believe that emerging segments of continuity were expressed in our data in a combination of higher peak counts coupled with lower proportion of time below $10\mu\text{V}$. Understanding the functional characteristics of discontinuous signal generated early in gestation and differences in function as the shorter segments seen later during cycling will provide insight into brain development and sleep processes. There are surely complex relations within these patterns that may be best explored using alternative analytic approaches. Some authors recommend nonlinear analyses to better explore the multi-level

signals that make up neonatal brain function (Janjarasjitt, Scher, & Loparo, 2008; Pereda et al., 2006; Vanhatalo & Kaila, 2006).

We recognize the following limitations related to instrumentation, analysis and sampling procedures. CFM signal is obtained from a single channel EEG without the ability to assess focal information including symmetry, synchrony across hemispheres or detailed frequency (without raw EEG analysis). Further, the development of CFM in the adult population and translation to neonates, especially premature neonates may contribute to difficulties in differentiating subtleties unique to neonatal brain function. However, the non-invasive nature of obtaining limited channel EEG in the NICU and maintaining long recordings over hours is a major benefit.

We did not approach this analysis with ardent attention to artifact reduction. There are without doubt issues associated with skin-electrode interface resulting in high impedance, and movement artifact included in our data set. We chose instead to conduct an overview analysis to explore methods to begin quantification of the encompassing patterns of continuity and discontinuity over hours of time our recordings. The recordings are of varied lengths and the use of median aggregates may help to ameliorate the effect of artifact within subject by reducing the outlier effect of short bursts of extreme amplitude within each epoch of data. Future studies should more directly address impedance, motion and behavioral state of the infant (Suk, Krauss, Engel, & Perlman, 2009).

This analysis was limited by our aim to simply quantify and describe the characteristics of continuity and discontinuity. Our peak counting variable was designed upon an empirical process to estimate a baseline (moving median average window). Recognizing that this approach may over or underestimate peaks, we chose it as a starting place for our exploration. Further, our

process to estimate proportion less than $10\mu\text{V}$ is not as sophisticated as measuring the intervals between bursts of electrical signal, but our variables do not require agreement of a common definition for bursts among expectedly non-stationary data or extremely short data blocking strategies with resultant computational burden (Chalak, et al., 2011; Klebermass, et al., 2011; Victor, Appleton, Beirne, Marson, & Weindling, 2005; Wikström, et al., 2008).

The small study sample comprised a wide range of developmental ages. This offered opportunities for exploration using a descriptive approach and obvious drawbacks for generalization. The limitations of recruiting vulnerable infants receiving intensive care and a naturalistic design to capture exposure to opioids (not reported here) necessitated expansion of the inclusion criteria to infants closer to term development. Future studies to further develop or validate quantitative techniques for limited channel EEG interpretation would be best served by purposive sampling of a homogenous group with respect to maturation or resource to obtain a larger sample and conduct more detailed analysis controlling for maturational effects.

In conclusion, these data were examined in order to identify additional strategies for quantification of limited channel EEG signals. Our findings indicate that it is possible to make determinations of continuous and discontinuous patterns with simple quantifiable measures, and that this approach behaves in accordance with expected maturational changes. Taken together with quantified measures of the lower border and bandwidth of CFM signal output, counting peaks and estimating proportion below $10\mu\text{V}$ may be useful for future researchers to explain effects of interventions, medications, and brain function disruption related to injury even among infants of gestation less than 30 weeks.

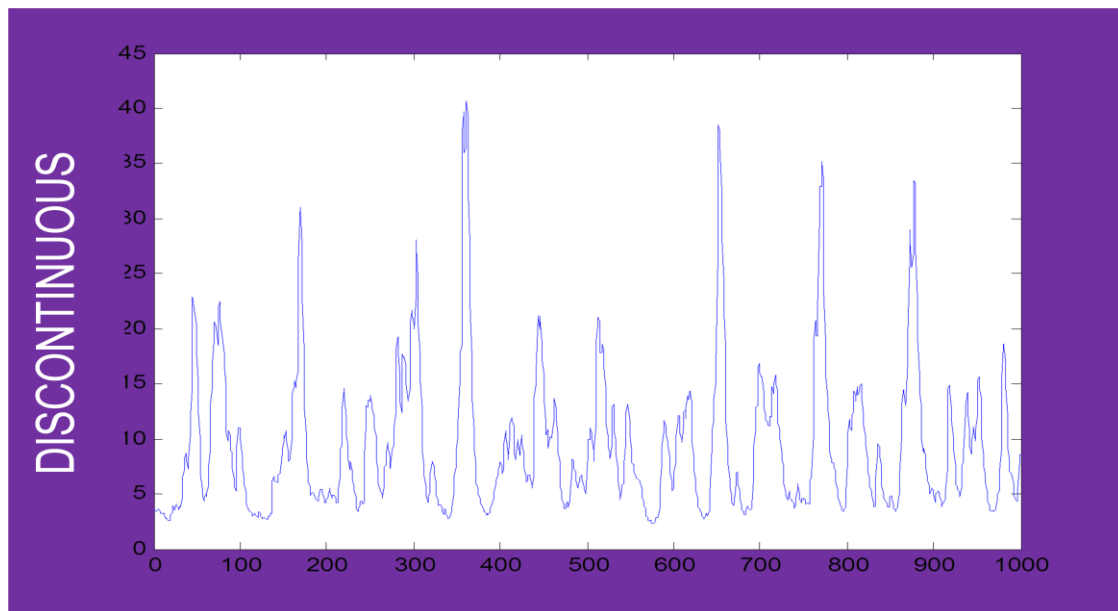


Figure 2.1a Example of a Discontinuous Pattern Note. 250 seconds of discontinuous CFM signal (4 samples/second); y axis is Hz

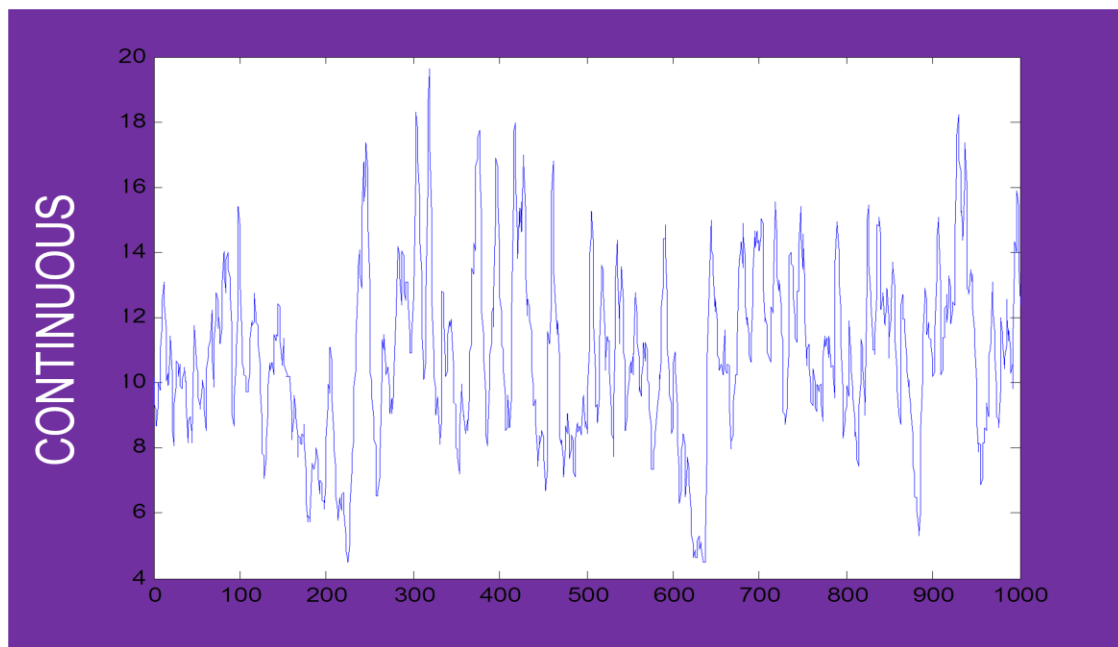


Figure 2.1b Example of a Continuous Pattern (same infant)
Note. 250 seconds of continuous CFM signal (4 samples/second); y axis is Hz

Table 2.1

Conceptual Framework for Limited Channel EEG Interpretation

Brain Function Pattern	Operational Variables	Derived by	Definition by Key Characteristics	Developmental Changes
Discontinuity	Bandwidth	Difference between CFM max and min	Wide bandwidth	<i>Initial pattern of very early gestation typical pattern at <30 weeks PMA</i> <i>After 29-30 weeks PMA, pattern alternates with continuity and becomes a precursor to quiet sleep</i>
	Proportion less than 10 μ V	% of each minute <10 μ V amplitude	Bursts of high amplitude interspersed with low amplitude “quiescent intervals”	
	Peak count	Each point 10 μ V over baseline		
	Lower border	CFM min	Lower border 3-5 μ V	
		Standard deviation of CFM min	Lower border near flat or with low variance across recording	
Continuity	Bandwidth	Difference between CFM max and min	Narrow bandwidth	<i>Develops over time, usually seen by 29 weeks PMA</i> <i>When alternating with discontinuity is considered to be active sleep or wake</i> <i>Pattern may regress to discontinuity after injury or medication</i>
	Proportion less than 10 μ V	% of each minute <10 μ V amplitude	Dense frequency of steady amplitude	
	Peak count	Each point 10 μ V over baseline		
	Lower border	CFM min	Lower border >5 μ V	
		Standard deviation of CFM min	Lower border shows variance across recording	
Cycling	Bandwidth variance across recording	Standard deviation of bandwidth	Change between discontinuity and continuity in the same recording	<i>Initially presents as slight variation in lower border visible as early as 25-26 weeks PMA</i> <i>By term, progresses to smooth cycles by term, quiet sleep \geq20 minutes</i>
	Lower border variance across recording	Standard deviation of CFM min		

Note. PMA = Postmenstrual Age, CFM=Cerebral Function Monitor, Min=Signal minimum, Max=Signal maximum

Figure 2.2 Effect of PMA on CFM Signal Characteristics

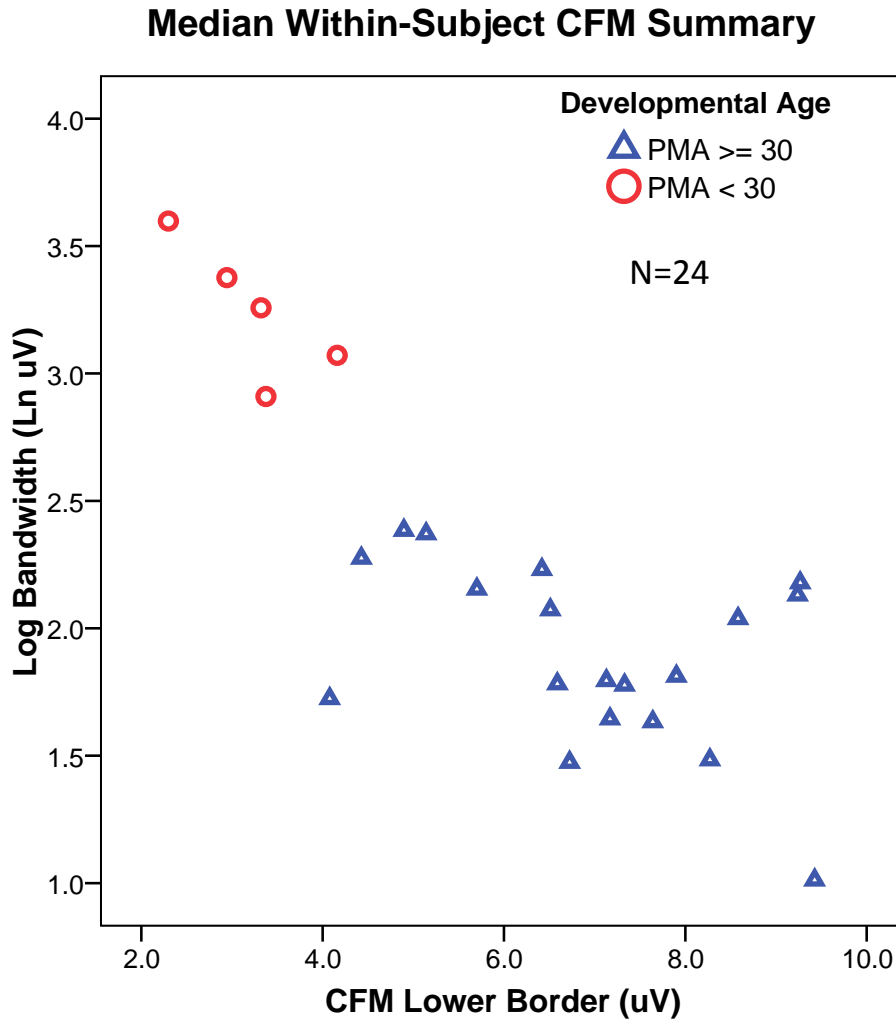


Figure 2.2. Effect of postmenstrual age (PMA) on Cerebral Function Monitoring (CFM) Signal Characteristics. y axis is log transformed to better reflect distribution of bandwidth values, also from CFM signal.

Figure 2.3 Log Bandwidth (individual median) by Postmenstrual Age (PMA)

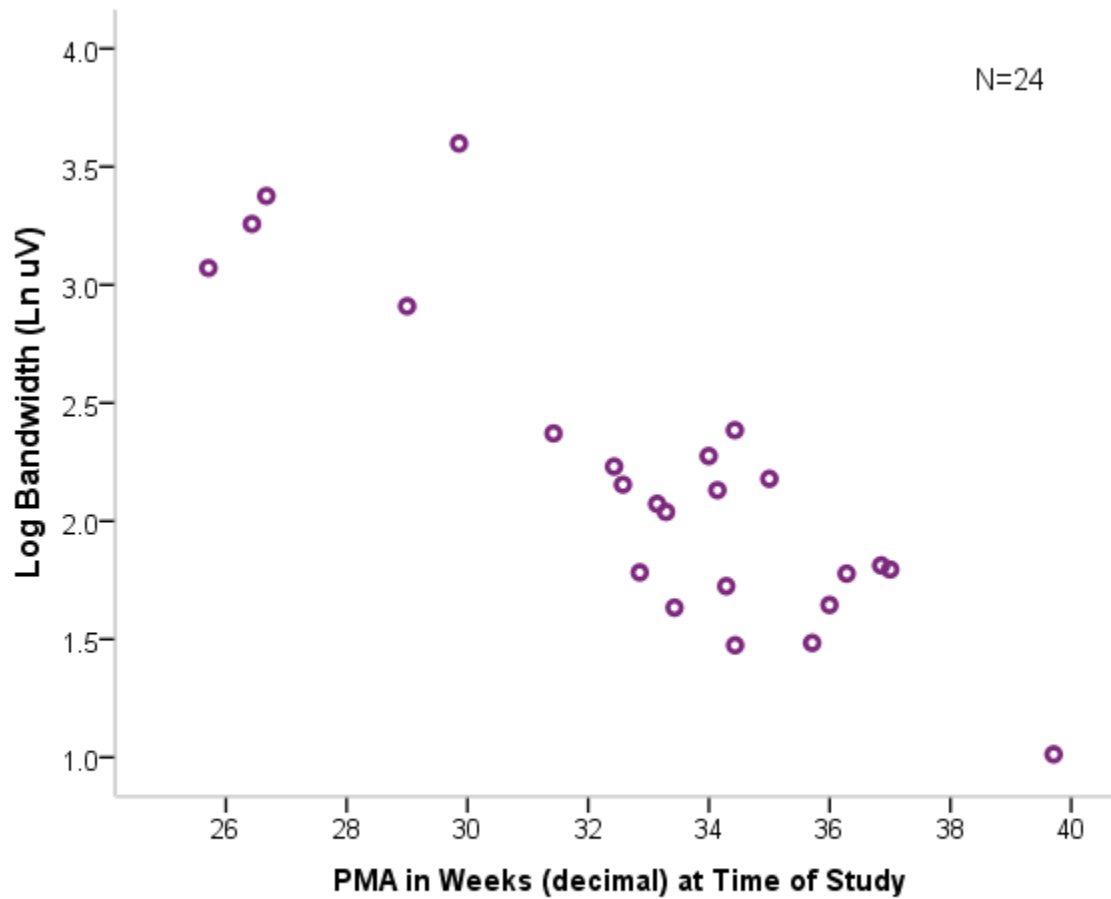


Figure 2.3. Log Bandwidth (individual median) by Postmenstrual Age (PMA). Each dot represents the median log Bandwidth for an individual infant (N=24). There is a natural separation between the younger (<30 weeks PMA) and older (>30 weeks PMA) infants.

Table 2.2

Descriptive Summaries of Continuity with Comparison by low PMA (<30 weeks)

Characteristic	All Ages n=24		Infants <30 weeks PMA n=5		Infants >30 weeks PMA n=19	
	Mean	SD	Mean	SD	Mean	SD
	Peak Count (number)	3.83	1.53	4.65	0.94	3.37
Proportion less than 10 μ V (%)	0.24	0.17	0.48	0.13	0.14	0.13
Lower Border (μ V)*	6.19	2.13	3.22	0.68	6.97	1.62
Lower Border Variance (μ V)**	3.96	3.15	1.65	0.42	4.57	3.28
Bandwidth (μ V)*	52.96	27.62	92.56	14.27	42.56	19.48

Note. PMA = postmenstrual age

* group mean calculated from median aggregate within-subject

** group mean standard deviation of signal from median aggregate within-subject

Chapter 3: Spectral Analysis of Neonatal Brain Function: Effect of Opioids

Opioids, though a mainstay of adult pain care, may not provide the analgesic effect once assumed for neonates. While opioids are commonly ordered in the neonatal intensive care unit (NICU) to provide sedation to promote optimal mechanical ventilation and analgesia, randomized controlled trials have raised concern about lack of analgesia (Cignacco et al., 2008) and absence of protection for neurologic outcomes, such as severe intraventricular hemorrhage and periventricular leukomalacia, in premature infants after routine opioid infusions (Anand et al., 2006; Anand et al., 2004; Simons et al., 2003). Well documented maturational differences in pharmacokinetics, side effect profiles, and wide variations in inter-individual pain behavior reduction create controversy among clinicians about optimal pain management approaches for term and premature neonates (Allegaert, Simons, Vanhole, & Tibboel, 2007; Anand et al., 2008; Knibbe et al., 2009). Concerns about potential neurologic and sleep disruption after opioid exposure during development further contribute to questions about utility of opioids in the neonatal setting (Axelin, Kirjavainen, Salanterä, & Lehtonen, 2010). Neurophysiologic measurement of sleep and brain function may give insight into the effects of opioids on the developing brain.

The electroencephalogram (EEG) measures the neuronal signal at the surface of the brain as it is transmitted across the cranium and skin of the scalp. EEG is sensitive to changes in blood pressure, oxygenation, and other physiologic events including pharmacologic effects. EEG signal, like heart and respiratory rates is cyclic, resulting in rhythmic waves occurring at different frequencies depending upon the infant's state. The spectrum of brain frequencies can be analyzed by transforming the EEG signal through fast Fourier transformation (FFT) and

evaluating the proportion of power that is found in particular frequency bands of interest.

Neonatal brain frequencies vary by sleep state and post menstrual age (PMA), and are made up of amplitude expressed in units of voltage usually between 50-100 μV for term gestation infants. Premature infants may have background amplitude as high as 300 μV (Boylan, Murray, & Rennie, 2008). The predominant frequency range for neonatal EEG is from 0.5 Hz to 30 Hz, representing a spectrum of EEG intensity occurring at different frequencies. The lowest frequency band termed delta, contains waves or oscillations of as low as 0.5 Hz to roughly 4Hz. In adults and older children these slow waves generally indicate deeper sleep states as compared to theta, alpha and beta bands. The faster frequencies are found respective to awake and highly active neurologic states. Both frequency and amplitude attenuate with maturation into brain function patterns that form the basis for sleep states.

Brain function is typically described in terms of two dynamic patterns that arise from the oscillation of neuronal firing in the thalamic and cortical regions of the brain. Complex sequences of recurrent neuronal connections with excitatory and inhibitory neurotransmission processes generate varied frequencies of electrical signal. When synchronized across hemispheres of the brain, neuronal transmission results in periods of wakefulness and arousal with cognition and levels of sleep (Steriade, Gloor, Llinas, Lopes de Silva, & Mesulam, 1990). Brain activity at rest is termed background brain function, and represents the ongoing neuronal activity in the absence of arousal. The earliest pattern, *discontinuity*, is the primary condition of fetal and early neonatal life. Discontinuity refers to periods of quiescence in the electrical signal interspersed with high amplitude bursts. The bursts usually occur at low frequencies but may have higher frequency rhythms nested within the burst (Amzica & Steriade, 2000; Parmelee, et al., 1969; Vanhatalo & Kaila, 2006). The second brain activity pattern called *continuity* is

characterized by a higher frequency, lower voltage, uninterrupted electrical signal that generally represents brain organization with more synchronized neuronal firing. With increasing maturation, the developing infant brain generates a more prominent pattern of continuity, eventually resulting in a normal alternating cycle of short periods of discontinuity that reflect quiet sleep. Longer periods of continuity represent active sleep and wake times. However, true sleep state differentiation is not strongly evident until approximately 30 weeks post menstrual age (PMA) and beyond.

The immediate effect of opioid therapy has not been fully explored with regard to brain function in early life. The effects of sufentanil, a potent synthetic opioid on premature infant EEG, have been studied for the purpose of avoiding false interpretation of neurologic assessment after sedation (Nguyen The Tich, Vecchierini, Debillon, & Pereon, 2003). No qualitative changes in EEG background patterns or unusual waveforms were reported, although discontinuity was increased after opioid bolus infusion, as measured by the intervals between amplitude bursts. Similar increases in discontinuity and burst suppressive patterns were found in a prospective study of infants 30-42 weeks gestation while morphine was administered by continuous infusion (Young & da Silva, 2000). Another polysomnographic study in premature infants 28-32 weeks PMA compared opioid (oxycodone) to nonpharmacologic pain management approaches and highlighted changes in EEG sleep architecture, specifically the cyclic changes of sleep state patterns. The researchers expressed concern about a reduction of rapid eye movement (REM) and the timing of non rapid eye movement sleep (NREM) occurring before REM sleep after opioid administration (Axelin, et al., 2010). Systematic evaluation of opioids in the context of neonatal intensive care is needed to develop an understanding of brain function patterns,

extent of opioid effect and potential for sleep pattern disruption in order to inform clinical dosing and pharmacologic sedation decisions for premature infants.

The present hypothesis generating study explored immediate opioid effects on brain function in a mixed gestational age group of critically ill neonates. In particular, the aim was to describe changes in brain wave signal variability by extracting raw EEG signal from a limited channel aEEG recording and examine differences in spectral density distribution before and after standard morphine administration.

Methods

Participants.

Participants were recruited from two level III NICU settings if they were 24-36 weeks PMA, expected to receive morphine by bolus for standard comfort management and had undergone mechanical ventilation for at least 24 hours. Neonates were excluded while on high frequency ventilation and if they had known neurologic alterations (grade III or greater intraventricular hemorrhage, periventricular leukomalacia, congenital malformations involving neural structures, previously diagnosed seizures). A second research site was added after the study had been open for a year, inclusion criteria was expanded to 42 weeks PMA. Post operative conditions were included and mechanical ventilation was optional in the second site. Recruitment and enrollment in the study was as follows: 60 infants were screened for inclusion, of those 37 met criteria for inclusion, 10 parents declined consent while 19 parents gave consent for infant participation, 13 were not studied for various reasons (transferred to another unit or hospital, required surgery, did not receive morphine, respiratory support issues, parent indecision, a participant expired after enrollment and equipment failure occurred during one recording). Data were collected between July, 2010 and April, 2012, with approval from the institutional review boards for both clinical sites.

Study design and measures.

This was a naturalistic experiment to describe brain function before and after intravenous morphine administration given in the context of routine NICU care. We chose a small n within subjects ABAB design due to the known inter-individual variation in opioid metabolism and neurophysiologic signal. The design provided two opportunities for each neonate to serve as his/her own control. The replication of two consecutive doses (when possible) facilitated continuous monitoring with a single application of the electrodes and minimal handling, thus decreasing the burden of measurement. The peak therapeutic effect was expected within 30 minutes of IV administration (Lemyre, Doucette, Kalyn, Gray, & Marrin, 2004). While timing of the dosing could not be precisely predicted, the analysis plan was to divide the recording into segments prior to and following the dose. We expected the recording interval to last much longer than the baseline because it was dependent upon the needs of the neonate and the nursing decision points in administering the bolus. This was a known limitation of the natural experimental clinical setting.

Limited channel brain function monitoring.

Conventional EEG recordings are the gold standard for electrical brain monitoring, however they are expensive to obtain and interpret. Full EEG is costly to the vulnerable premature infant due to time and physical handling requirements for multiple electrode placements. Long term (greater than 2 hours) continuous EEG recordings are difficult to obtain within the NICU care setting (Hellstrom-Westas, et al., 2008). Limited channel EEG also known as amplitude-integrated EEG (aEEG) is a direct method of cerebral function monitoring requiring fewer electrodes (3-5 depending upon the number of channels) and is well tolerated by critically ill

infants (Foreman, et al., 2011). Further, options exist for non-invasive sensors using hydrogel adhesive which are preferred for research over clinically accepted needle electrodes.

Validity of aEEG is established by the concurrent recording of raw EEG signal (de Vries, Ter Horst, & Bos, 2007), whereby users can differentiate artifact in the signal from true cerebral activity. Additionally, continuous electrode impedance values recorded during aEEG provide assurance of signal integrity. aEEG technology has been validated in varied age groups of neonates by comparison to conventional EEG. Clancy and colleagues (2011) demonstrated a significant moderate positive association between conventional EEG and aEEG background for infants after cardiac surgery. Normative values of aEEG document maturational changes in brain function of premature infants without injury (Burdjalov, et al., 2003; Kato et al., 2011; Olischar, et al., 2004; Zhang, et al., 2011). In addition to a body of work supporting aEEG as a prognostic tool for outcome after ischemic brain injury among term infants, studies recently established predictive value for intraventricular hemorrhage and brain injury in premature infants (Bowen, et al., 2010; Chalak, et al., 2011; Inder et al., 2003; Klebermass, et al., 2011). Bowen and colleagues (2010) identified a neuroelectric marker for short term outcome among premature infants <29 week PMA. They reported EEG continuity less than 80% at amplitude below $10\mu\text{V}$ per 2 hour epoch, predicted 73% of severe brain injury or death, with an 86% negative predictive value.

We chose aEEG as a measure to reduce physiologic distress for the neonate and simplify the process of assessing brain function without disrupting standard NICU care. Direct, non-invasive physiologic data was desired to describe opioid effect in this vulnerable population of infants. While single channel aEEG does not provide data on morphology, regional/focal detail, symmetry or synchrony of brain function, it records general background EEG, and offers access

to a channel of raw EEG signal data. Diffuse electrical signal as derived from the aEEG cross cerebral channel was thus appropriate for generalized assessment of neonatal cerebral function in the context of this exploratory study.

Study protocol.

After receiving informed consent, a single limited channel EEG recording (CFM 6000, Natus Medical, Inc., San Carlos, CA) was obtained from each neonate. Recordings ranged from 3-11 hours depending upon sedation or analgesic needs. All procedures were conducted in the incubator or radiant warmer in conjunction with necessary NICU care. Skin was prepared with 30 seconds of gentle abrasion using NuPrep (D.O.Weaver, Aurora, CO) in accordance with a standard protocol we previously described (Foreman, et al., 2011). Three hydrogel scalp electrodes were then applied to standard locations (P3-P4 and ground) by International 10-20 system landmarks modified for neonates. aEEG, CFM graphic output, raw EEG signal, and electrode impedance were continuously sampled and recorded at 100/second. Major body movements, care activities and timing of morphine doses were digitally notated by the investigator. Behavioral state was observed if detectable and noted. Data were later downloaded to disk for signal processing and statistical analysis. Total lifetime opioid dosing was also calculated for each participant to include previous intermittent and in some cases continuous infusions (prior to the study period), and ranged from 0.1-3.2 mg/kg since birth. This study was not designed to demonstrate dose effect.

Method of analysis.

Data preparation and signal processing.

In order to evaluate possible changes within the spectrum of brain function frequencies, after morphine administration, it was necessary to design a custom signal processing approach. This included filtering, smoothing, and assessing for artifactual effects of movement, followed by FFT exploration of structure within the frequency domain. The CFM 6000 device standard algorithm selectively filters the spectral information to remove low frequencies (<2 Hz) and high frequencies (>15 Hz) in order to reduce artifact from sweating and other adult oriented facial movement. To maximize the descriptive approach for premature infant brain function, where most signal power is found in the very low frequencies, we chose to custom filter the raw EEG data to encompass a wider frequency bandwidth (<0.5 Hz – 30 Hz). Impedance values were monitored to identify major motion artifact and poor electrode adherence.

We blocked the data by short (6 second) epochs prior to artifact removal to reduce data loss and to account for non-stationarity of signal. Stationarity refers to a sequence of data that has similar structure across time regardless of where the analysis begins (Bakeman & Gottman, 1997). Stationarity is important when conducting time series analysis that involves predicting how data will behave based upon previous segments. EEG data are labile and irregular, thus creating challenges for analysis by sequence, including FFT. *See Figure 3.1. Example of Neonatal EEG Signal.* Six second epochs, including data acquired during impedance outside of a consistent threshold, were removed. Relative time segments, representing pre and post morphine bolus were identified. Data were later aggregated over each time segment by participant for statistical comparison. We limited the pre-morphine segment to 15 minutes before the dose due

the logistics of electrode application and clinical timing of drug administration. For this report the post-morphine segments were evaluated at 30, 60, and 120 minutes post bolus.

Spectral analysis.

Spectral analysis offers a way to assess cycles of brain electrical activity that repeat over time in a given frequency or spectrum of multiple frequencies. Power spectral densities are computed functions evaluating the amount of variance contributed by cycles in the data as compared to noise if there were no true cycles (Bakeman & Gottman, 1997). For EEG data, the power refers to EEG intensity (EEG amplitude squared measured as voltage, reported as μV^2) generated by brain wave activity as distributed over particular frequency bands (delta, theta, alpha, beta).

Two calculated values used in this study to summarily quantify within each subject the total power across the full spectrum include the spectral edge frequency (SEF) and the power scale slope. Authors have described SEF as a simple compilation to reduce large data sets with variable frequencies that can be applied in determining maturation of brain function in animals and premature human infants (Bell, McClure, McCullagh, & McClelland, 1991; Szeto, 1990). The SEF50 represents the midpoint of frequency power expressed by the brain function signal. Similarly, the SEF90 represents the frequency at which 90% of the total power or EEG intensity falls below (Inder, et al., 2003; Szeto, 1990). SEF50 is comparable to a median point in the spectrum whereas SEF90 is more like the 90th percentile and refers to all amplitude generated power (brain activity) below that point. A high SEF50 means that the midpoint of total power in the spectral density occurs at faster frequencies. The high SEF50 indicates an increased amount of higher frequency cycles within the recording. Waves of brain activity are cycling at generally faster rates at least 50% of the time. In contrast, a lower or a decrease in the SEF50 reflects more power on the lower half of the spectrum, in the slower delta bandwidths. Spectral edge

frequency assessment must be interpreted within the context of the infant's developmental trajectory. Brain function frequencies vary with maturation, and are disrupted by brain injury and possibly opioid administration (Bell, et al., 1991; Inder, et al., 2003; Saito et al., 2012; Victor, et al., 2005).

The power scale slope is a calculated value representing the entire spectrum of brain electrical function frequencies. The shape and height of all peaks of power, after they are sorted by frequency, are summarized by taking the slope of the highest points to calculate the power scale slope. Power scale slope offers additional differentiation of power under frequency bands than SEF which reflects the cumulative spectral power at a given threshold (e.g. 50%, or 90%). Negative slope values indicate more total power in the low frequency bands and less spread across the full spectrum, or less power in the higher frequencies. A flat power scale slope reflects equal distribution of power across low and high frequencies. Conversely, a positive slope would occur if there was high power in the high frequencies and less power in the low frequencies. For a premature infant high power in the low frequency range produces a steep slope (negative number), although with maturation, normal brain function will include higher frequencies and less power in the low range, resulting in a flatter slope with a less negative value. Moreover, with injury to the brain, power densities may regress from higher or mixed frequencies to primarily low frequencies, reflected by an increasingly negative power scale slope value.

Statistics.

We conducted signal processing including custom filtering, smoothing and power spectral density distributions in MatLab R2011a (The MathWorks, Inc. Natick, MA). After processing and transformation in MATLAB, data elements were exported to SPSS 15 (IBM, Chicago, IL)

for further exploration. The data were examined visually through pattern analysis for each participant with comparison tests between segments for variables of interest. Mean and median differences in SEF and power scale before and after morphine segments were compared across subject by non-parametric Friedman rank order test. A liberal significance level of $p < 0.05$ was set, due to the exploratory nature of our inquiry.

Results

Nine neonates were recruited and studied by limited channel continuous EEG before and after morphine doses administered for standard NICU comfort management. Data were excluded from analysis for one infant due to high impedance values for over 95% of the recording. This infant was born at 24 4/7 weeks and underwent a long course of ventilation (70 days) prior to the date of study (at 34 4/7 weeks), including a brief pulse of postnatal steroids. Skin maturation over his 10 weeks in the NICU may have contributed to failure to obtain acceptable skin electrode interface. Of the remaining eight (two male) infants, the median PMA was 29.4 weeks (range 25.7-39.7 weeks), and median weight 835 grams (range 623-3490 grams). See Table 3. 1. Two infants were within 48 hours post surgical intervention for gastroschisis, the remainder received mechanical ventilation for prematurity and respiratory distress during the study period. SNAPPE-II (Score for Neonatal Acute Physiology Perinatal Extension-II) is a robust, simply administered scale of neonatal severity and has retained reliability over time without deterioration despite changes in practice (Zupancic et al., 2007). Physiologic data and baseline birthweight, APGAR scores are collected within 12 hours of admission/birth, with the intent of predicting risk of mortality. For the current study, calculated SNAPPE-II scores demonstrate a range in mortality risk at birth. Aside from maturation, there were no conditions

expected to affect brain function. All were treated with bolus morphine given intravenously for sedation or painful procedures.

Morphine doses across all neonates ranged from 0.03-0.18 mg during the study recordings for a median of 0.05 mg/kg. During the 10 recorded morphine doses no visual pattern differences in SEF50, SEF90 or power scale slope were detected between segments of signal analysis immediately before and after the morphine dose within subject by 30 minutes. *See Figure 3.2. Example of Pre/Post Morphine Times.* Longer segments (60 and 120 minutes after morphine) of processed signal were evaluated to describe changes in spectral densities. Patterns varied by participant. Some had increased SEF90 at 30 minutes after morphine, and continuing increase at 60 minutes. Others showed a decrease in SEF 90 at 60 minutes with rebound at 120 minutes. Still others showed slight increases followed by later decrease in power edge and slope. In general, the most consistent finding included patterns reflecting expected features related to maturational status of each neonate. *See Figure 3.3. Maturation Effects on SEF50.* Neonates with higher PMA demonstrated flatter power scale slope values and lower SEF50 and SEF90 values irrespective of morphine administration during the study recording period.

After visual pattern evaluation, we conducted across subject Friedman nonparametric rank order tests on aggregated median values derived within each subject by morphine time point to determine if a difference could be found between the pre-morphine time and any other post-morphine time period (the first [30 min], second [60 min] or third segment [120 min]) of the recording after morphine. Median power scale slope showed no difference for morphine times ($n=8$, $\chi^2=1.050$, $df=3$, $p=0.789$). Similarly, for SEF90 we did not find a significant difference between morphine times ($n=8$, $\chi^2=2.885$, $df=3$, $p=0.410$) whereas, SEF50 differed significantly across morphine times ($n=8$, $\chi^2=8.923$, $df=3$, $p=0.03$). All infants tolerated the application of

aEEG electrodes and recording procedures well, clinical status remained stable throughout the study. No skin irritation was found upon removal of the electrodes or during application.

Discussion

The effect of morphine on the developing brain is of serious concern for neonatal providers, especially as related to the relief of pain and alleviation of stress during intensive care. Pain management strategies for the population of premature infants vary widely from unit to unit, and by provider. The present study offers an initial exploration of physiologic response to opioid administration through a direct measure of brain function.

These data were surprising in light of previous descriptions of profound disruptions in EEG after morphine administration in infants (mostly 30-35 weeks PMA) (Young & da Silva, 2000) and synthetic opioid effect on less mature neonates (Nguyen The Tich, et al., 2003) and oxycodone effect on premature infant sleep architecture (Axelin, et al., 2010). We anticipated a decrease in background brain function amplitude or a consistent change in background pattern reflected in the spectral frequency distribution immediately after morphine administration but did not find this in the present report. Similarly, while not a primary outcome, Victor and associates (2005) also did not find consistently significant differences in relative delta power (a component of spectral analysis) in premature infants administered morphine. They did not report spectral edge frequencies for the morphine exposed infants. They identified a narrow window of early maturity 24-28 weeks gestation as a possible rationale for the absence of significant differences by development.

A Japanese group recently utilized spectral analysis combined with qualitative description to delineate disorganized patterns of EEG in premature infants with brain injury at varied developmental ages (Saito, et al., 2012). It is possible that morphine disrupts brain

function leading to a dysmature pattern that is EEG features not expected at a given age of maturity. The return to a primarily discontinuous pattern after morphine administration in an infant of gestational age sufficient to generate continuity of brain function or higher spectral densities could be construed as dysmature. Although in our study immature patterns of brain function were seen throughout most of the recordings, we simply may not have had the precision of measurement to differentiate further dysmaturity in spectral density summarization. The EEG signal of mixed frequencies also varies in relation to state, and shape and quality of waves, within a single recording over time. As part of a systematic establishment of quantitative modeling to capture complexity of brain function and account for maturation, a dysmaturity index based on 7 specific sleep measures was developed (Scher et al., 2003). Spectral energies are a part of the reported model; however the index also requires several aspects of sleep physiology not available when using limited channel EEG methods as in the present study. Eye movement, arousal counts, sleep cycle lengths, regional spectral correlations, and respiratory ratios were combined in multivariate analyses. Receiver-operating characteristic curves demonstrated that differentiation by maturational groups would have been lost by reduction of the variable set. Thus, it may be that dimensions of opioid induced changes within our data would be more evident if analyzed in combination with additional pieces of physiologic information. Individual spectral energy measures may not have the depth of complexity to expose maturational patterns and effects of opioids within the primarily discontinuous, low frequency cycles of premature infant brain function.

Brain function is compiled of complex spikes and bursts of varying amplitude layered upon rhythmic processes, all orchestrated across intricate thalamic and neocortical networks with regulatory functions in the brainstem (Amzica & Steriade, 2000; Vanhatalo & Kaila, 2006).

Functional evolution of brain activity is affected by maturation as the brain develops more fully, by use and reactivity to environmental stimuli, by injury or illness. For the premature infant, neuronal development and brain activity is most certainly disrupted by the unexpected timing and environment of neonatal intensive care, the extent of which is extremely individualized. The effect of neuroactive agents including morphine adds another dimension.

Definition of a true baseline for comparison of morphine effect requires an intricate balance of physiologic and signal stability not easily achieved in a clinical setting. Application of the electrodes involves handling the infant, which although not painful, may have contributed to a heightened alert state or arousal. Also in the present study, morphine was administered by nursing decision. The clinical signs taken by nurses to indicate need for analgesia or sedation such as irritability, movements, or crying are more likely to represent arousal than sleep states. For analysis, we assumed a linear progression of effect after opioid administration, comparing spectral information before morphine was given to time points after administration of the dose. Our findings may have included larger differences or common patterns of change had we compared data collected several hours after the morphine to data obtained more immediately after the dose. Thus avoiding possible disruptions related to arousal or stress of handling the infants.

This exploratory study has raised questions about morphine effects on neonates. We recognize several shortcomings. Primarily, limited channel EEG was chosen due to simplicity of use in a clinical setting and reduced cost, both physiologic and economic. A broader montage of EEG channels and eye movement measurement would add to the strength of this study by providing the ability to assess for synchronization and symmetry of brain function by region, and categorization of state changes. A more robust dataset that included detailed description of

infant state or perhaps video of bedside activities and infant response would also improve the ability to readily identify artifact. In addition, the algorithm built into the CFM 6000 device is not optimally suited for evaluation of spectral densities generated by early born infants.

Immature patterns of brain function are heavily based in the lower frequencies; the CFM algorithm was initially derived from adult EEG and attenuated to higher frequency bands to avoid muscle artifact and to reflect the expected frequencies of adults. Standard CFM data are therefore filtered to remove all frequencies below 2 Hz which is where a great deal of power exists for premature infants. We addressed this issue through the use of custom signal processing. A specific bandpass filter for the raw EEG data was designed to capture frequencies above 0.5 Hz as appropriate for immature brain function patterns.

Further, while our study was designed to describe effects within subjects during a single continuous recording and thus to reduce the effect of maturation, the convenience sample does include infants of varying gestations. A tighter recruitment of like gestational ages, or alternatively a larger sample suitable for group comparisons would allow analysis with relation to development and maturation and potentially wider generalization of findings.

Finally, the use of FFT although commonly reported for frequency domain analysis of electrophysiologic data was not ideal for this dataset. Victor and colleagues (2005) noted that spectral analysis may not be an appropriate method for the analysis of higher frequencies due to non-stationarity. Fourier transforms rely on the assumption that the data are stationary at least for a short window of the time series. In this study, the immature patterns of EEG prevailed, primarily in a discontinuous background state, with irregular bursts of high amplitude interspersed with quiescent periods. We attempted to deal with this analysis problem by choosing short block lengths, resulting in a bulky and at times unwieldy data set. Although

improved over longer blocks, non-stationarity persisted. Strategies for optimal analysis for future studies and secondary analysis include use of nonlinear dynamics, and alternative transformative approaches such as Gabor transform for time-frequency analysis, or discrete wavelets to decompose the complexities of brain function signals.

In conclusion, spectral density measures did not demonstrate obvious changes in neonatal brain function after morphine administration. Taken together with outcomes of other studies, these data contribute to the unfolding story of inter-individual difference in both brain function and physiologic response to opioids. Our description of opioid effect includes minimal immediate change within 30 minutes, and inconsistent changes in slope and SEF90 at 60 minutes with further individual variation after two hours. When within subject median data were aggregated across infants, only SEF50 showed a significant change in rank order across morphine times at 30 and 60 and 120 minutes. It is not known which time points contributed to the change, due to the nature of the Friedman test. In effect, we postulate that the median power shifted from mixed to primarily lower frequencies immediately after morphine, with a gradual return to a more mixed frequency spectrum. In our analysis and previous studies, morphine effects mimic immaturity with a reduction in higher frequency cycles resulting in a transiently less organized brain function pattern. Only the measure more sensitive to high power in the upper half of the spectrum (SEF 50) illustrated clear evidence of the change. We believe that the immaturity of the current sample may not show effect based upon the measurement chosen. More detailed analysis within specific bandwidths (e.g. theta and beta) may generate further understanding of opioid effects. If possible to capture, mid and high frequency bands may hold keys to subtle shifts within the spectrum despite the prevalence of low frequency signal before

term gestation. The critical vulnerability of brain development for premature infants remains a high priority for further exploration.

Table 3.1.

Demographic Characteristics of Participating Infants (n = 8)

Characteristic	At birth		At time of study	
	Median	Range	Median	Range
Age (weeks)				
GA	27.9	24-38.6		
PMA			29.4	25.7-39.7
Weight (kg)	0.78	0.62-3.49	0.84	0.62-3.49
Ventilator days			9	1-19
SNAPPE-II score	21	0-57		
Gender (n)	Female	6		
	Morphine			
	Median	Range		
Study dose (mg/kg)	0.05	0.03-0.08		
Total (mg/kg)	0.41	0.10-3.19		

Note. GA = gestational age; PMA = postmenstrual age;
SNAPPE-II = Score for Neonatal Acute Physiology Perinatal Extension-II
Total morphine dose includes previous infusions and bolus doses to date



Figure 3.1. Example of Neonatal EEG Signal

Fifteen seconds of raw EEG tracing from CFM 6000. From a premature neonate at 29 weeks postmenstrual age. Note non-stationarity of signal across the epoch.

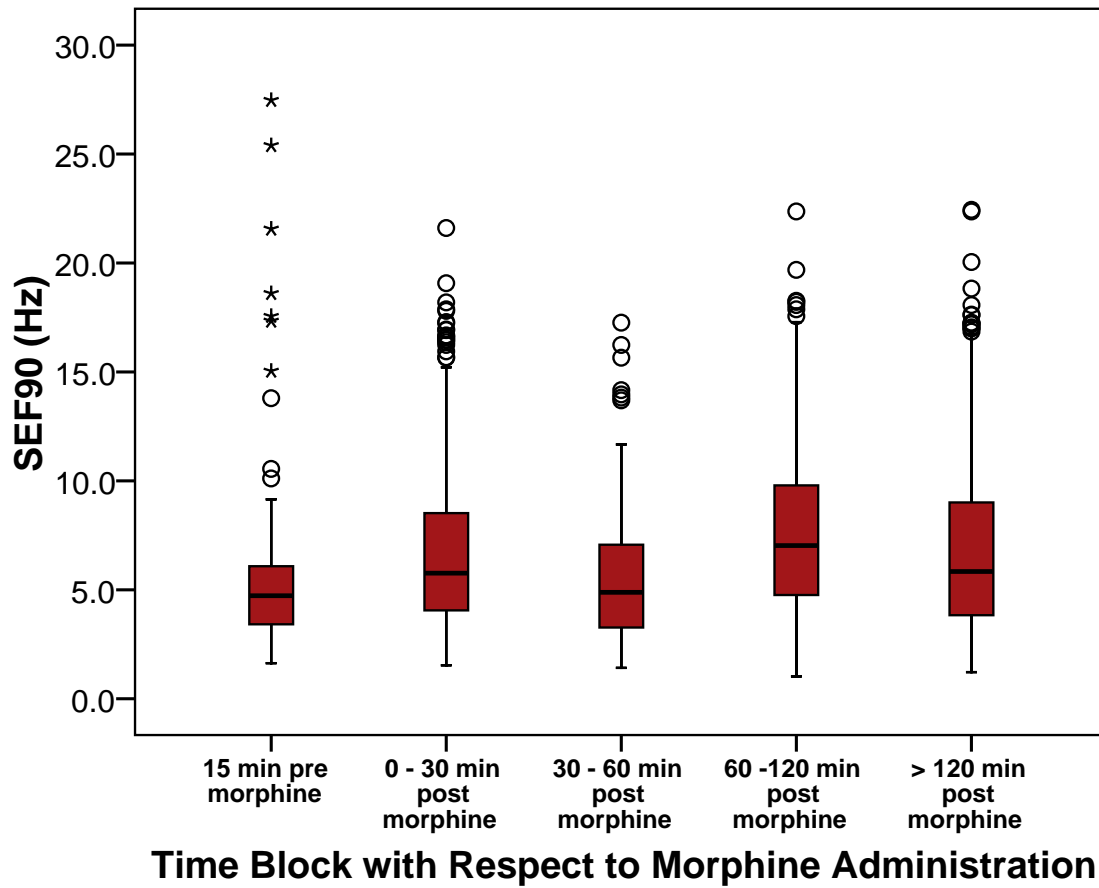


Figure 3.2. Example of Participant Box Plot for Visual Analysis (SEF 90); n=1, Box plot of SEF90 (Hz) across morphine time blocks. Plot shows high variability at each time point, with minimal difference between median (solid lines) SEF90 values across time. Example is from a premature infant approximately 27 weeks PMA at time of recording.

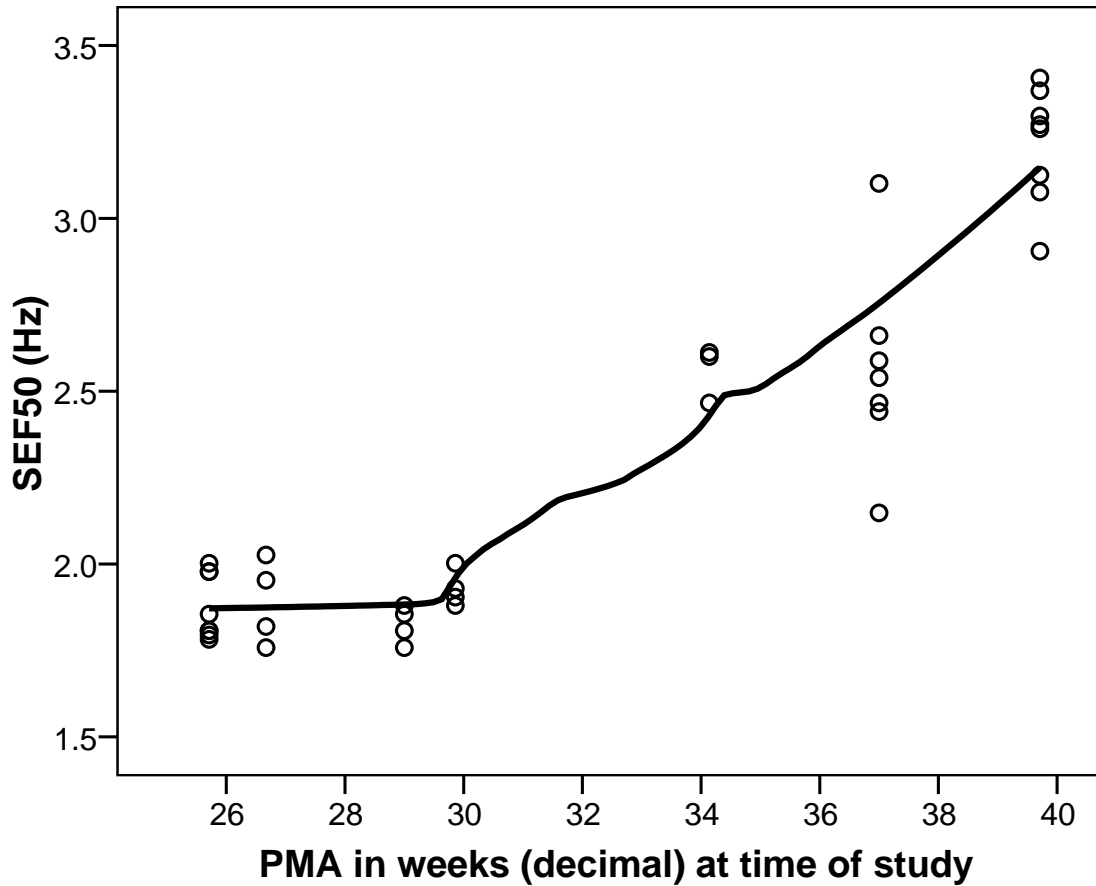


Figure 3.3. Effect of Maturation on SEF50(Hz); $n=7$, Each vertical cluster shows variance of SEF50 by morphine time point for a single participant. Median SEF50 values increase as a function of maturation by week of post menstrual age. Line is drawn as a fit of all data. One participant did not receive morphine during the recording. Participants with more than one morphine dose have more data points recorded. Data was removed for artifact in some cases.

Chapter 4: Under the Spectrum: Searching for Cyclic Effects of Opioid on Premature Infant Brain Function

Early pain creates lasting changes in pain perception, physical sensitivity, and may disrupt brain development (Brummelte et al., 2012; Grunau et al., 2005; Leslie et al., 2011). On the other hand, there is rising concern that opioids may not be effective at relieving procedural pain or reducing the transmission of pain signals to the brain (Carbajal et al., 2005; Cignacco, et al., 2008; Fitzgerald & Walker, 2009) and that morphine use is not protective against negative neurodevelopmental outcomes (Anand, et al., 2004). Of additional concern, pain management techniques, including opioids, resulted in marked alteration of brain function patterns within the span of sleep structure among infants 28-32 weeks postmenstrual age (PMA) (Axelin, et al., 2010). However, the intent to relieve pain, provide sedation and optimize therapeutic effects of mechanical ventilation continues to drive the clinical use of opioids among premature infants. This paper presents data from a descriptive study of brain function before and after standard opioid administration in premature infants. Spectral analysis of EEG will be explored to search for changes in brain function patterns and cyclicity within the developing brain.

Brain function and development of sleep.

Physiologic function is governed by rhythmicity, whether imposed or endogenous. This is especially true for the vulnerable premature infant, with respect to brain function and development of sleep processes. Healthy sleep and wake development is dependent upon complexity within brain function waves and organized patterns of evolving brain waves. Premature infant brain function is dominated by a pattern that is referred to as discontinuous, and reflects interruptions or nearly isoelectric intervals in the signal captured by electroencephalogram (EEG), aptly described as bursts and flats in early work by Parmalee et al

(1969). In comparison, infant brain function at term gestation is primarily continuous, made up of the oscillation of neuronal firing and inhibitory feedback through a more mature loop of thalamic and cortical neurons. Once normal developmental brain structural changes have occurred, continuity will encompass cross-hemispheric synchronization of the bursts found in the premature discontinuous brain function patterns. Synchrony of the neuronal firing along with changes in burst duration contribute to a lower voltage signal without breaks, thus the term continuity (Vanhatalo & Kaila, 2006). Further, rhythmic cycling between the two patterns of continuity and discontinuity will form the basis for active and quiet sleep in the newborn (Mirmiran, Maas, & Ariagno, 2003; Scher, Johnson, & Holditch-Davis, 2005). Decades of basic science and limited clinical application have built upon the early descriptive ideas, yet the mechanisms of premature infant brain function and sleep development are not fully understood.

Although immature brains present immature patterns of EEG, a steady oscillation of slow frequency waves is readily apparent, whether in immature animal or human signal (Amzica & Steriade, 2000; Steriade, 1994; Vanhatalo & Kaila, 2006). Slow waves are classified by the nomenclature delta, usually defined as less than 2-4 Hertz (Hz) in frequency and, with development, will make up the major component of the quiet sleep state. Faster frequencies contribute to the active state of sleep, an intense time of rapid neuronal firing and complex feedback loops generated by the thalamo-cortical relay system in the brain. Researchers no longer think of sleep as a passive state of rest for the brain (Peirano & Algarin, 2007), rather both quiet and active sleep are known to play functional roles in brain growth and maturation. The weeks prior to term gestation (24-40 weeks) are a critical time for both rapid physical and functional changes within the brain. Key functions of cortical firing, neurotransmission and inhibition through thalamo-cortical circuits, and laying down collective neuronal networks will

become the neurophysiologic foundation to support cognition, sleep, and lifelong functional health.

The emergence of sleep and wake organization is one of the most significant aspects of newborn development and should be a primary concern for care providers and families of early born infants (Peirano, et al., 2003). Sleep disturbances experienced in the neonatal intensive care unit (NICU) are thought to contribute to behavioral and physiologic consequences well into adulthood (Mirmiran & Ariagno, 2003). The premature brain is undergoing transition from fetal to neonatal life in a developmentally unexpected environment. Environmental noise, painful procedures, unanticipated physical handling and physiologic stresses are commonplace. As introduced above, the use of opioids for sedation and procedural pain management is likely to disturb the development of sleep cycles with unknown consequences.

Limited channel EEG.

Although full multi-channel EEG is the gold standard for neurophysiologic monitoring and diagnostics in the neonatal population, limited channel EEG systems are approved for routine clinical use and screening in the NICU (El-Dib, Chang, Tsuchida, & Clancy, 2009; Rosen, 2006). Limited channel EEG offers a noninvasive mechanism to monitor brain function including sleep cycling. Limited channel EEG is also known as amplitude integrated EEG (aEEG). The original device, called CFM for cerebral function monitor was developed to assess neurologic function in adults after cardiovascular collapse and resuscitation (Maynard, et al., 1969; Prior, et al., 1970; Prior, et al., 1971). At present varied options are available by device and manufacturer using the original CFM algorithm.

Although now adapted for neonatal use, in general, currently available monitors use the same approach as the adult device of the early 1970's. A single EEG channel is recorded; the

signal is then filtered to between 2 and 15 Hz and smoothed, to reduce noise. It is attenuated for higher amplitudes, especially those generated by premature infants that might reach amplitudes greater than 300 μ V. An EEG signal has deflections downward as well as upward. For aEEG, the absolute value is taken to avoid negative values; this is known as rectifying the signal. Finally the signal is graphically presented as a compressed time trend. Three hours of signal is visible on the device monitor, to provide a convenient display for clinical evaluation as compared to reams of paper that used to be generated by analog EEG.

In the NICU, aEEG is used clinically to screen infants with hypoxic-ischemic injury prior to hypothermic treatment. Prognostic value for neurologic outcome is well demonstrated for term infants (Klebermass, Kuhle, Kohlhauser-Vollmuth, Pollak, & Weninger, 2001; ter Horst, van Olfen, Remmelts, de Vries, & Bos, 2010; Toet, van Rooij, & de Vries, 2008; van Rooij et al., 2005). A meta-analysis of aEEG use for monitoring infants with hypoxic-ischemic encephalopathy reported an overall sensitivity of 91% (95% CI 87-95) and a negative likelihood ratio of 0.09 (95% CI 0.06-0.15) for aEEG to accurately predict poor outcome (Spitzmiller, et al., 2007). When simultaneously compared to the gold standard conventional EEG in 637 paired epochs, aEEG demonstrated a significant moderate positive association to determine background pattern across a range of normal to severely abnormal. The authors noted that knowledge of patient age and presence of seizures strengthened the ability of the reviewer to accurately predict moderately abnormal or severely abnormal background on conventional EEG from moderately abnormal aEEG background (Clancy, et al., 2011).

Recent data in premature infants shows promise over more traditional ultrasound for prediction of outcome by Bayley developmental scale at 3 years (Klebermass, et al., 2011). Also in premature infants, researchers have looked at continuity and sleep wake cycling by aEEG with

percent of time in low voltage for prediction of death, intracranial hemorrhage and other short term negative neurodevelopmental outcomes (Bowen, et al., 2010; Chalak, et al., 2011; El-Dib et al., 2010).

Brain function develops and changes with age. Reference standards of aEEG patterns with regard to maturational norms are now well established for term and premature gestation infants (Burdjalov, et al., 2003; Olischar, et al., 2004; ter Horst, Mud, Roofthoof, & Bos, 2010; Vecchierini, et al., 2007; Victor, et al., 2005; Zhang, et al., 2011). Researchers have also defined dysmature (Okumura, Hayakawa, Kato, Kuno, & Watanabe, 2002) and disorganized (Hayakawa, et al., 1997; Saito, et al., 2012; Selton et al., 2010) as abnormal patterns for brain function changes after injury or associated with chronic morphologic changes in EEG wave patterns that do not accurately reflect gestational age. Infants born at or near term gestation who maintain a highly discontinuous pattern more reflective of early prematurity after injury exhibit a dysmature pattern. Among infants <29 weeks PMA the absence of even an immature cycling pattern between discontinuous and continuous patterns (development of obvious mature cycles occurs after 30 weeks) as measured by visible variation in the lower border of the aEEG graphic tracing less than 2 μ V was predictive of poor long term outcome (Bowen, et al., 2010).

Opioids and brain function.

Opioids are used to support pain management and to provide sedation during NICU care, especially for mechanically ventilated infants. While the effect of opioids on the developing brain has not been widely studied, several studies suggest that opioids may disrupt EEG patterns. Adult patients receiving remifentanil versus saline as part of an anesthetic regimen showed modifications of the spectral characteristics of EEG signal in proportion with the opioid infusion rate (Kortelainen, Koskinen, Mustola, & Seppanen, 2008). The study was designed to explore

frequency power progression during anesthesia. Adults followed a general pattern of biphasic frequency shifting from initially high to low frequency power and reached clinical endpoints, such as loss of verbal command in less time when the opioid was included in the anesthetic regimen. Infants and children undergoing anesthesia, including some opioids were recently studied to assess a frequency domain parameter known as spectral edge frequency (SEF90) and amplitude-integrated EEG (aEEG) (*See detailed descriptions of SEF and aEEG in Data Analysis/Instruments*). The authors describe a paradoxical response of high SEF90 when infants under six months of age were anesthetized as compared to awake periods (McKeever, Johnston, & Davidson, 2012). In a study of premature infants who received sufentanil, EEG signal was strongly affected by bolus and continuous infusion of the opioid (Nguyen The Tich, et al., 2003). The researchers report a significant increase in features associated with discontinuous brain function pattern, increased length of intervals of low amplitude between high amplitude bursts, and increased percentage and duration of bursts. They contend that to avoid misinterpretation of brain function changes, sedation must be considered when EEG is used. A single case report of morphine overdose in a term infant being monitored with aEEG after hypoxic-ischemic encephalopathy demonstrated a dramatic change from a continuous pattern on the tracing to a discontinuous pattern and subsequent reversal after administration of naloxone, an opioid antagonist (Niemarkt, Halbertsma, Andriessen, & Bambang Oetomo, 2008). Similar findings of reversible periods of increased electrical quiescence within the EEG recording were reported after morphine administration to premature infants (Young & da Silva, 2000). The long term effects of opioids on the developing human brain are far from understood.

Problem.

The use of opioids in the NICU to reduce procedural and postoperative pain while important to optimize comfort and minimize negative outcomes, may contribute to untoward patterns of brain function seen as EEG changes. The full consequence of brain function disruption upon neurodevelopment and sleep state progression early in life is not known.

Purpose.

The purpose of this study was to evaluate changes in brain wave signal variability with quantification of signal cyclicity before and after standard morphine administration.

Specifically, we examined differences in spectral density distribution after extracting raw EEG signal from limited channel EEG recordings in the NICU.

Methods**Design.**

The study made use of a naturalistic experiment to describe effects of morphine given as part of standard NICU care on power spectral densities of brain function. We proposed an ABAB within-subject design to compare data from time blocks before and after opioid administration. This strategy helped to address the tremendous inter-individual variation that was expected between infants and the strong effect of maturation on brain function patterns during the critical period prior to term. To build strength into the small n study, we intended to measure the before and after signal for two consecutive morphine doses. This also afforded the opportunity for extensive data collection with application of a single set of electrodes to minimize handling of the infant. Timing of the blocks was flexibly designed to reflect the known variation in dose timing that would be encountered in the NICU based upon patient indications and nursing judgment for intermittent administration of the opioid.

Setting and sample.

The setting for this study included two Level III NICUs in the Pacific Northwest. Each provides care for premature infants who may undergo opioid administration as part of standard comfort care or sedation to promote physiologic stability during mechanical ventilation, although the second site also includes a population of infants requiring general surgery.

The sample comprised infants at 24-42 weeks postmenstrual age (PMA), expected to receive morphine by bolus for mechanical ventilation or painful procedure, with English speaking parents. Infants were excluded while on high frequency ventilation and if they had known neurologic alterations (grade III or greater intraventricular hemorrhage, periventricular leukomalacia, congenital malformations involving neural structures, previously diagnosed seizures).

We received approval for this study from the institutional review boards of the respective clinical research sites. After screening and medical team approval, parents were approached and gave their informed consent for participation of their infant. Tracking of enrollment revealed the following: 60 infants were screened for inclusion, 37 met criteria for inclusion, 10 parents declined consent while 19 parents gave consent for infant participation. Thirteen infants were not studied for various reasons: transferred to another unit or hospital, required surgery, did not receive morphine, respiratory support issues, parent indecision, a participant expired after enrollment and equipment failure occurred during one recording. Data reported in this study were collected between July 2010, and April, 2012.

Instruments and measures.

Limited channel EEG.

This study employed the Olympic CFM 6000 (Natus Medical, San Carlos, CA) to collect background brain function data as aEEG signal. Concurrent acquisition and recording of raw EEG data in the CFM 6000 enhance the potential to identify artifact for optimal interpretation. Additionally, the device maintains a continuous impedance meter that allows the operator to assess signal fidelity in real time. Data were sampled at 100 times/second.

Demographics.

Demographic information for this study was obtained from the medical record of the infant and included: age, weight, gender, primary diagnosis, exposure to ante or postnatal steroids, thermal support, respiratory support, inotropic support, and other environmental factors that may contribute to artifact. In addition, the infants were scored for acuity at birth by the SNAPPE-II scale a robust, simply administered scale of neonatal severity that has retained reliability over time without deterioration despite changes in practice (Zupancic, et al., 2007).

Procedures.

A single recording of aEEG was obtained from each infant after parental consent. Study procedures were conducted in the NICU, within the incubator or radiant warmer without disrupting normal care activities by the nurses, providers, or families. We made standard measurements for placement of three non-invasive hydrogel sensors in the international 10-20 locations modified for neonates. One electrode served as the ground on the forehead, the other two were placed in P3-P4. The parietal location is recommended for limited channel aEEG, to avoid muscle artifact from the face and forehead and to obtain a broad signal of background EEG

(Hellstrom-Westas, et al., 2008; McKeever, et al., 2012). We used our previously reported protocol for skin preparation and hydrogel sensor placement (Foreman, et al., 2011). While recording the aEEG signal, infant behaviors, nursing care activities including morphine administration times, and sleep or wake states were digitally annotated when evident. Most recordings were conducted between feedings or immediately after handling for standard NICU caregiving, and ranged from approximately 3-12 hours, depending upon infant activity and need for sedation or pain management.

Data analysis.

Signal Processing.

Raw EEG signal and impedance data were downloaded to disk from the CFM device then evaluated visually and processed according to the following custom signal processing approach. As reported in our previous paper, the data were filtered and smoothed, then assessed for presence of artifact. We initially blocked the data into short, 6 second epochs to reduce loss due to impedance, and to minimize effect of non-stationarity. The principle of stationarity refers to the similarity of data structure within a segment, regardless of at what point in the segment one begins the analysis (Bakeman & Gottman, 1997). EEG signal is known to be non-stationary, and the extremely labile signal that is immature EEG creates challenges for Fast Fourier transformation (FFT). All data were evaluated for the presence of impedance above 19.9 kOhms, due to major motion artifact or poor electrode function or negative impedance deflections below 0.1 kOhms, occurring when the amplifier was saturated by very high amplitude bursts above the manufacturer's threshold. Epochs were removed if found to include impedance values outside this range.

Spectral Analysis.

EEG is typically interpreted by expert qualitative review that encompasses multiple channels and montages of signal from many scalp electrodes. Symmetry, synchrony, wave shape, morphology, topography of where brain waves are generated and cyclic frequencies contribute to the multifaceted interpretation. Detailed information about synchronous patterns and wave morphology are not available through limited channel EEG, although symmetry across hemispheres is accessible if dual channel EEG is used. Single channel raw EEG does provide the information needed to conduct frequency analysis. The evaluation of brain wave frequency and strength of particular frequencies is achieved by breaking the signal spectrum of waves into bands by frequency measured in Hz, and analyzing the signal power within each band or part of the full spectrum. In order to compare data across bands or within the spectrum, signal power was computed by determining the contribution to variance by cycles in the data as compared to noise if there were no true cycles (Bakeman & Gottman, 1997). EEG power comes from the square of signal amplitude, reported as μV^2 , and equates to EEG intensity. Power across the full spectrum can also be summarized by use of a calculated variable called spectral edge frequency (SEF). The edge frequency refers to a point (frequency) at which the total power in the spectrum is contained below, and then is broken out as a percentage. For example, SEF50 represents the frequency that delimits 50% of the total power below it, similar to a median. SEF90 is a useful measure of the 90th percentile of total power below that particular frequency. We report on SEF50 and SEF90 across the spectrum of 0.5-35 Hz as filtered from the raw EEG data obtained by the CFM device.

Our data were transformed by FFT and separated analytically into standard traditional bands for delta (0.5-4 Hz), theta (4-7 Hz), alpha (7-13 Hz), beta (>13 Hz) for each subject.

Absolute and normalized values were calculated for each of the spectral entities. Normalization takes the ratio of power under a given band to the total power, providing a reference with regard to the rest of the spectrum, whereas absolute power is an estimate of the power under each band regardless of the rest of the spectral structure. Additionally, the data were log transformed to improve distribution. We then further broke the spectrum into a series of ten 2Hz “mini-bands” to give precision to our exploratory analysis of spectral shifts after morphine administration.

Morphine Times.

We created time blocks for segments of data relative to time of morphine administration. Median values of data within block by participant were calculated and compared by visual review of plotted differences. Logistics of electrode application during the early part of the recording and clinical timing of administration guided our decisions to structure the time blocks relative to morphine. This primarily affected the pre-morphine block which we set to 15 minutes before the dose, named Time 1. Time 2 was 0-30 minutes after the dose, Time 3 was 30-60 minutes after, Time 4 was 60-120 minutes after and the remainder of the recording was blocked to Time 5 (not analyzed for this report).

As in our previous paper, we conducted visual pattern assessments of each variable, for this report the variables included log transformed spectral power divided into 2 Hz frequency mini-bands between 0.5 and 35 Hz, along with SEF50 and SEF90 and normalized and absolute power for each traditional band. Then a series of nonparametric tests were performed on aggregated median within-subject values blocked by morphine time points to evaluate the morphine effect.

This small n study was designed for exploration and description primarily within subject, devoid of intent to generalize our findings beyond the development of future hypotheses.

Without prior data, we did not have a good estimation of effect size for changes before or after morphine dosing, and thus did not approach the study with an expectation of power. For this discussion, we have set a liberal alpha level at $p < .05$, primarily as a gauge for which tests and combination of variables might be useful for future exploration.

Additional Analyses.

The effect of morphine administration within individuals was explored by plotting the participant's grand median of EEG signal voltage in various spectral bands across all morphine time blocks. The percent above each individual's median value gives a comprehensive view of changes in the data over time without loss to group or within-subject aggregation.

Because the presence of large age effect was expected due to the known developmental trajectory and the wide spread of gestational ages in the sample, a preliminary age grouping was formed to compare young premature infants to those greater than or equal to 30 weeks PMA. We chose this cut-off to reflect the dynamic changes in continuity of brain function occurring at approximately that time in development.

In order to uncover possible effects of morphine on our spectral variables, we considered a repeated measures ANOVA for spectral edge frequencies, mini-bands, and standard bands both normalized and absolute values. In light of our small study numbers, standard corrections for violated assumptions of sphericity and homogeneity were not sensible. Thus we chose to use the multivariate test, accepting a less powerful statistical test with a conservative assumption for significance. Although the sample is small, the analysis was extended to within subject median aggregated values for each of the morphine time points and with a test of age effect and an interaction between age and time (morphine effect), using a cutoff of 30 weeks PMA to assess

age effect between subjects. We chose 30 weeks PMA to align with the developmental progression of beginning to generate continuity of signal.

Statistics.

Signal processing was conducted in MatLab R2011a (The MathWorks, Inc. Natick, MA) for custom filtering, smoothing, and power spectral density distributions. Data elements were then exported to SPSS 15 (IBM, Chicago, IL) for statistical testing and graphic exploration.

Morphine time blocks were used to explore differences in the frequency domain. Tests of difference across morphine time points were conducted by non-parametric Friedman rank order test. Repeated measure ANOVA were conducted to test for interactions with respect to age.

Results

Sample characteristics.

Nine infants were recruited for this study. One male infant was studied at 34.57 weeks PMA however his data were not included due to high impedance over the majority of his recording. He was a long term NICU patient with chronic lung disease following many weeks on high frequency oscillatory ventilation as treatment for respiratory failure and postnatal steroids to optimize successful weaning from the ventilator with extubation after 70 days. Skin maturation may have contributed to difficulties achieving good contact and an appropriate skin electrode interface.

The resulting eight (two male) infants age between 25.71 and 39.71 (mean 29.89 SD 4.89) weeks PMA, were studied in either of two NICUs. Birthweights ranged from 0.54 to 3.49 kg (mean 1.27 SD 1.03), with average weight at time of study 1.32 kg (SD 1.02). Mean number of days on the ventilator was 8.65 days (SD 4.93, range 1-19). The SNAPPE-II standardized measure of illness acuity at birth was calculated for each infant; scores ranged from 0-57 (mean

30.30, SD 23.88). The majority of infants (73%) received two doses of antenatal steroids, and none of the infants in the analysis received postnatal steroids. Parents were invited to participate in care during the study, of those who chose to be present, most were there less than 1/3 of the study recording time.

Data reduction due to artifact (impedance).

Mean impedance across the entire data set was 9.97 kOhms (SD 5.67), down from 13.18 kOhms (SD 7.48) before processing. Epochs were removed if impedance was outside the stated range (below 0.1, or above 19.9 kOhms), resulting in recordings of usable data between 1.22 and 12.24 hours in length. Total epochs removed equaled 11% of the full dataset, for a loss of 5.61 hours of recorded signal across all participants. Two of the 8 recordings carried the greatest loss; the remainder averaged 3% reduction per recording. There was a minimal reduction in data mean values after processing to remove blocks of data with either high or low impedance with overall standard deviation of EEG voltage recorded as 17.34 μ V (SD 17.82) before processing and 15.07 μ V (SD 12.91) afterward. This relates to reduction of high amplitude, often occurring due to motion artifact which saturates the amplifier and gives a low or negative impedance value and high impedance, due to poor electrode conductance quality.

Morphine characteristics.

Dosing.

Morphine doses across all neonates were low and ranged from 0.03-0.18 mg during the study recordings (mean 0.05 mg/kg, SD 0.05), this equates to 0.04 mg/kg mean (SD 0.03). Total lifetime opioid dosing was also calculated for each participant to include previous intermittent and in some cases continuous infusions (prior to the study period), and ranged from 0.1-3.19 mg/kg since birth (mean 0.71, SD 0.86). This study was not designed to demonstrate dose effect.

Morphine effects across time.

Friedman tests were conducted between morphine time points, starting with SEF 50 which identified a significant difference in rank order among the four time points: 15 minutes prior, 30 minutes after, 60 minutes after and 120 minutes after morphine administration ($n=8$, $\chi^2 = 8.923$, $df = 3$, $p < 0.030$), the total power of the spectrum was log transformed to improve distribution, and tested, there was no significant difference found among the four morphine time points by rank order Friedman test. Finally, we tested our “mini-bands”, all were log transformed to reduce heterogeneity. Several of the mini-bands in the spectral ranges above delta showed significant differences with regard to morphine administration times, *see Table 4.1. Differences in Median Aggregated Power Spectral Bands by Morphine Time Point.*

Friedman tests were also done to explore significant changes between two time points: time point 1 (15 minutes prior to morphine administration) and time point 3 (60 minutes after morphine administration). We collapsed the higher frequency bands into a single variable called “Non-Delta” representing all spectral power bands above 4 Hz. The two point Friedman did not demonstrate significance between morphine time 1 to morphine time 3 for “Non-Delta” ($n=9$, $\chi^2=2.778$, $df=1$, $p < .096$), however a specific component of higher frequency, the log transformed mini-band of 14-16 Hz did show a significant difference in mean rank ($n=9$, $\chi^2=5.444$, $df=1$, $p < .020$) between time 1 and time 3.

Individual participants.

A within-subject grand median for high and low spectral power (absolute delta and absolute beta power) across the whole recording for each participant was plotted. We then evaluated the percent of each high and low frequency data points above the median within each time block. This simple statistic (% above the individual subjects’ median value of each spectral band)

provided a standard way to explore possible shifts within the spectrum across the morphine time points, while retaining variability. *See Figure 4.1. and Figure 4.2. by individual participant.*

More mature infants had higher overall power in both median absolute delta power and higher median absolute beta power than younger infants, and a proportionally lower ratio of delta power compared to beta power. Each showed variation in the changes across time. The first example is a term gestation infant (39.71 weeks PMA) whose median absolute delta power was 2268.4 μV^2 , and median absolute beta power was 342.2 μV^2 . Whereas, the second example is a premature gestation infant (26.67 weeks PMA) whose median absolute delta power was 1302.05 μV^2 , and median absolute beta power was 57.83 μV^2 .

Effect of age.

A repeated measures ANOVA was conducted across all four morphine time points, SEF50, SEF90 and one of the mini-bands showed significantly different values across the morphine time points *See Table 4. 2. Effect of Morphine and Age on Variability of EEG Spectral Density.* Of the mini-bands, only low delta (0-2 Hz) showed a significant difference, while the mini-band of 8-10 Hz showed a trend toward significant differences across time points for morphine effect. We then looked closely at only time points 1 (15 minutes prior to morphine administration) and 3 (60 minutes after morphine administration) again by repeated measures ANOVA to explore within-subject effects of morphine versus between-subject effects of grouping by age <30 weeks PMA. Morphine effects were seen to trend toward significance for mini-bands on the low and mid parts of the spectrum (0-2Hz and 8-10Hz), and age group effect was significant for full bands of Alpha and Beta frequencies (normalized across the spectrum), and SEF90, as well as the two mini-bands described above. *See Table 4.3. Effect of Morphine at Two Time Points and Age on Variability of EEG Spectral Density.*

Discussion

This paper offers an expansion of the current knowledge of effect of morphine or other opioids on spectral features of brain function in premature infants. Previous authors have reported opioid effects on premature infant sleep structure, EEG voltage and wave patterns in the time domain (Axelin, et al., 2010; Nguyen The Tich, et al., 2003; Young & da Silva, 2000). We believe that frequency information provides deeper insight into possible disruptions of sleep states and developing sleep architecture.

The intent with this study was to collect data around two morphine doses per infant, to verify direction or effect size within-subject if differences were found after opioid administration. Unanticipated practice changes in the NICU reduced the number of infants receiving morphine, and thus our data reflect a small sample of which only three infants received two doses of morphine. One infant did not receive morphine during the recording, but did receive it two hours prior, this data was included in the analyses that did not include morphine time points. We analyzed 10 total morphine events from 8 infants; some had limited data at either time point 2 or time point 3 due to handling, procedures, or electrode issues resulting in high impedance.

As reported in the earlier paper *Spectral Analysis of Neonatal Brain Function: Effect of Opioids*, (see *Dissertation Chapter 3*) spectral edge frequencies across time points within-subjects were explored and we did not find significant differences at SEF90, although SEF50 showed some differences between time points relative to morphine administration. SEF50 is more sensitive to higher frequency changes, which were not evident with SEF90 which was likely dominated by the heavy low frequency presence of immature brain function. The development of the partial spectral variable, called “non-delta”, which included a log

transformed compilation of all frequencies above 4Hz helped to explore this line of thinking. If there were shifts among the frequencies from high to low frequency, we thought they might be more evident when pooled together. This led us to create the mini-bands and look for cyclic differences within smaller sections of the spectrum, and ultimately to other analytical tactics. In this analysis we found that the delta (2-4 Hz) and low delta (0-2 Hz) dominated over higher frequencies, especially for younger infants, where older infants had more room to shift among frequencies under the spectrum.

The Friedman test uses a simple ordinal ranking within subject, such that the mean and standard deviation of the data are completely discarded in favor of a ranking order, dismissing all but the minimum variability within the data. It is a test suitable for small numbers. We initially tested for morphine effect across four time points, but through visual analysis of the plots, we ascertained what seemed a stronger difference at time three (60 minutes after morphine administration). Also, it was impossible to tell the order or direction of differences identified by the Friedman test. In the case of our non-delta compiled variable, the data demonstrated a strong trend toward a difference between Time 1 and Time 3 and the mini-band in the beta range was significant. It is our general assumption that morphine effects may be masked by the heavy presence of power in the delta range. If the primary frequency is very low at baseline, standard spectral measures will not show further power in the low frequencies.

The significant changes seen in the higher frequency mini-bands may be carried by a few mature infants in our data set. When only summarized data were examined, the morphine effect did not emerge. Our process to explore the effect of age on spectral changes was undertaken in part because of our belief that the “floor” effect of low gestation and low frequency might not be also true for more mature infants. A recent exploratory study using aEEG and spectral densities

to assess depth of anesthesia in children and infants described the SEF90 measure as inconsistent possibly due to a mixed group of infants and older children (McKeever, et al., 2012). Between-group analysis was not an intended aim of this study, and our small sample was in no way powered to answer questions about gestational age. However like other physiologic processes, EEG interpretation during the neonatal period must account for the developmental continuum. Future studies of larger size should be designed to sample more consistently with regard to gestational maturation.

In addition to sample size and heterogeneity, this study was limited by several factors related to the naturalistic setting, to our rudimentary process for addressing artifacts within the EEG data, and especially to adaptation of the clinical tool aEEG for research uses. As described above, morphine was not administered on a regular schedule, and thus, the timing of our electrode placement and data capture was one mostly of convenience. The absence of a true baseline period without morphine or handling to place electrodes may have contributed to our mixed findings.

Furthermore, the study did not include a measure for pain intensity, and it seems intuitive that infants may have experienced increased pain prior to the morphine administration decision by the nurse. Pain and arousal as evidenced by elevated aEEG amplitude or total spectral power may have occurred at time 1 for some infants. Several infants received morphine during the study to reduce procedural pain, for example, central line placement. The procedural handling occurred during the recordings and likely contributed to variations in EEG signal, both as artifact, and possibly as arousal or sleep interruption.

The reduction of artifact in EEG research is usually managed by careful selection of short data segments or aggressive removal of affected data points. As an exploratory study of

morphine effects over time, we are very interested in the use of the full recording, rather than segmenting the data. We recognize the disadvantages of not taking a stronger approach to artifact removal, and might plan for it in a future study design.

The use of aEEG, originally designed for adult brain function patterns in the infant and particularly, premature infant population brings a host of translational issues. The algorithm is attenuated for adult ranged voltage, the internal filtering of CFM signal eliminates most of the low delta range of frequencies, and the single channel summarily limits the ability to assess synchrony, symmetry, or morphology of brain waves. The drawbacks however, do not outweigh the benefit of gaining access to continuous EEG signal with minimal physiologic cost to the infant.

In conclusion, the exploration of spectral densities offers a framework for improving our understanding of how brain function frequencies shift within the spectrum after morphine. Spectral details, such as the structure of power in the higher frequencies also provide an avenue to tease apart the complexities of mixed frequencies that are occurring during maturation in the weeks before and after term gestation. The link between brain function frequency, developing patterns and sleep processes is a critical area for further work. This paper has introduced some basic analytic approaches to processing and exploring EEG signal as derived non-invasively from limited channel EEG in the setting of the NICU. Future techniques might include alternative transforms to the FFT, which is less than ideal for chaotic EEG signal. The ability to explore both time and frequency domains through phase-space transformations also generate intrigue in the context of complex mixed frequency signals coupled with the effect of intervention or medication.

Table 4.1.

Differences in Power Spectral Mini-Bands Across Morphine Time Points

Spectral Power "Mini" Band (Log transformed)				
	n	χ^2	df	Significance*
10-12 Hz	8	9.450	3	.024*
12-14 Hz	8	14.550	3	.002*
14-16 Hz	8	13.500	3	.004*
16-18 Hz	8	7.050	3	.070
> 18 Hz	8	13.050	3	.005*

Note. Friedman Rank Order Test across four time points:

T1=15 min prior, T2=30 minutes after, T3=60 minutes after, T4=120 minutes after

n refers to number of total morphine events with available data at each of four time points:
(3 infants had more than 1 morphine dose and some recordings had no data at a given time point due to artifact)

Calculated from median aggregate within-subject by time point across the sample

* $p < 0.05$

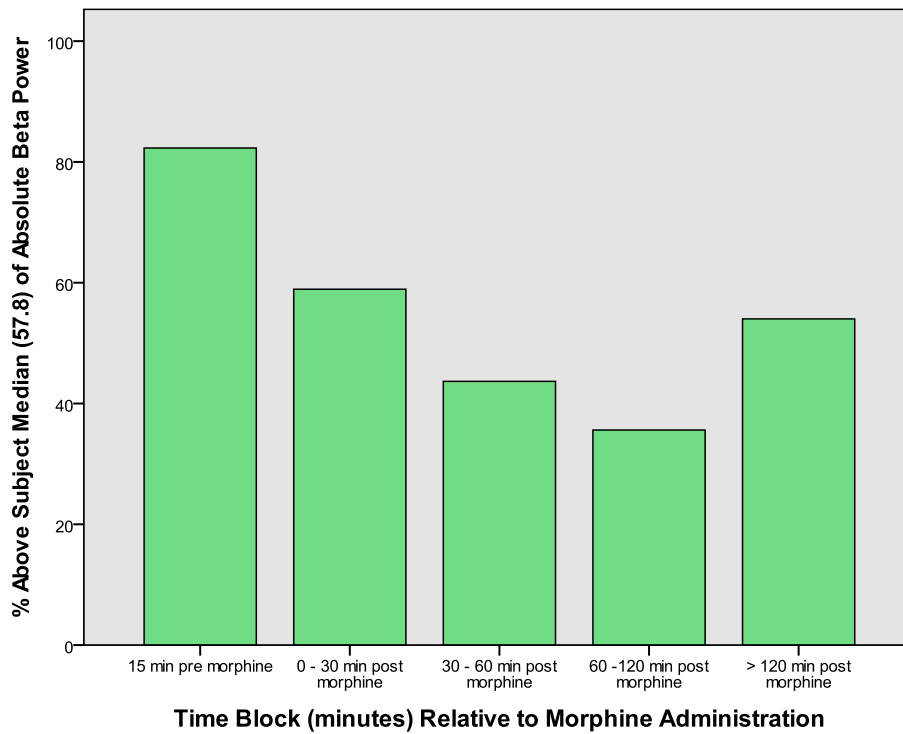
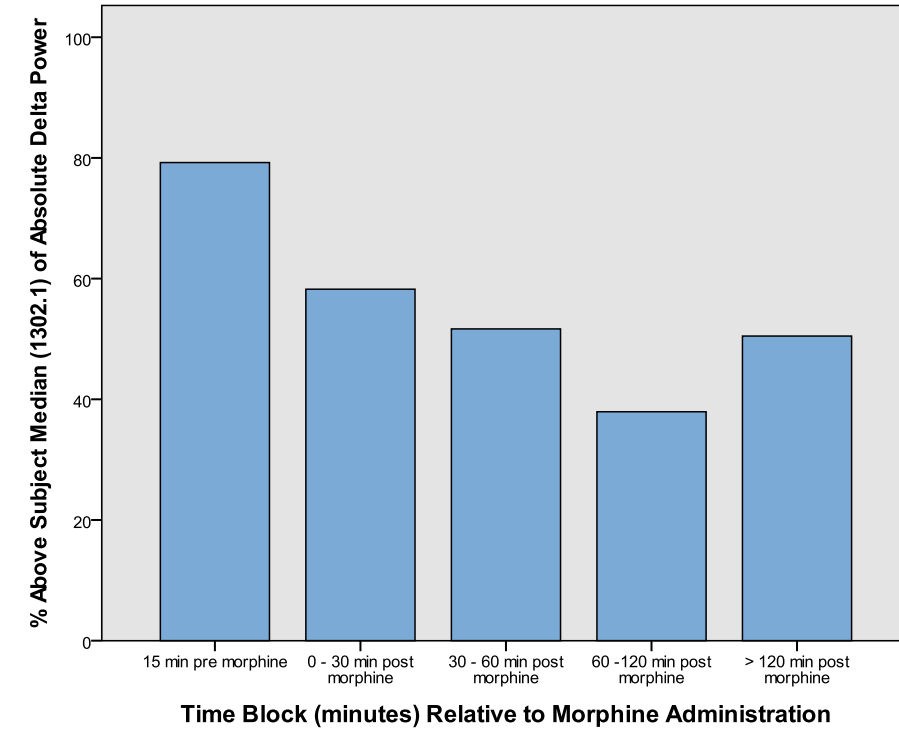


Figure 4.1. Spectral Power Shifts by Morphine Time for a Premature Infant (PMA26.67 weeks) n=1. Plots show similar patterns of power shift after morphine.

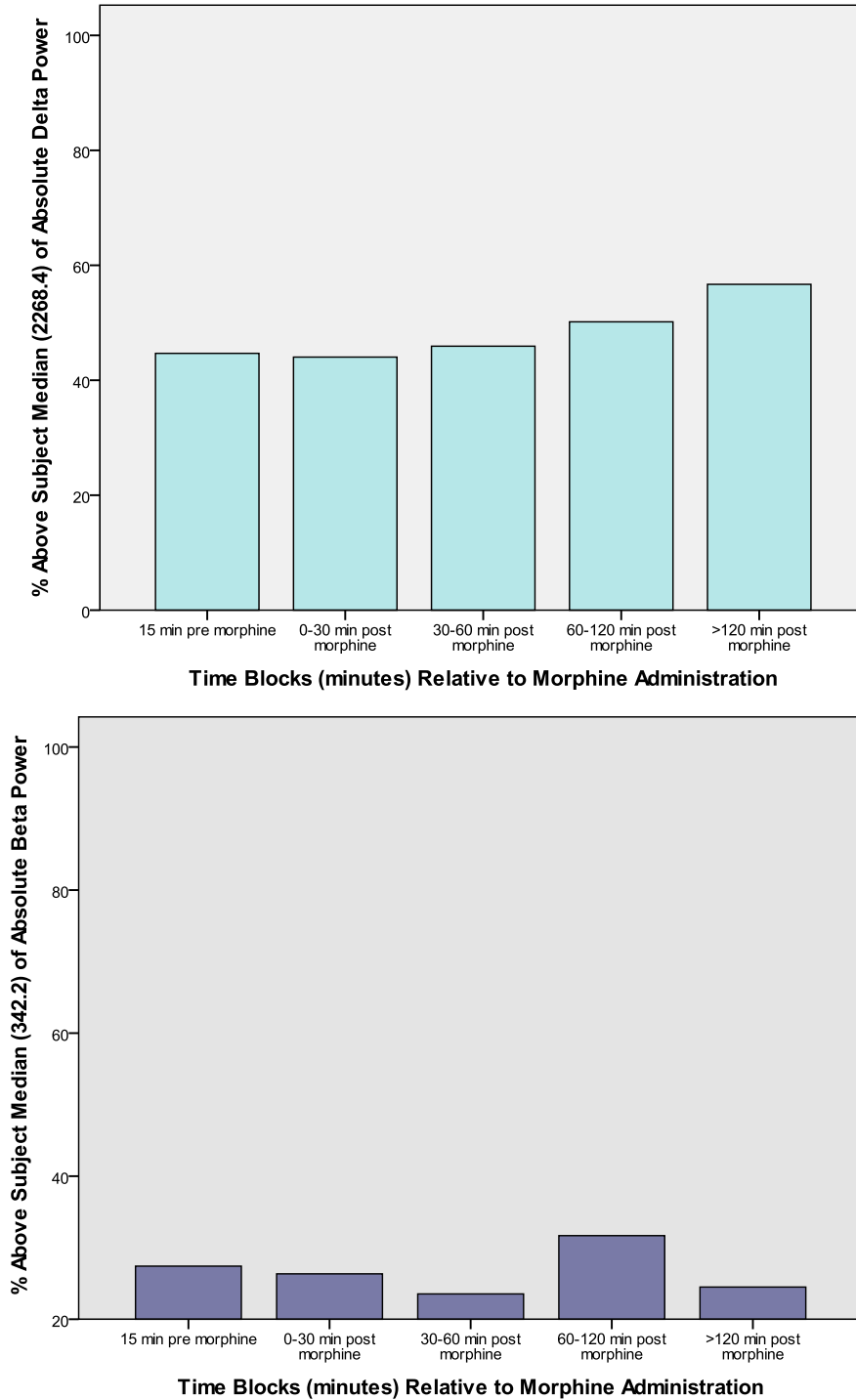


Figure 4.2. Spectral Power Shifts by Morphine Time for a Term Infant (PMA 39.71 weeks) n=1. Plots show large difference between delta and beta power above median.

Table 4.2.

Effect of Morphine and Age on Variability of EEG Spectral Density

Repeated Measure ANOVA for 4 Morphine Time Points

n=8 (5<30 weeks PMA/3>30 weeks PMA)

Variable	Morphine Effect		Age Group Effect		Interaction Effect	
	F	p-value	F	p-value	F	p-value
SEF50	21.625	.006*	72.345	<.0001*	5.115	.074
SEF90	6.045	.057	48.157	<.0001*	11.047	.021*
0-2 Hz Low delta mini-band	.879	.523	9.185	.023*	5.781	.062

Note. Multivariate repeated measures ANOVA across 4 Time Points

T1=15 min prior, T2=30 minutes after, T3=60 minutes after, T4=120 minutes after

n refers to number of total morphine events with available data at each of four time points:
(3 infants had more than 1 morphine dose and some recordings had no data at a given time point due to artifact)

Calculated from median aggregate within-subject by time point across the sample

* p< 0.05

Table 4.3.

Effect of Morphine at Two Time Points and Age on Variability of EEG Spectral Density

Repeated Measure ANOVA for 2 Morphine Time Points

n=9 (5<30 weeks PMA/4>30 weeks PMA)

Variable	Morphine Effect		Age Group Effect		Interaction Effect	
	F	p-value	F	p-value	F	p-value
SEF90	3.388	.108	42.125	<.0001*	5.256	.056
Alpha Normalized	3.694	.096	104.639	<.0001*	5.991	.044*
Beta Normalized	.586	.469	11.349	.012*	4.627	.069
0-2 Hz Low Delta mini-band	5.341	.054	14.756	.006*	9.506	.018*
8-10Hz Alpha mini-band	5.159	.057	8.215	.024*	5.790	.047*

Note. Multivariate repeated measures ANOVA across two time points:
T1=15 min prior, T3=60 minutes after

n refers to number of total morphine events with available data at each of two time points:
(3 infants had more than 1 morphine dose and some recordings had no data at a given time point due to artifact)

Age group is (<30 weeks PMA)

Calculated from median aggregate within-subject by time point across the sample

* p< 0.05

Chapter 5: Summary and Conclusions

The study of vulnerable infants brings challenge of scale and complexity, this research has not differed. The depth of information hidden within the neonatal brain is barely captured by the signal that is EEG and yet it provides a complex layering of variation by time, by frequency, by individual, and most evidently by maturation and developmental trajectory. The work presented in this dissertation is but the beginning of what can be learned through the exploration of premature infant brain function and opioid effect upon it.

In the first paper, aEEG signal was investigated to fill a gap in the literature with regard to interpretation and simple approaches for quantifying aEEG data. The primary contribution of this part of the dissertation study was to introduce and test two measures for quantifying discontinuous signal. A straightforward process to reliably count amplitude peaks and capture the low voltage component within the signal may be of use to future researchers with interest in the early gestation infant. Determining the point at which continuity emerges within the primarily discontinuous aEEG signal, representing increasing cortical function and the initiation of cycling has strong implications for the measurement of interventions to nursing care, for example comfort measures, positioning and support, parental involvement including skin to skin holding, as well as further study of opioids or pharmacologic agents that may interfere with normal sleep development. In addition, the findings from this section validated the strength of the bandwidth of aEEG signal amplitude in relation to maturation. Calculation of the bandwidth through analysis and signal processing provides precision to the interpretation, and precludes the difficulties of interpreting the piece-wise logarithmic scale available through visual analysis alone.

The dissertation does not report on the morphine effect with respect to the time domain variables. The use of a combination of the peak count and proportion less than $10\mu\text{V}$ may provide an analytic tool useful in identifying the emergence of continuity from the primarily discontinuous immature signal or in reverse, the loss of cycling in a formerly healthy pattern. Early exploration of factor analysis to use these and other simple measures in combination showed promise and would be worth further exploration. Just as the developmental effects are not unilateral, or linear in progression, the opioid effects are most likely multidimensional. Multi-level analysis although beyond the scope of this dissertation would be a logical next step.

Examining the frequency domain to assess for differences in spectral density with respect to morphine administration brought challenges requiring intentional decisions related to signal processing and data management. This paper generated more questions than answers. The use of summary measures power scale slope and spectral edge frequency offered an initial approach to meeting the aim of quantifying signal cyclicity. However, unlike reports in the literature of profound changes in EEG voltage and increased discontinuity, these configurations did not detect obvious morphine effects. The curious finding that SEF50 showed morphine effect when SEF90 did not, led us to the further exploration in the final paper. EEG signal presents challenges for the use of the FFT due to the inherent non-stationary nature of EEG data. Blocking the data into short segments (6 seconds) was a strategy used to cope with the erratic primarily discontinuous signal of early gestation infants. Future researchers or secondary analysis of this data set might consider alternative transforms.

Analysis for the final paper justly cemented the observation that age of the infant with its strong influence on spectral features was likely masking the effect of morphine on brain function. The spectrum of early gestation infants is strongly skewed toward the low frequencies

with a heavy amount of delta power and limited higher frequencies before 30 weeks. The observation of changes in power of some of the mini-bands over time, provide evidence of morphine effect especially in the higher frequencies such as alpha and beta. The overlay of maturation and the highly immature sample may have created a “floor” phenomenon within the spectrum whereby the power shifts had no room to move further downward after morphine administration. While the small sample precluded generalizing from the data, the decision to test for age and age by morphine time interaction served to uncover the shifts of higher frequency in the older infants. Searching beneath the cover of spectral structure and within the context of expected maturation driven power segmentation proved valuable in this paper. Investigating individual variation and intensive exploration of multiple frequencies and the shape of the spectral information are natural extensions of this work. Further analysis through the use of discrete wavelet transforms or time-frequency approaches or entropy based approaches could be applied to the existing data for deeper understanding of the emerging rhythms and possible disruptions due to opioids.

Many questions remain after this initial expedition into EEG interpretation and developing brain function. A few are outlined below; all represent avenues for further study:

- An inquisitive theme is raised by the visual review of individual signal plots across morphine time points, in that some of the infants had elevated delta power and total power at the Time 1 (prior to morphine administration). Is this a reflection of arousal due to handling, e.g. electrode application, pain, or other? What is the appropriate baseline for comparison of morphine effects, perhaps it should be hours after administration rather than shortly before.

- The middle range frequencies between beta and delta were not evaluated by this work, and the subtle shifts in spectral power may provide insight into sleep processes in the very young.
- The mechanisms of morphine and other opioids with respect to development are key factors in better understanding the potential for function disruption. If the high frequency signal represents cortical function, and is then disassociated by morphine, leaving a discontinuous signal, is it transient, are neurons quieted, disinhibited, or simply disconnected and for how long? This work highlighted the complexities of investigating immediate responses of brain function to opioids, in addition, studies of long term effects and sleep processes are critically needed.
- Would agents, other than morphine generate similar spectral shifts or voltage reduction (by aEEG signal analysis)? If development of the GABAergic system of inhibition in the brain contributes to brain function patterns (prolonged discontinuity), what effects might be seen on brain function after benzodiazepine infusion?
- If a fully homogeneous sample of 28 week infants were readily available for study, would morphine effects be more evident?

In conclusion, this dissertation brings to light the importance of understanding and promoting optimal brain function patterns in development. The valuable exploration of underlying rhythms as the foundation for sleep patterns coupled with growing concerns about effectiveness and safety of opioids in the NICU afford us the incentive to undertake the complexities of collecting, interpreting and translating neurophysiologic data in perhaps the most vulnerable human population.

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CURRICULUM VITAE

LAUREN THORNGATE PhD, RN, CCRN

Former name: Lauren Thorngate Taquino

EDUCATION:

- 2006-2012 Doctor of Philosophy (PhD) Nursing Science
University of Washington, School of Nursing, 2012
- 1987-1989 Master of Science, (MS) Community Health/Nursing Administration
University of California, San Francisco, School of Nursing, 1989
- 1980-1984 Bachelor of Science, Nursing (BSN)
University of San Francisco, 1984

FACULTY APPOINTMENT:

Clinical Faculty, University of Washington, School of Nursing, Department of Parent-Child Nursing. May, 1992 – September, 2006.

PROFESSIONAL EXPERIENCE:

- Current Position: Staff Nurse, Per Diem
12/11-ongoing Intermediate Care Nursery
Tacoma General Hospital
- Recent Position: Clinical Nurse Specialist (part time)
8/05-9/11 Neonatal Intensive Care Unit
University of Washington Medical Center

2/04-7/04

Interim Nursing Director, Infant Intensive Care Unit
Children's Hospital & Regional Medical Center
Seattle, WA

2/94 – 4/05

Neonatal Clinical Nurse Specialist
Children's Hospital & Regional Medical Center
Seattle, WA

11/90 - 2/94

Neonatal Clinical Nurse Specialist
Tacoma General Hospital
Tacoma, WA

3/89 - 10/90

Neonatal Outreach Coordinator
Perinatal Outreach Program
Children's Hospital and Medical Center
Oakland, CA

11/84 - 3/89

Staff Nurse II and III
Intensive Care Nursery
Children's Hospital and Medical Center
Oakland, CA

6/84 - 11/84

Charge Nurse
Long Term Care Facility
St. Francis Pavilion
Daly City, CA

HONORS/AWARDS

Dissertation Award, University of Washington School of Nursing, 2012
Magnuson Scholar, 2009-2010
March of Dimes Scholarship, 2008
American Association of Critical Care Nurses Scholarship, 2006-2007

LICENSURE AND CERTIFICATION:

Registered Nurse, State of Washington
Registered Nurse, State of California, (1984-2000)
Regional Instructor, Neonatal Resuscitation Program (AAP/AHA)
CCRN, Neonatal Exam, August 1992

RESEARCH EXPERIENCE:

Research Assistant Feasibility of Telehealth Technology for Pain Assessment in Adult Family Homes. University of Washington School of Nursing. (PI: Anne P. Poppe PhD, MN, RN; Co-investigator: George Demiris PhD). March, 2012-present.

Principal Investigator (with Karen Thomas PhD, RN mentor) Opioid Effects on Premature Infant Brain Function Measured by Limited Channel EEG. University of Washington Medical Center and School of Nursing. Project dates 9/09-present. Funded by 1F31NR011365-01 National Institute of Nursing Research (NINR), NIH.

Research Associate (with Shuyuann Wang Foreman PhD, RN & Karen Thomas PhD, RN)
Preterm Infant Brain Study. University of Washington Medical Center and School of Nursing.
 Project dates 3/09-2/11.

Principal Investigator Prevalence Study of Opioid Administration Practices in one NICU.
 University of Washington Medical Center. Project dates 1/09-3/09.

Principal Investigator (PNANN Chapter Research Project) The Effects of Thermocouple Sensor Placement on Neonatal Skin Temperature Measurement. Children's Hospital Medical Center and Madigan Army Medical Center. Project dates 7/96 –6/00.

Co-investigator (with Lori Milkovich, RN, BSN) (Clinical research/product evaluation) Effects of the 'Wee Thumbie' Pacifier on Self Calming and Oral Feeding Skills. Children's Hospital Medical Center. Project dates 3/96-9/97.

Principal Investigator (Quality Improvement project) One Touch II Blood Glucose Monitoring System: Accuracy and Clinical Applications in the NICU. Tacoma General Hospital. Project dates 12/93-2/94.

Co-investigator (with Susan Blackburn PhD, RN,C, FAAN) Infant's Responses to Stressful Nursing Care Activities. Tacoma General Hospital and University of Washington School of Nursing. Project dates 6/92 -12/93.

FELLOWSHIP:

Pre-doctoral trainee, National Research Service Award (NRSA), 1F31NR011365-01, NIH/NINR (9/2009-8/2011)

Pre-doctoral trainee, Biobehavioral Nursing Research Training Program, T32NR007106, NIH/NINR (2006-2007, reappointed 2008-2009)

RESEARCH GRANTS:

Sigma Theta Tau, Psi Chapter, (\$1500), PI, September, 2009

American Nurses Foundation, Hyundai Motors Scholar, (\$7500), PI, October, 2009

Integra/Neuroscience Nurses Foundation, (\$9988), PI, December, 2009

TEACHING RESPONSIBILITIES

Lectures: Neonatal Pain Assessment and Management, Neonatal Skin and Wound Care, Clinical Nurse Specialist/Advanced Practice Nurse Roles: Master's and Doctorate nursing and midwifery students (2003-2011)

Mentoring: Honors undergraduate nursing students in research design and implementation (2009-2011)

Clinical Precepting: Neonatal Clinical Nurse Specialist students, Neonatal Nurse Practitioner students; 30-90 hours/quarter (2001-2011)

Program and Curriculum Development: Interprofessional clinical simulation curriculum designed and implemented for Neonatal Resuscitation Program (NRP) certification: nurses, respiratory therapists, physicians in training; monthly programs including high and low fidelity simulation, team communication, high risk/low volume skill development, program evaluation (2010-2011)

PROFESSIONAL ORGANIZATIONS/ACTIVITIES:

Western Institute of Nursing (2009-present)

American Nurses Association (2009-present)

American Pain Society (2009-present)

American Association of Critical Care Nurses (2006- present)

Pacific Northwest Association of Neonatal Nurses

President Elect 5/91-12/92

President 1/93-12/93

Past President 1/94-12/94

Chair, Program Committee 5/91-12/92

Chair, Conference Planning Committee 8/91-3/93

Chair, Community Service Committee 3/95- 6/99, 1/06-present

Member, Research Committee 6/92-present

Editorial Board, PNANN Newsletter 1/00-12/05

National Association of Neonatal Nurses (1990-present)

Peer Reviewer, *Advances in Neonatal Care, Official Journal of the National Association of Neonatal Nurses (Elsevier)*

Special Interest Group Facilitator: Home Health (2001-2002)

Sigma Theta Tau, Alpha Eta (1988-1997)

Sigma Theta Tau, Psi Chapter at Large (1997-present)

American Public Health Association (former member)

PUBLICATIONS: * Former name Lauren T. Taquino

Peer Reviewed Manuscripts

1. Thorngate, L., & Foreman, S. W. (2012). Practical issues of physiologic measurement for clinical research with children Part I: Measurement accuracy and precision. Journal for Specialists in Pediatric Nursing, 17, 70-75. DOI: 10.1111/j.1744-6155.2011.00298.x

2. Thorngate, L., & Foreman, S. W. (2011). Practical issues of physiologic measurement for clinical research with children Part II: Establishing feasibility. Journal for Specialists in Pediatric Nursing, 16, 320-324. DOI: 10.1111/j.1744-6155.2011.00306.x.
3. Foreman, S.W., & **Thorngate, L.** (2011). Amplitude-integrated electroencephalography: A new approach to enhancing neurologic nursing care in the Neonatal Intensive Care Unit. Newborn & Infant Nursing Reviews, 11, 135-140. DOI:10.1053/j.nainr.2011.07.005.
4. Foreman, S.W., **Thorngate, L.**, Burr, R.L. Thomas, K.A., (2011). Electrode challenges in amplitude-integrated EEG: Research application of a novel noninvasive measure of brain function in preterm infants. Biological Research for Nursing, 13(3), 251-9. DOI: 10.1177/1099800411403468.
5. Mokhnach, L., Diercks, K., Anderson, M., Berkan, M., Glorioso, R., Hou, S., Loeffler, K., Millar, A., Shinabarger, K., Thomas, K., **Thorngate, L.**, Walker, W., Yates, M., Zburin, I.(2010). NICU procedures are getting sweeter. Neonatal Network, 29(5): 271-279. *Excellence in Writing Award, 2009, by Neonatal Network*
6. Thomas, K., Magbalot, A., Anderson, M., Berkan, M., Diercks, K., Dilback, N., Hou, S., Millar, A., Mokhnach, L., Shinabarger, K., **Thorngate, L.**, Walker, W. (2010). Seasonal mapping of NICU temperature. Advances in Neonatal Care, 10 (2): 83-87.
7. Thorngate, L. & Rios, CAEV. (2008). Clinical care at the genomic interface: Current genetic issues in neonatal nursing. Newborn and Infant Nursing Reviews, 8 (1): 36-42.
8. Blackburn, S., DePaul, D., Loan, L.A., Marbut, K., **Taquino, L.T.**, Thomas, K.A., Wilson, S.K. (2001). Neonatal thermal care, part III: The effect of infant position and temperature probe placement. Neonatal Network, 20 (3):25-30.
9. Blackburn, S., DePaul, D., Loan, L.A., Marbut, K., **Taquino, L.T.**, Thomas, K.A., Wilson, S.K. (2001). Neonatal thermal care, part II: Microbial growth under temperature probe covers. Neonatal Network, 20 (3):19-23.
10. Blackburn, S., DePaul, D., Loan, L.A., Marbut, K., **Taquino, L.T.**, Thomas, K.A., Wilson, S.K. (2001). Neonatal thermal care, part I: Survey of temperature probe practices. Neonatal Network, 20 (3):15-18.
11. Brown, P. & **Taquino L.T.** (2001). Designing and delivering single room neonatal care. Journal of Perinatal Neonatal Nursing, 15 (1): 68-83. *Named in top 10 articles of the decade by JPNN (1996-2006)*
12. **Taquino, L.T.** (2000). Promoting wound healing in the neonatal setting: Process versus protocol. Journal of Perinatal Neonatal Nursing, 14 (1):104-118.

13. **Taquino, L.T.** & Lockridge, T.M. (1999). Caring for critically ill infants: Strategies to promote physiological stability and improve developmental outcomes. Critical Care Nurse, 16(6): 64-79.
14. Lockridge, T.M. & **Taquino, L.T.** (1999). Back to sleep: Is there room in that crib for both AAP recommendations and developmentally supportive care? Neonatal Network, 18(5): 29-33.

Letters to the Editor/Commentary

1. Thorngate, L & Coker, K (2008). The Universe of Developmental Care (Letter to the Editor). Advances in Neonatal Care, 8 (6): 299-300. DOI: 10.1097/01.ANC.0000342754.18568.d5
2. Thorngate, L. (2008). Letters to the editor...Sato and Senesac (2007), "Imaging nursing practice: The Roy Adaptation Model in 2050". Nursing Science Quarterly, 21 (1): 91.

Book Chapters

1. Lockridge, T., Podruchny, A. & **Thorngate, L.**, (2010). Infant sleep position protocols. In, Kenner, C. & McGrath, J.M. (Eds.) Developmental Care of Newborns & Infants: A Guide for Health Professionals (2nd Edition). Elsevier (Mosby): St. Louis. pp.263-286.
2. Thorngate, L., & Lockridge, T. (2009). Parenting in the NICU. In Newborn Intensive Care: What Every Parent Needs to Know (3rd Edition). J. Zaichkin,(Ed.); American Academy of Pediatrics, pp. 152-175
3. Lockridge, T. & **Taquino, L.T.** (2004). Infant sleep position protocols. In, Kenner, C. & McGrath, J.M.(Eds.) Developmental Care of Newborns & Infants: A Guide for Health Professionals. Elsevier (Mosby): St. Louis. pp. 183-196.
4. Thorngate, L. & Herzog, B. (1990). Standardized Patient Care Plan for the Infant with Bronchopulmonary Dysplasia. In Lund, C.H. (Ed). Bronchopulmonary Dysplasia: Strategies for total patient care. Petaluma, CA: Neonatal Network.
5. Thorngate, L. & Jereb, R. (1990). Discharge Teaching Plan for the Infant with Bronchopulmonary Dysplasia. In Lund, C.H. (Ed). Bronchopulmonary Dysplasia: Strategies for total patient care. Petaluma, CA: Neonatal Network.

ABSTRACTS PRESENTED

"Improving Measurement Precision for Premature Infant Brain Monitoring" in Innovations In Methods And Measures for Infants, Children, and Adolescents Symposium (podium presentation co-authored with Shuyuann Wang Foreman PhD, RN). *45th Annual Communicating Nursing Research Conference* (Western Institute of Nursing), Portland, OR, April, 2012

- “Explorations in aEEG Analysis: Searching for Cyclicity” (poster). *45th Annual Communicating Nursing Research Conference* (Western Institute of Nursing), Portland, OR, April, 2012
- “Refinement of a Scoring System for Amplitude-Integrated EEG in Preterm Infants” (poster co-authored with Shuyuann Wang Foreman, PhD, RN, Karen Thomas, PhD, RN). *25th Annual Meeting of Associated Professional Sleep Societies*, Minneapolis, MN, June, 2011.
- “Opioid Effect on Premature Infant Brain Function: A Preliminary Analysis” (poster co-authored with Shuyuann Wang Foreman PhD, RN, Karen Thomas PhD, RN & Robert Burr MSEE, PhD). *44th Annual Communicating Nursing Research Conference (Western Institute of Nursing)*, Las Vegas, NV, April, 2011.
- “Amplitude-Integrated EEG: A Measure of Sleep in Preterm Infants” in Symposia: Assessing Sleep-Wake Patterns Across the Life Span (podium co-authored with Shuyuann Wang Foreman, PhD, RN & Karen Thomas, PhD, RN.) *44th Annual Communicating Nursing Research Conference (Western Institute of Nursing)*, Las Vegas, NV, April, 2011.
- “Noninvasive Brain Monitoring In Preterm Infants: Amplitude-Integrated EEG” (podium co-authored with Shuyuann Wang Foreman, PhD, RN, Karen Thomas, PhD, RN & Robert Burr, MSEE, PhD). *44th Annual Communicating Nursing Research Conference (Western Institute of Nursing)*, Las Vegas, NV, April, 2011.
- “Opioid Effect on Premature Infant Brain Function Measured by Limited Channel EEG” (poster co-authored with Karen Thomas, PhD, RN). *National Graduate Student Research Festival (National Institutes of Health)*, Bethesda, MD, October, 2010.
- “Amplitude Integrated EEG: A Novel Measure of Premature Infant Brain Function”. (poster co-authored with Shuyuann Wang Foreman, PhD, RN and Karen Thomas, PhD, RN). *43rd Annual Communicating Nursing Research Conference (Western Institute of Nursing)*, Glendale, AZ, April, 2010.
- “Feasibility of Hydrogel Electrodes for Amplitude-integrated EEG (aEEG) Monitoring on Preterm Infants at Bedside”. (podium co-presented with Shuyuann Wang Foreman, PhD, RN). *5th Annual International Conference on Brain Monitoring and Neuroprotection in the Newborn (University of South Florida)*. Clearwater, FL, January, 2010.
- “Got Drugs? Assessment of Opioid Administration Practices in one NICU”. *Research Summit (National Association of Neonatal Nurses)*. Scottsdale, AZ, April, 2009.
- “NICU Procedures are Getting Sweeter: Development of a Sucrose Protocol for Neonatal Procedural Pain”. (poster co-authored with Mokhnach, L., Berkan, M., Diercks, K., Thomas,

K., Anderson, M., Dilback, N., Hou, S., Millar, A., Shinabarger, K., **Thorngate, L.**, Walker, W.) *Nursing Research Consortium*, Seattle, WA January, 2009.

“Safety Driven Practice Changes for use of Heparin in the NICU” (poster co-authored with Millar, A., Anderson, M., Berkan, M., Diercks, K., Dilback, N., Hou, S., Mokhnach, L., Shinabarger, K., Thomas, K., **Thorngate, L.**, Walker, W.) *Nursing Research Consortium*, Seattle, WA January, 2009.

“Weaning to Crib: Evidence Based Practice Change” (poster co-authored with Diercks, K., Anderson, M., Lingen, L., Millar, A., Mokhnach, L., Page Krussel, S., Shinabarger, K., Thomas, **Thorngate, L.**, Walker, W.). *Nursing Research Consortium*, Seattle, WA, January, 2008. *Poster winner of People’s Choice Award*

“NICU Nurses’ Thermal Environment: I Thought it was a Hot Flash!” (poster co-authored with the NICU Local Practice Council). *Pacific Northwest Association of Neonatal Nurses Fall Conference*, Seattle, WA, October, 2007.

“Beyond the Patient Satisfaction Survey: The Ways, Why’s and Means of Engaging Families in NICU Quality Improvement” (co-presented with Laurie Reed, NICU parent). *The 3rd International Conference for Patient and Family Centered Care*. Seattle, WA, August, 2007

“Implementing a Successful Transition to Single Room Neonatal Care” (co-presented with Phyllis Brown, RNC). *The Physical and Developmental Environment of the High-Risk Infant*. Clearwater Beach, FL, January, 2003.

“Incorporating AAP Sleep Recommendations into Developmentally Supportive Care” (co-presented with Terrie Lockridge, MS, RNC). *New Rhythms in Neonatal Nursing, 18th Annual Conference of the National Association of Neonatal Nurses*, Nashville, TN, November, 2002.

“Single Room Care from Concept to Outcome” (abstract presentation, co-authored by Phyllis Brown, RN; Sandra M. Miller, AIA; Adam Kerner, AIA) *National Association of Neonatal Nurses, 16th National Meeting: Celebration of Excellence: The total Neonatal Nursing Experience*, San Antonio, TX, September, 2000.

“The Effects of Thermocouple Sensor Placement on Neonatal Skin Temperature” (presented on behalf of PNANN Research Committee), *Bridging the World: International Neonatal Nursing Conference*, Harrogate, Yorkshire, United Kingdom, April, 1999 (international audience).

“The Effects of Thermocouple Sensor Placement on Neonatal Skin Temperature” (presented on behalf of PNANN Research Committee), *National Association of Neonatal Nurses, 14th Annual Meeting*, Cincinnati, OH, September, 1998 (poster presentation, national audience).

“Neonatal Opiate Weaning Protocol” (co-presented with Ann McKinstry RPh), *National Association of Neonatal Nurses, 14th Annual Meeting*, Cincinnati, OH, September, 1998 (poster presentation, national audience).

“One Touch II Blood Glucose Monitoring System: Accuracy and Clinical Applications in the NICU” *National Association of Neonatal Nurses, 11th Annual Meeting*”. San Antonio, TX, September, 1995 (poster presentation--national audience).

"Empowerment of a Community". *American Public Health Association National Meeting*. Boston, MA, 1988. (national audience).

Published Abstracts

1. Thorngate, L. (2009). Got drugs? Assessment of opioid administration practices in one NICU. (abstract) *Advances in Neonatal Care*, 9 (4): 185.
2. Klee, K.M.; **Taquino, L.T.** & Baroni, M.A. (2002). Lactation Support: A Collaborative Model (abstract). *Clinical Nurse Specialist: The Journal of Advanced Nursing Practice*, 16 (3): 132.
3. **Taquino, L.T.** & Blackburn, S. (1994). The effects of containment during suctioning and heelstick on physiological and behavioral responses of preterm infants (abstract). *Neonatal Network*, 13(7): 55.

INVITED PRESENTATIONS:

“Pain in Neonates”. *Nursing Update. Airlift Northwest flight nurse staff*. Seattle, WA, February, 2012.

“It’s NOT Only Skin Deep: Neonatal Skin and Wound Care” *.6th Annual Professional Outreach Education Conference, Promoting Excellence in Perinatal and Neonatal Care, sponsored by Providence Sacred Heart Medical Center & Children’s Hospital Professional Outreach Education Program*, Spokane, October, 2011.

“Amplitude Integrated EEG (aEEG): Implications for the Care of Premature Infants”. *Developmental Interventions in Neonatal Care, sponsored by Contemporary Forums*. San Francisco, CA, November, 2010.

“Prevention and Management of Neonatal Pain: At What Developmental Cost?”

Developmental Interventions in Neonatal Care, sponsored by Contemporary Forums. San Francisco, CA, November, 2010.

“Infant pain experience: Details, Definitions, and Development”. *Neonatal Nursing: Their Brain is in Your Hands (Pacific Northwest Association of Neonatal Nurses)*. Renton, WA, June, 2009.

“It’s a plastic world: DEHP and chemical awareness in the perinatal setting (co-presented with Terrie Lockridge). *Many Voices, One Mission 2008 AWHONN Convention (Association of Women’s Health, Obstetric and Neonatal Nurses)*, Los Angeles, CA, June, 2008.

“First, Do No Harm” (co-presented with Terrie Lockridge, MSN, RNC). *Neonatal Nursing Excellence, Growing and Knowing, 22nd Annual Conference of the National Association of Neonatal Nurses*, Nashville, TN, November, 2006

“Reducing Hazards and Patient Complications in the NICU” (co-presented with Linda Lefrak, MSN, RN), *Fetus and Newborn, sponsored by Contemporary Forums*, San Francisco, CA, October, 2006

“Management of Pain and Agitation in the Neonate” *Fetus and Newborn, sponsored by Contemporary Forums*, San Francisco, CA, October, 2006

“Weaning Sedatives and Narcotics from the Neonate” *Neonatal Drug Therapy, sponsored by University of Washington Continuing Nursing Education*, Seattle, WA, March, 2006.

“Neonatal Opiate Withdrawal: Is There a Yellow Brick Road?” *Neonatal Pharmacology, sponsored by Contemporary Forums*, Seattle, WA, June, 2005.

“Palliative Care in the NICU: Making the Shift to Comfort Care”. *Loss and Grieving in Pregnancy and the First Year of Life, sponsored by Inova Health System*, Fairfax, VA, May, 2005.

“Palliative Care in the NICU: Making the Shift to Comfort Care”. *High Risk Perinatal-Neonatal Care, sponsored by World Research Group (WRG)*. Boulder, CO, January, 2005.

“Palliative Care in the NICU: Making the Shift to Comfort Care”. *Developmental Interventions in Neonatal Care, sponsored by Contemporary Forums*, Seattle, WA, November, 2004.

“Creating Family Life in the NICU” (co-presented with Phyllis Cartmel, RNC). *Developmental Interventions in Neonatal Care*, sponsored by Contemporary Forums, Seattle, WA, November, 2004.

“Redesigning our Infant Intensive Care Unit: Moving Forward in Partnership with Families” (co-presented with Louine U. Verneuil, LICSW, MPH, Bill & Kathy Nichol, parents). *First Annual International Family Centered Care Conference*, sponsored by Institute for Family Centered Care, Boston, MA, September, 2003.

“Promoting Family Centered Care in the NICU: Practical Tips for Design and Delivery” (co-presented with Phyllis Brown, RN) *Perinatal Symposium; Family Centered Maternal and Newborn Care, Principles of Practice*, sponsored by Hill-Rom/Air Shields, Cincinnati, OH, September, 2002.

“Ooooo!! That Smarts: Pain Management Strategies for Newborns” *27th Annual Bill Young Perinatal Conference*, sponsored by Rogue Valley Medical Center, Medford, OR, July, 2002.

“Dermal Do’s and Don’ts: Neonatal Skin Care and Wound Healing” *27th Annual Bill Young Perinatal Conference*, sponsored by Rogue Valley Medical Center, Medford, OR, July, 2002.

“Some Like it Hot: Newborn Thermoregulation Revisited” *27th Annual Bill Young Perinatal Conference*, sponsored by Rogue Valley Medical Center, Medford, OR, July, 2002.

“Designing and Delivering Neonatal Care in Single Rooms” (co-presented with Phyllis Brown, RN) *Agency for Health Care Administration Office of Plans and Construction (AHCA) 17th Annual Seminar*, Orlando, FL, October, 2001.

“Opiate Use and Weaning: The Good, the Bad, and the Ugly” *2001 NANN’s Neonatal Odyssey, 17th Annual Conference of the National Association of Neonatal Nurses*, Salt Lake City, UT, October, 2001.

“Designing and Delivering Single Room Neonatal Care: New Unit Presentation (co-presented with Phyllis Brown, RN), *The Physical and Developmental Environment of the High Risk Infant*, St. Petersburg, FL, January, 2001.

“Designing and Delivering Care in Single Rooms” (co-presented with Phyllis Brown, RN), *Developmental Interventions in Neonatal Care Preconference: NICU Design: Designing Dreams and Delivering the Future*, New Orleans, LA, November, 2000.

“Suggestions for Success: Tips that Will Make Your Project Less Stressful”, *Developmental Interventions in Neonatal Care Preconference: NICU Design: Designing Dreams and Delivering the Future*, New Orleans, LA, November, 2000.

“Strategies to Prevent and Manage Pain in the Developing Neonate” (co-presented with gretchen Lawhon, RN, PhD), *Developmental Interventions in Neonatal Care*, New Orleans, LA, November, 2000.

“Wound Care: The Hit Parade of Skin Care Products”, *Timely Topics in Neonatal Care, Pacific Northwest Association of Neonatal Nurses Biannual Neonatal Conference*, Seattle, WA, May, 1999.

“Neonatal Skin Care: How to Promote healing and Make Sense of the Wound Care Product Parade” *Charting Your Course in the NICU, National Association of Neonatal Nurses, Clinical Update*, Honolulu, HI, March, 1999 (national audience).

“Is That Fungus Among Us: Differentiating between Skin Infections” *Charting Your Course in the NICU, National Association of Neonatal Nurses, Clinical Update*, Honolulu, HI, March, 1999 (national audience).

“Neonatal Skin and Wound Care” *University of Washington, Neonatal Pharmacology Conference*, Seattle, WA, March, 1998.

“Opiate Weaning Protocol: Early findings” (co-presented with Ann McKinstry RPh), *Northwest Pediatric Critical Care Consortium*, Seattle, WA, October, 1997.

“Effects of the ‘Wee Thumbie’ Pacifier on Self Calming and Oral Feeding Skills” (co presented with Lori Milkovich, RN, BSN), *National Association of Neonatal Nurses, 13th Annual Meeting*, Phoenix, AZ, September, 1997 (poster presentation-national audience).

“CAUGHT IN THE NET: Old Fish and New Fish Swimming the NICU-NET”, *Pacific Northwest Association of Neonatal Nurses, Regional Conference*, Bellevue, WA, February, 1997 (national audience).

“Click here: An Interactive Tour of the Intensive Care Nursing”, *Children’s Hospital Board of Directors, Nursing Committee*, Seattle, WA, February, 1997.

“Neonatal Sepsis”, *Yakima Valley Memorial Hospital*, Yakima WA, September, 1996

“PICC Lines: Care and Feeding” *Ross Nursery Manager's Meeting*. Seattle, WA, 1995.

"Kangaroo Care: Snuggling Down Under" *Future Perspectives in Perinatal Nursing (SW Washington Regional Perinatal Conference)*. Tacoma, WA, 1994.

"Assessment and Treatment of Pain in Newborns" *Seattle Area Society of Hospital Pharmacists*. Seattle, WA, 1994.

"Infant's Responses to Stressful Nursing Care Activities" *Celebration of Clinical Research*. Tacoma, WA, 1994.

"Preterm Infant Responses to Nursing Care Activities: Research Results" *National Association of Neonatal Nurses 9th Annual Meeting*. Orlando, FL, 1993. (national audience)

"Neonatal Assessment and Transition" *SW Washington Regional Perinatal Program, Inpatient OB Certification Review Course*. Tacoma, WA, 1992.

"Introduction to Nursing Research" *MultiCare Medical Center Nursing Residency, Core Curriculum*. Tacoma, WA, 1992.

"Neonatal Respiratory Topics" and "Neonatal Multisystem Topics" *Children's Hospital/MultiCare Medical Center's Neonatal/Pediatric CCRN Review Course*. Seattle and Tacoma, WA, 1992.

"Neonatal Skin Care: Panel Presentation" *Pacific Northwest Association of Neonatal Nurses*. Seattle, WA, 1991.

"High Risk Infants: Multisystem Care Needs" *Pacific Lutheran University, School of Nursing*. Tacoma, WA, 1991.

"Persistent Pulmonary Hypertension of the Newborn" *Sonora Community Hospital*. Sonora, CA, 1990.

"Presentation Graphics" *University of California, San Francisco, School of Nursing, Faculty Meeting*. San Francisco, CA, 1988.

OTHER

Network of Doctoral Students in Nursing, *Charter Member*, University of Washington, 2010

Institute for Healthcare Improvement Open School, *Member*, University of Washington Chapter, 2010

