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Ollscoil na hÉireann, Corcaigh

National University of Ireland, Cork



Sleep and Developmental Outcome of the Moderate to Late Preterm infant

Thesis presented by

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Declaration

This is to certify that the work I am submitting is my own and has not been submitted for another degree, either at University College Cork or elsewhere. All external references and sources are clearly acknowledged and identified within the contents. I have read and understood the regulations of University College Cork concerning plagiarism and intellectual property.”

A handwritten signature in cursive script that reads "Mary Anne Ryan".

Mary Anne Ryan

Date 7.4.23

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Abbreviations

aEEG Amplitude-integrated electroencephalogram

AS Active sleep

ASQ Ages and Stages Questionnaire

Aw Awake

BW Birth weight

CNS Central nervous system

CP Cerebral Palsy

CUMH Cork University Maternity Hospital

cSWC Complete SWC

DQ Developmental quotient

ECG Electrocardiogram

EEG Electroencephalogram/ graph

cEEG Continuous EEG

vEEG video EEG

EHR Electronic Health Record

EMG Electromyogram

EOG Electrooculogram

G Gram

GA Gestational age

Hrs Hours

Hz Hertz

IBI Inter-burst interval

IQ Intelligence quotient

ID Identification number

IQR Interquartile range

IS Indeterminate sleep

Kg Kilogram

LMPT Late and Moderate Preterm

LPT Late preterm

MDS Mental Development scales
Min Minutes
MLP Moderate to late preterm
MOD Mode of delivery
MOF Mode of feeding
MPT Moderate preterm
MRI Magnetic resonance imaging
Mths Months
NGT Naso-gastric tube
NICU Neonatal intensive care unit
NIDCAP Neonatal Individualised developmental care and assessment program
NNU Neonatal Unit
NREM Non-rapid eye movement
Hz Hertz
PMA Post menstrual age
qEEG Quantitative EEG
QS Quiet sleep
REM Rapid eye movement
Resp Respirations
Sec Seconds
SAT Spontaneous activity transient
SD Standard deviation
SWC Sleep-wake cycle
TA Tracé Alternant
TD Tracé Discontinu
TST Total Sleep Time
TTN Transient tachypnoea of the Newborn
µV Microvolt
WHO World Health Organisation
Wks Weeks
Yrs Years

Abstract

Background

Sleep is the primary activity during early brain development and an essential part of healthy cognitive, physical and psychosocial development. Electroencephalography (EEG) provides detailed information about brainwave activity during sleep which changes in different sleep states and advancing gestational age (GA).

The moderate to late preterm (MLP) infant is defined as an infant born between 32-36 +6 weeks GA. MLP's are under-researched in terms of developmental outcome.

We hypothesise that:

- The sleep architecture of healthy MLP infants at 36 weeks may differ according to birth GA, birth weight, sex, mode of feeding or location (cot/incubator) at time of monitoring.
- The developmental outcome of healthy MLP infants may be different to that of a term control group at 4 months and 18 months PMA.

Aims

- To describe the sleep architecture of healthy MLP infants in the neonatal unit at 36 weeks and the frequency of sleep interruptions using continuous EEG monitoring with video.
- To describe parameters for the main EEG feature of quiet sleep i.e. inter-burst intervals (IBI) of MLP group with a normal developmental outcome at 18 months

- To compare neurodevelopmental outcome of the MLP infant group to that of a term control (TC) infant group at 4 months and 18 months.

Methods

MLP infants recruited in the neonatal unit had overnight continuous EEG monitoring (12 hours) with video at 36 weeks post menstrual age (PMA). Post-acquisition, sleep states and sleep interruptions were annotated and quantified based on visual analysis of EEG, behavioural observation and cardiorespiratory parameters.

Using an inter-burst interval (IBI) detection algorithm five IBI features of QS were extracted from MLP infants with a normal developmental outcome at 18 months.

Outcome of MLP infant group was compared to a healthy term control (TC) group based on scores achieved in the Griffiths III mental development scales at 4 and 18 months PMA.

In comparing outcome of MLP and TC infant groups, the Mann–Whitney U test was used for continuous variables and the Chi-squared test or Fisher’s exact test was used for categorical variables. A p -value <0.05 is considered to be statistically significant. Cohens d (the standardized mean difference between the MLP and TC groups) was used as the measure of effect size.

Results

Ninety-eight infants had overnight EEG's included in this study. In the neonatal unit 23.3% of sleep cycles were interrupted primarily for feeding. The total overnight sleep time (TST) was 7.09(6.61-7.76) hrs including 4.58(3.69-5.09) hrs in active sleep (AS), 2.02(1.76-2.36) hrs in quiet sleep (QS) and 0.65(0.48-0.89) hrs in indeterminate sleep (IS). The total duration of AS was significantly lower in infants born at lower GA ($p=0.007$) whilst the duration of individual QS periods was significantly higher ($p=0.001$).

Sixty infants had a normal outcome at 18 months and were included in the QS analysis study using the IBI detection algorithm. Normative data for five IBI features was extracted from QS. All IBI features were significantly longer for infants cared for in incubators although these infants were chronologically younger ($p<0.001$).

When neurodevelopmental outcome of the MLP and TC groups were compared at 4 and 18 months PMA, the MLP infant group achieved lower scores than in overall general development. The greatest differences were in the area of gross motor development ($p<0.001$, with a Cohen's d effect size of -0.665), eye-hand coordination ($p<0.001$, with a Cohen's d effect size of -0.648). Using the reported Griffith's cut off of < 90 for delayed development, 7% (5/75) of the MLP group had delayed development at 18mths compared to 2% (2/92) of the TC group.

Conclusion

EEG provides an objective insight into sleep organisation and may be considered a biomarker of brain development. This thesis provides detailed analysis of the sleep EEG of a cohort of healthy MLP infant at 36 weeks PMA and provides useful reference criteria for studies that may assess brain maturation in the future, particularly for those infants in neonatal intensive care units.

Covid impact statement

This study commenced in July 2017 with recruitment completed in September 2018. The 18-month follow-up developmental assessments commenced February 2019 and were due for completion September 2020. The Covid-19 pandemic reached Ireland in February 2020. In March 2020 the Irish government introduced public health measures to mitigate its impact including restricted (non-emergency) access to health care facilities, the closure of schools and colleges and all research activities were paused. A stay-at-home order banned all non-essential travel and a national 'lockdown' commenced. Follow up assessments for this study at 18 months were delayed and challenged by these pandemic restrictions.

With an easing of restrictions delayed 18month follow-up assessments were relocated to a facility off the hospital campus. This required a risk assessment analysis and guidelines and policies to be developed to minimize risk to staff, parents and their infants. The pandemic may also have contributed to more infants lost to follow- up.

Chapter 1

1.1. Introduction

1.1.1 Background to this research study

Preterm birth is defined as birth before 37 weeks gestation.(1) The American Medical Association Workgroup defined the subcategory of moderate to late preterm(MLP) as preterm infants born between 32- 36+6 weeks GA.(2, 3) Globally the MLP infant group account for approximately 85% of all preterm births.(4) Despite representing the largest group of preterm infants, there is a paucity of literature about developmental outcome of this group.

There is increasing clinical evidence supporting the importance of sleep in the maintenance of neural networks, supporting connectivity and brain plasticity. This in turn promotes optimal neurosensory and neurocognitive development (including learning and memory), behavioural functioning, physical growth and overall health and wellbeing. (5, 6, 7) This is particularly relevant during a critical period of development that occurs in the last trimester.(8)

Sleep and brain development may be disrupted because of preterm birth. Sleep architecture refers to the structural organisation of sleep and how sleep cycles change from one sleep state to another throughout the night. (9) An environment that supports the preservation of sleep will facilitate neural maturation and provide the scaffolding for future higher-order functions. Admission to the highly stressful environment of the neonatal unit may disrupt and fragment sleep and negatively impact the developing brain. Protecting sleep is a critical component of neonatal care. The quality of sleep in early life has been identified as a predictor of physical, cognitive, motor, neurologic, and psychosocial development. (10, 11, 12)

EEG provides detailed insight into the microstructure of sleep and sleep organisation providing objective information regarding ongoing sleep quality and quantity. (13, 14) Accurate interpretation of EEG considers normal sleep state alterations that occur with advancing GA and the appearance of EEG features unique to each gestational age (GA). Inter-burst intervals (IBI) are periods of discontinuity or low voltage activity which is a normal feature of QS in the preterm infant. With maturation, the duration of IBI's decrease and the EEG becomes more continuous. This makes EEG a valuable tool for confirming a normal trajectory of brain maturation and cerebral functioning whilst highlighting those infants who warrant further follow up.

For the most part, EEG in the neonatal unit is associated with the critically ill infant at risk of seizures, typically undergoing hypothermia treatment. EEG monitoring and sleep state analysis is not routinely performed in the healthy MLP infant group prior to

discharge, hence little is known of the potential prognostic value of EEG at this time point amongst this specific cohort.

Preterm infants are at risk of increased mortality and morbidity in comparison to term-born infants (15, 16) which decrease with advancing gestational age. (17, 18) Developmental outcome of the healthy MLP infant has been explored less in the literature in comparison to the very preterm infant or the term infant with hypoxic ischaemic encephalopathy (HIE). Accurate assessment of outcome requires standardised and validated tools with developmental assessment carried out by trained professionals.

I devised the name **SLEEPi** for this study based on the acronym for Monitoring **SL**eed through **EE**g in **P**remature infants.

1.1.2 Aims

This study has the following aims:

- To describe the nocturnal sleep organisation of MLP infants in the neonatal unit at 36 weeks PMA using continuous video EEG and cardio respiratory parameters.
- To quantify the number of overnight sleep interruptions, the state from which sleep state the interruption occurs and the purpose of same.
- To provide a detailed analysis of the proportion of sleep states overnight and investigate if sleep measures are influenced by birth gestational age(GA), birth

weight, sex, location (cot/incubator) and mode of feeding at the time of monitoring

- To describe detailed normative data regarding features of inter-burst intervals (IBI) in quiet sleep (QS) of a MLP group with a normal developmental outcome at 18 months using an adapted IBI detection algorithm. I will also investigate if factors such as birth GA, birth weight, sex, sleep location (cot/incubator) and pregnancy type are associated with IBI features.
- Finally, this study will investigate the developmental outcome of a healthy uncomplicated MLP infant group (admitted to the neonatal unit at birth) at 4 months and 18 months post menstrual age (PMA) compared to that of a healthy term control group.

1.1.3 Scope of this thesis

Chapter 1 provides introductory literature relevant to the study including physiological of brain development with an emphasis on the moderate to late preterm (MLP) infant group. The physiological principles of EEG will be outlined and the sleep architecture of the MLP infant will be discussed.

Chapter 2 describes the methods used in this study. It describes the inclusion/exclusion criteria and procedures to which the infants were subjected.

Chapter 3 is the first of the results chapters and describes the main findings in relation to the quantification of overnight sleep cycles and interruptions to sleep in the neonatal unit at 36 weeks PMA and the purpose of same.

Chapter 4 provides a detailed analysis of overnight sleep in the neonatal unit and the proportion each sleep state based on visual analysis, behaviour observation and ECG/ respiratory parameters using the same overnight EEG.

Chapter 5 describes the analysis of inter-burst intervals a feature of quiet sleep in preterm infants. An adapted IBI detection algorithm will used quantify five features of quiet sleep amongst MLP with a normal developmental outcome at 18 months.

Chapter 6 describes the comparison of Griffiths III scores between MLP admitted to the neonatal unit and term controls and a term control group at 4 months and 18 months post menstrual age (PMA).

Chapter 7 is the concluding chapter that provides a brief summary of the contribution of this study to the literature and identifies the strengths, limitations and areas requiring further research.

1.2 An introduction to early brain development

Development of the central nervous system (CNS) is a highly organised process guided and shaped in the early stages by genetic factors, while later development is also modulated by activity and external input. (19, 20, 21) Characterizing patterns and timing of normative brain development is fundamental to understanding the neurodevelopmental processes, disorders of development and diseases that may occur in infancy and early childhood (22, 23, 24). In this section early brain development will be described and subsequently, development unique to the MLP will be described in more depth (section 1.2) (25).

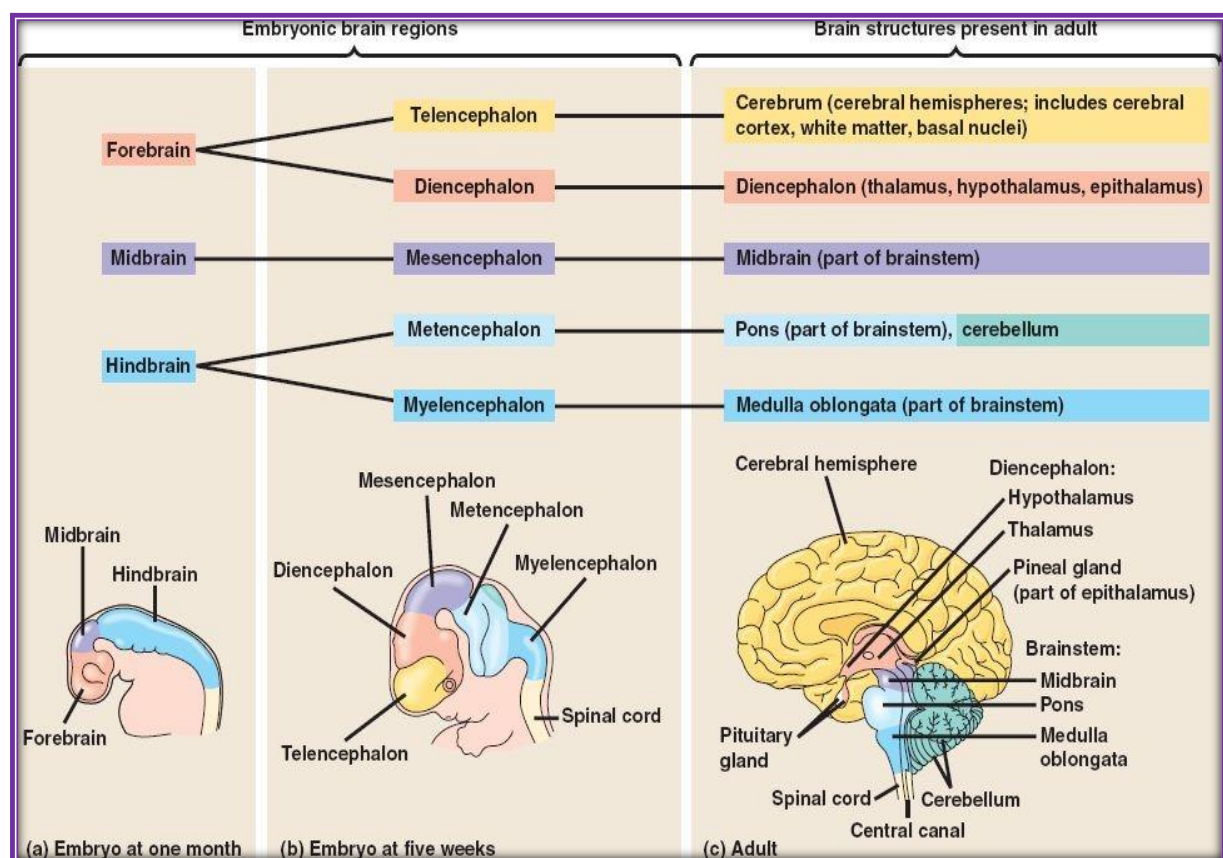


Figure 1.1 From early embryonic development to the mature brain. Downloaded from <https://www.sutori.com/en/item/embryonic-brain-region-brain-structure-in-adu>

1.2.1 Embryology and early CNS development

Early brain development is primarily genetically driven during the first and second trimester, hence gene irregularities will influence early brain morphology and the developmental outcome of the newborn.(26) Two weeks after fertilization, the rapidly growing embryo has implanted. Gastrulation results in the development of three germ layers including ectoderm, endoderm, and mesoderm. (27) Each layer will differentiate and grow into specific cell bodies which will lead to all types of human tissue. (28) Ectodermal cells thicken and flatten forming the neural plate(neurulation). Folding of the plate forms the neural tube which closes (rostral end first followed by caudal region) during week five GA (approx. day 30). (29) This structure forms the basis of CNS development.(30)Failure of the neural tube to close may lead to abnormal development e.g. anencephaly or spina bifida.

Three primary vesicles are formed in the anterior end of the neural tube which develops into the forebrain (prosencephalon), the midbrain (mesencephalon) and the hindbrain (rhombencephalon) from which all components of the brain are derived (Figure 1.1). Further subdivisions are each associated with the development of specific structures of the brain and complete by 7 weeks GA.(31)

Once the general structure of the neural tube has been laid out, proliferation occurs in the periventricular zone with each cell dividing in two, repeatedly adding a layer of cells with each division. From day 42 post-conception, cells multiply at a rate of up to 250,000 neurons a minute. Disorders of proliferation and a reduction in the duration of proliferation impacts CNS development and the overall size of the brain.

The 12th week of gestation is considered the timepoint for the start of neuronal migration from the germinal zone. Migration occurs to predetermined sites where differentiation into the specific type of cell appropriate to that location occurs. Early migrating cells occupy the deeper cortical layers whilst later migration passes through previously formed layers and ultimately form a multi-layered cortex (inside-out development) e.g. neurons formed in the ventricular zone migrate to their final locations in one of the six layers of the mature cortex. By 25 weeks gestation the characteristic six layers of the cortex will have formed (Figure 1.2).

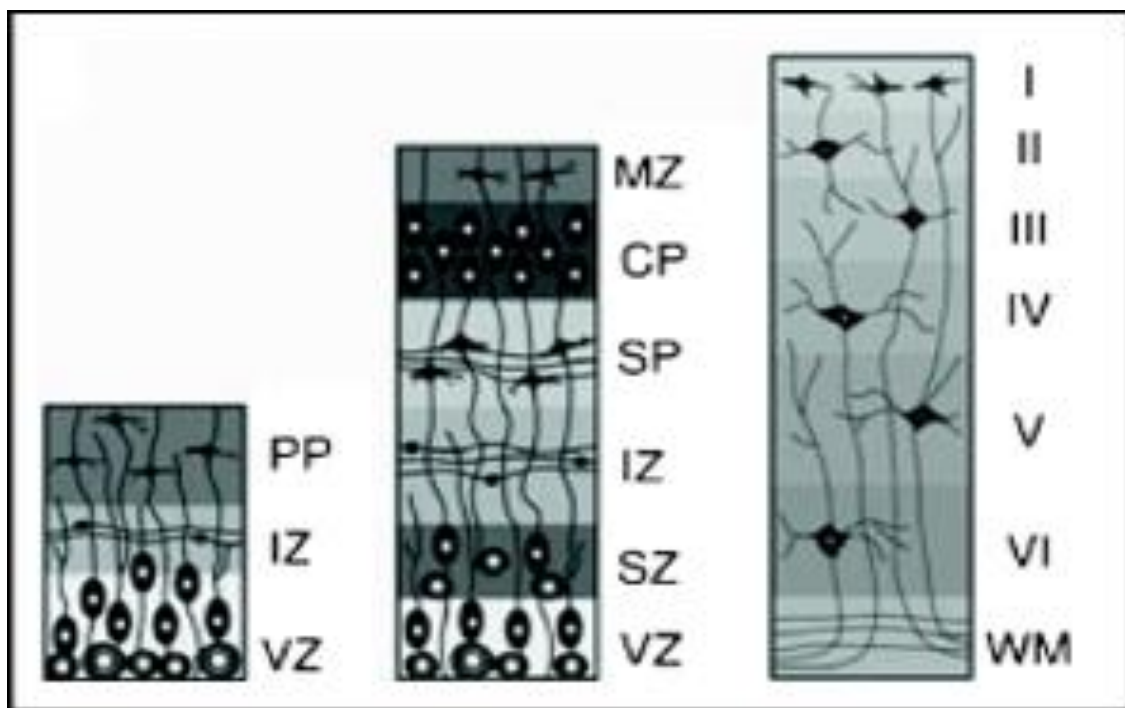


Figure 1.2 Schematic representation of the development of the 6 layers of the mature cortex. The first neurons migrate from the ventricular zone (VZ) to form the preplate (PP). As shown in the second panel, the next neurons split the PP into the marginal zone (MZ) and the subplate (SP), both transient brain structures. The mature brain, shown in the third panel, has six well developed cortical layers (I-VI), but none of the embryonic structures (MZ, SP, VZ). The intermediate zone (IZ) has become a mature white matter layer (WM). From Stiles and Jernigan(22)

During the MLP period, migration continues to support the gathering of similar cell types forming connections across many distinct regions and thereafter forming functioning neural circuits.(32) Failure or delay in neural migration may be caused by antenatal exposure to toxic substances, which is associated with a number of conditions, including autism and developmental delay. (33, 34)

Under genetic control, neuronal fate can proceed along one of two routes. Firstly, with adequate neurotrophic factors the neuron can mature and develop axons and dendrites, or it can be retracted through apoptosis. Apoptosis, the natural process of programmed cell death, is a prerequisite for normal neurodevelopment. This process of controlling cell survival continues during the MLP period and beyond, ensuring the appropriate cell populations are present in each area of the brain. (35) Apoptosis is an essential process required to balance cellular homeostasis needed to maintain immune responsiveness.(36) Apoptosis in early development may remove 40-60% of unnecessary, surplus, aged or damaged cells.(37)

The central nervous system continues to build on early fetal development and maturation toward term GA and beyond. Billions of neurons form synapses or connections with other neurons, leading to trillions of connections between cells.(38) Commencing in the third trimester (28 weeks) synapse formation may occur between the axon of one neuron and the dendrite of another or between the axon of one neuron and the cell body of another.(39) Achieving intracellular connections is a prerequisite for communication and the driving principle of CNS multicellular organization. Impaired connectivity is associated with disability. (40) It is important to note that the

various structures of the brain reach their peak synaptic activity at different time points. In contrast to the prefrontal areas, in which synaptic production does not peak until 15 months after birth, the visual cortex reaches its peak production four or five months after birth.(25)

Brain development results in a complex structure of more than 100 billion neurons that communicate through trillions of connections or synapses.(41) Maximum global synaptic density occurs by 3mths post-term and continues until 2 years of age. (42) The difference of timing in peak synapse production is important because it affects the timing of the plasticity of these regions; the later the peak synapse production, the longer the region remains plastic.

Synapse over-production is a normal aspect of brain development.(43) The final number of neurones or glia cells is genetically controlled through pruning. The more active a cell is, the less likely it is to be pruned. The timing of pruning varies within different brain areas. The brain also retains some unused synapses which will allow the brain to evolve, adapt and switch or develop functions as the need arises. Traditionally the literature supports the concept of the more synapses the better; however, when considering that pruning is part of development, increased synaptic activity could also be attributed to the failure of the process of pruning. (44) Pruning may also be considered the body's way of maintaining more efficient brain function as we get older and learn new complex information. (45) Altered synaptic pruning is associated with neurodevelopmental disorders including autism, attention-deficit hyperactivity disorder, and schizophrenia based on histological studies.(46)

Despite being described individually, processes of brain development occur in overlapping phases (Figure 1.3). These phases include migration or the gathering of similar cell types forming connections, and ultimately functioning neural circuits occur during the MLP period and is complete by term gestational age.(32) Brain-derived neurotrophic factors play a vital role in neural development determining neuronal survival (i.e. which neurons continue to mature with axons and dendrites or are retracted through apoptosis (Figure 1.3).(47, 48) Synaptic and axon pruning also leads to the degeneration of excessive axon branches while maintaining the integrity of the cell body.(48) It is estimated that anywhere between 40% to 60% of neurons are retracted.(49) Neurotrophins also increase myelination during the MLP period improving the speed of impulse conduction

Myelination generates multiple layers of insulating myelin membrane around axons, supporting long-range cortical connectivity and the rapid transmission of information between neurons.(50) Myelination occurs during a critical period of development when the brain is most vulnerable to environmental influences peaking in the last trimester. (51) Whilst it commences in the visual system it extends to the other sensory systems over the first year of life, progressing at different rates in different areas of the brain largely in a caudo-rostral direction. (52) The process continues toward adolescence and to a lesser extent throughout adulthood (Figure 1.3). Disorders or delays in myelination are associated with adverse developmental outcomes including childhood cognitive processes, learning and processing speed. (53)

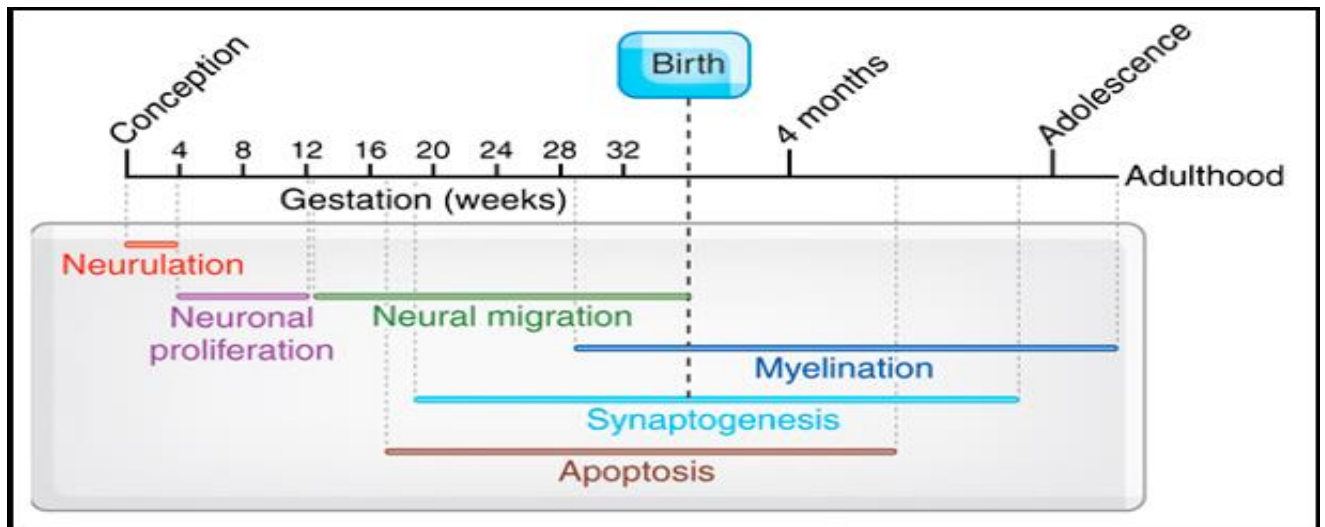


Figure 1.3 Timeline of human brain development from conception to adulthood. From Tau and Paterson⁵⁵

Synaptic passage of impulses or neuronal transmission, is mediated by chemical neurotransmitters which propagate signals from the presynaptic neuron to the postsynaptic neuron. Neurotransmitters play a vital role in brain development including neurogenesis, migration, differentiation and synaptogenesis. (54)

Neurotransmitters such as gamma-Aminobutyric acid (GABA) regulate neural migration by creating a chemical stimulus that alters intracellular calcium.(54) Changes in intracellular calcium result in neurotransmitter release, hormone secretion, cell fate and gene expression.(55) During the development of the CNS neurons may release transmitters targeting migrating neurons and growing axons before establishing contact and synapsing with their target cell.(56) Hence interference with the neurotransmitter system may lead to a range of developmental difficulties. (57)

1.2.2 The Macrostructure of the brain

Overall the brain triples in overall weight in the third trimester with an accelerated 35%-40% increase in brain weight and a 500% increase in brain volume in the last 6

weeks of pregnancy.(58) The transformation of the brain from a smooth surface into a convoluted dense structure with folds(sulci) and ridges (gyri) allows a large surface area of cortex to be fitted into the limited confines of the cranial vault. With advancing GA the cerebrum divides into a left and right hemisphere identical in appearance and separated by the cerebral or longitudinal fissure. Both hemispheres are divided into four separate lobes each associated with a different function (Figure 1.4).

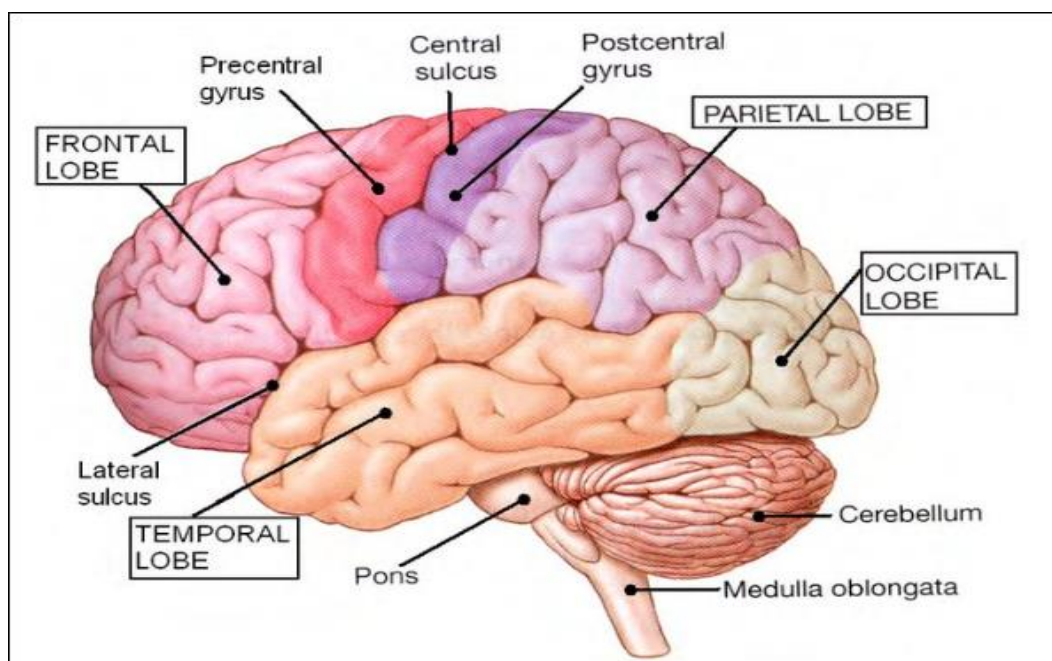


Figure 1.4 The macrostructure of the human brain From Tortora and Reynolds(59)

The protective cerebral spinal fluid (CSF) that circulates the brain and spinal cord is produced in the choroid plexus epithelium and ependymal cells of the ventricular system. CSF flows into the interconnecting chambers and the subarachnoid spaces. The vascular system of the brain becomes more complex and the blood vessels more robust with advancing GA, thus reducing the risks of intraventricular haemorrhage (IVH) associated with the fragility of capillaries in the germinal matrix.

1.2.3 Genetic influence of brain development

Brain development is the result of interactions of genetic and environmental influences. Whilst early brain development is predominantly genetically defined only one third (approx.10,000) of the whole genome is expressed during brain development(60). Age-specific gene expression plays an important role in several critical events during rapid fetal brain development, including neuronal cell migration and synaptogenesis.(61) Studies on adult twins have found that many aspects of brain anatomy are heritable, hence genetic factors contribute significantly to the variation in brain structure(62).

After birth development is predominantly experience dependant.(63) Experiences may also determine how genes are expressed or not expressed. Hence in early life the epigenome can be affected positively or negatively and have lifelong effects. Providing a positive supportive environment for the preterm infant in the NICU and prevent potential adaptations that may negatively impact an infant's life.

1.2.4 The environmental influence on brain development

It is not possible to consider brain development based on gene expression alone. Vasung et al describe genetic factors as being silenced in the last weeks of pregnancy with the environmental factors having the greatest impact on brain development.(25) Environmental influences and life experiences play a major role in shaping development. As embryological and fetal development is sensitive to maternal health

and biochemical make up, environmental interaction commences in-utero which may influence the genetically determined developmental processes.(64) (Fig 1.5)

Impaired maternal nutritional status in pregnancy will have knock-on effects on normal fetal growth and organ development especially during a vulnerable period of rapid development that occurs during the last ten weeks of pregnancy.(41) The timing of deprivation of specific nutrients during pregnancy e.g. proteins, fats, iron, zinc, iodine and vitamin B12 may potentially alter the trajectory of early fetal brain development.(65) This has led to a greater emphasis on maternal antenatal education and ultimately optimising the maternal nutritional status and that of the newborn. (66)

The spectrum of the teratogenic effects of toxin exposure such as nicotine and alcohol are well documented (Figure 1.5). (67, 68) Smoking may reduce fetal oxygenation which is associated with a variety of adverse fetal outcomes such as stillbirth, low birth weight (LBW), and being born small for gestational age (SGA). Both prescribed and illicit drugs may cross the placental barrier and impact fetal development leading to a variety of adverse outcomes and a prolonged stay in a neonatal unit. The nature and severity of alteration in brain architecture and the sequela of physiological and neurological adversities that may occur are dependent on the causative factor and the level, duration and timing of exposure during development.(41, 69)

Obstetric related medical conditions such as hypertension decrease the flow of nutrients and oxygen compromising synaptogenesis and axonal myelination.(70) Maternal infection and chorioamnionitis have a profound impact on developing neuronal circuits with the degree of physiological immaturity at the time of exposure reflecting the degree of morbidity particularly amongst the preterm infant

population.(71, 72) Obstetric complications such as placental abruption also increase the risk of early surgical delivery with associated complications of prematurity, low birth weight and small for gestational age.(73) Whilst maternal pregnancy-related complications generally resolve within the post-natal period the effect on the newborn may persist into adulthood.

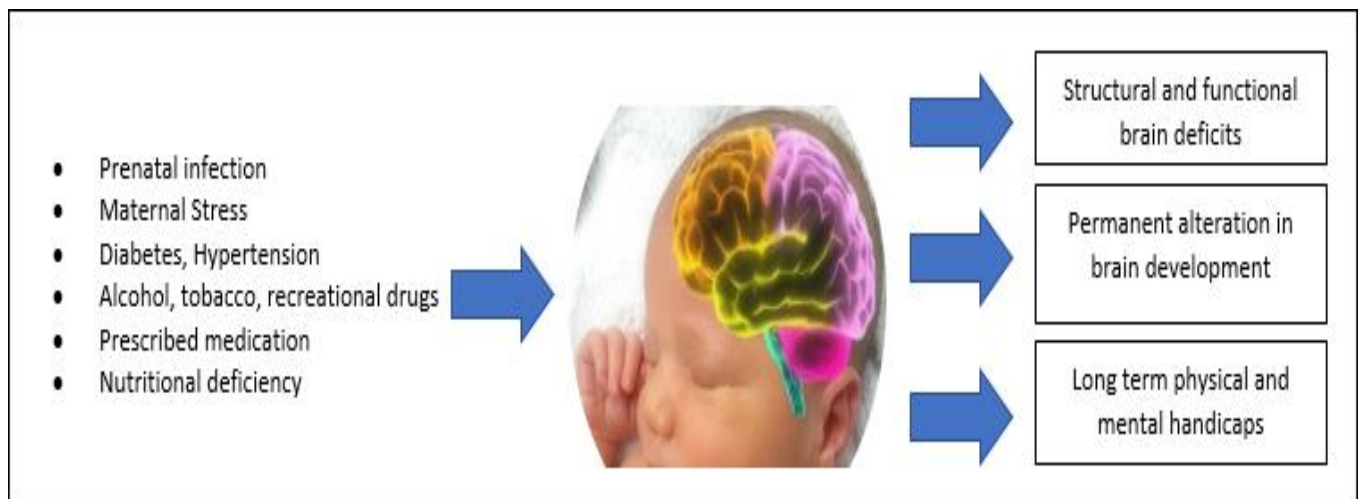


Fig 1.5 Antenatal factors that may influence developmental

1.2.5 Brain plasticity

The brain is an organ of adaptation, a feature termed plasticity.(74) Brain plasticity is considered the ability of the CNS to alter, rewire or reorganize its structure, functions and strength of connections in response to learning, environmental experiences/ stimuli or injury.(75, 76) Plasticity of the brain underlies much of the learning that occurs during the early newborn and childhood period.(48) This is especially relevant to the preterm infant who faces challenges of varying degrees (depending on the degree of prematurity) in the extrauterine environment for which they are poorly prepared.

The potential recovery from an alteration to the brain's microstructure following an insult (e.g. stroke, injury or environmental exposure to stress) is also facilitated through brain plasticity; however, the degree of plasticity is not unlimited.(77) Hence when severe damage occurs from a single event or continuously recurring negative events, the degree of the brain's plasticity may be insufficient to recover without development being compromised. As brain plasticity is greatest in the early newborn period, it also impacts the infant's vulnerability to early adverse experiences.

1.3 Preterm birth

The aetiology of preterm birth is frequently multifactorial. In many cases there may be no easily identifiable cause. Blencowe et al. describe preterm birth as a syndrome caused by both fetal and/ or maternal factors leading to spontaneous or provider-initiated delivery of a premature infant. (78) Goldenberg et al proposed that all factors that increase systemic inflammation and uteroplacental ischaemia/haemorrhage, infection (endometritis), uterine overdistension and stress may contribute to preterm birth.(79) Goldberg identifies maternal risk factors to include previous preterm birth, low interpregnancy interval, high levels of psychological stress, black race, low BMI, women with a short cervical length and a raised fetal fibronectin concentration. (79) Societal or maternal pressure may also influence the timing of delivery. However, the timing of a decision to electively deliver a preterm infant must be made on a case-by-case basis by expert obstetricians balancing the potential neonatal risks and maternal outcome.

1.3.1 Defining terms

Preterm birth is defined as birth before 37 weeks GA with subcategories of preterm birth categorized based on birth GA. For the purposes of this study, we used the World Health Organisation guidelines to define the subcategory of prematurity. These definitions include extremely preterm (<28 weeks birth GA), very preterm (28- 31+6 weeks birth GA) and moderate to late preterm (MLP) as preterm infants born between 32- 36+6 week's GA unless otherwise specified (2, 3). The MLP infant group may be further sub-grouped as moderately preterm (MPT) defined as infants born between 32 to 33+6 weeks GA and late preterm (LPT) infants defined by a birth GA of 34 to 36+ 6 weeks.

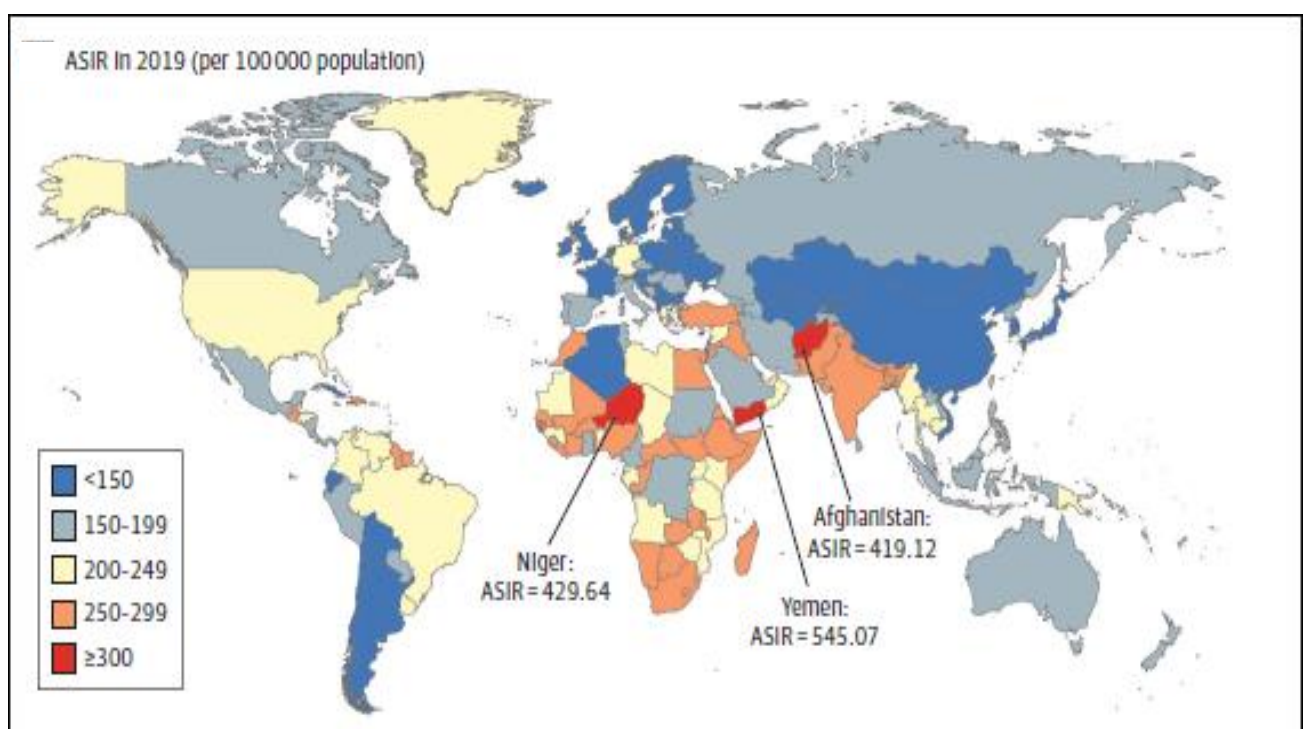
1.3.2 Incidence of preterm birth

The estimated global preterm birth rate is 10.6% and rising, resulting in 15 million babies born too soon each year. (80, 81) (Figure 1.6). The preterm birth rate has risen by 5.6% between 1990 and 2019(82) and is likely to continue due to the increased use of artificial reproductive technology and an associated increase in multiple births.(83, 84) There is an associated increase in fetal and maternal morbidity with multiple pregnancies and consequently an increase in spontaneous or surgically indicated delivery.(85) Up to 50% of multiple births occur prematurely. (86, 87)

Preterm birth is more common in boys (55% of all preterm births are male) but the cause of this is unknown.(88) Approximately 12% of babies born too soon are from lower-income countries compared with 9% in higher-income countries, (81) with an estimated 81.1% of lower income preterm births occurring in Asia and sub-Saharan

Africa.(80) Hence country of birth is in itself a risk factor with racial disparities remaining evident. (81, 89)

In the USA, an increase in the total preterm birth rate for 2017–2018 was exclusively due to the number of infants born late preterm (34–36 weeks). Based on data from 41 countries (n=131 296 785), 84.3% of all preterm infants were born between 32-36+6 weeks gestational age (GA). (81, 90) Globally moderate to late preterm (MLP, defined by Cheong et al as 32–36 weeks GA) account for approximately 80-85% of all preterm births.(4) Data from the UK indicate that the overall percentage of preterm live births decreased from 7.8% in 2019 to 7.4% in 2020.



ASIR: age-standardised incidence rates

Figure 1.6 Estimates of worldwide incidence of preterm birth. From Cao et al(82)

In 2019, 59084 infants were born in Ireland, 3657 were born prematurely equating to 6.2% of births (data provided by the National Women and Infants Health Programme).

This was a reduction from 4290 infants born prematurely in 2017 equating to 6.9% of births, 3550 or 82.8% occurring during the MLP period.(91)

In CUMH in 2020, 352 infants were born between 32-36+6 weeks GA, 269 of whom required admission to the neonatal unit. By comparison in 2021, 360 infants were born from the same GA group with 309 requiring admission to the neonatal unit. The greatest difference in admissions was evident amongst the late preterm (LPT) infant group defined as infants born between 35-36+6 weeks GA. In 2020 61% of LPT infants were admitted to the neonatal unit whilst in 2021 66% required admission. More infants with a birth GA of 36-36+6 weeks in 2021 also had a stay in the neonatal unit (n=122) compared to the same GA in 2020 (n=103). Interestingly in 2021 with the exception of one, all infants born <36 weeks required admission to the neonatal unit.

Table 1.1 Moderate to late preterm admissions to the neonatal unit by gestational age group in 2020 and 2021 in Cork University Maternity Hospital

Birth GA group (weeks + days)	2020 Number of infants born	2020 of Infants admitted to NNU	2021 Number of infants born	2021 of Infants admitted to NNU
32 to 32+6	22	22	13	13
33 to 33+6	37	37	35	35
34 to 34+6	46	46	57	57
35 to 35+6	71	61	83	82
36 to 36+6	176	103	172	122
Total	352	269	360	309

GA; gestational age, NNU: neonatal unit

Data obtained from the Maternal and Newborn Clinical Management System, Cork University Maternity Hospital

1.3.3 Preterm infant mortality

Preterm babies born before term are at a greater risk of increased mortality and morbidity in comparison to term-born infants. (15, 16) A progressive decrease in mortality occurs with advancing gestational age. (17, 18, 92, 93, 94) Preterm birth, intrapartum-related complications, birth defects, infections, (e.g. pneumonia) and diarrhoea are the leading causes of most neonatal deaths in 2019. Based on the CSO statistical publication in May 2021, neonatal mortality rates in Ireland (< 28 days) decreased from 2.4 to 2.0 deaths per 1.000 live births.(91)(Fig 1.7)

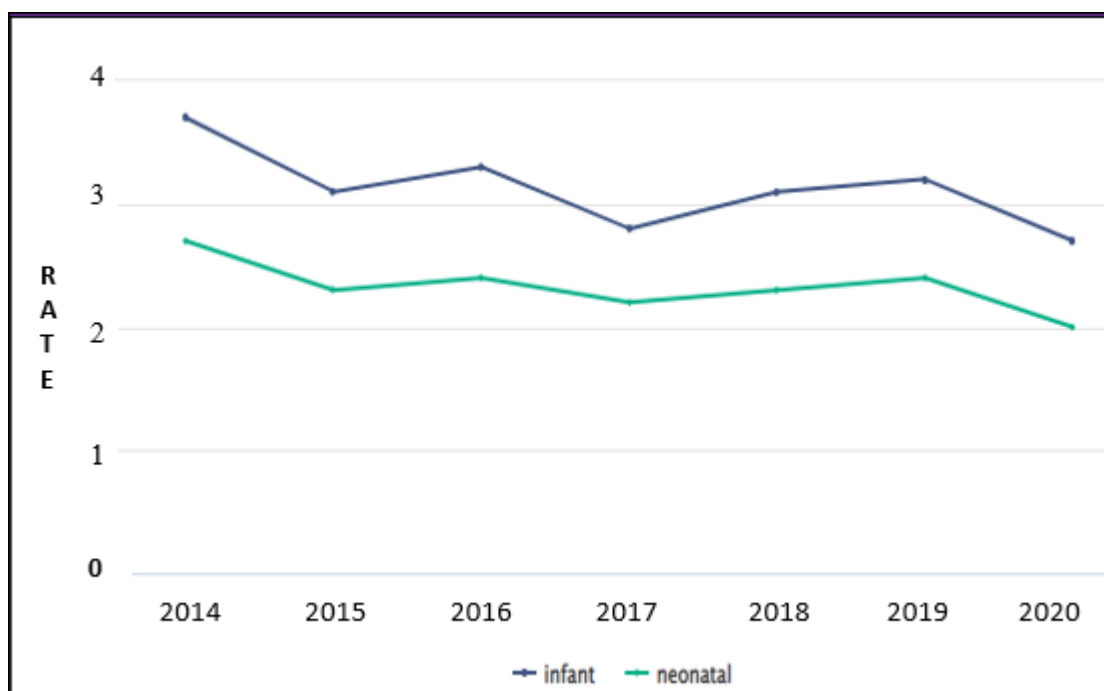


Figure 1.7 Mortality rate in Ireland per 1000 live births. Irish statistics relating to infant and neonatal mortality. From office of CSO 2020 statistics (91)

Globally, the vast majority of childhood deaths (85%) occur in the first 5 years of life which equates to 52 million children under 5 who will die between 2019 and 2030 (95) almost half (47 %) of which occur within the first month of life. Preterm birth remains the leading cause of mortality in children under 5 years with approximately 1 million children who die each year due to complications of preterm birth (many of which are preventable). (96, 97) A disparity is evident based on geographical location with the majority of deaths occurring in low & middle-income countries. (95) Ethnicity and sex

have also proved to impact mortality. Matthews reported that the preterm related infant mortality rate (defined as death before 1 year of age) for non-Hispanic black women was three times higher than that of non-Hispanic white women with preterm males noted to be at an increased risk in comparison to female infants.(98)

In 2017, infant mortality rates for preterm infants in the USA were 20.50/ 1000 births between 32-33 weeks GA falling to 8.50 /1000 births between 34-36 weeks GA and a further decreased to 2.10/1000 by term GA.(99) In an overall analysis of neonatal mortality (<28 days of age) amongst all infant groups i.e. term and preterm (n=21771) over an 18year period, McIntire et al. reported that birth during the late preterm period (identified as infants born at 34, 35, and 36 weeks GA) equated to 76% of all preterm births. Mortality rate associated with LPT at birth was reported as 1.2, 1.5 and 0.5 deaths per 1000 infants born. (100) Mortality rates are 25 times higher for low birthweight infants (less than 2,500 grams) (50.26 per 1,000) than for infants with birth weights of 2,500 grams or more (2.05 per thousand births).(98)Although the risks of mortality for the MLP infant group is low, there remains a higher risk in comparison to the term-born infants. (64)

Perinatal mortality audits on a national and worldwide scale that include a thorough investigation of contributing factors would assist in identifying potential treatments and the training required, possibly enhance the standard of care, and influence clinical and national policy.(101) Improving outcomes of newborn infants has become a priority of the United Nations Children's Emergency Fund (UNICEF) and the World Health Organisation (WHO) in the Every Newborn Action Plan which promotes accelerated efforts in reducing infant and child mortality by 2030. (95)

1.3.4 Short-term physiological morbidity of the MLP infant

All babies born before term are at a greater risk of short- and long-term physiological morbidity in comparison to term-born infants. (15, 16, 102) MLP's are at a higher risk of admission to the neonatal intensive care unit; the lower the GA and/or weight the greater the risk.(103) (Fig 1.8) Whilst some of the difficulties encountered by the MLP infant may be transient and require minimal medical intervention, there is a higher demand for NICU resources amongst MLP infants in comparison to term infants. (102, 104, 105).

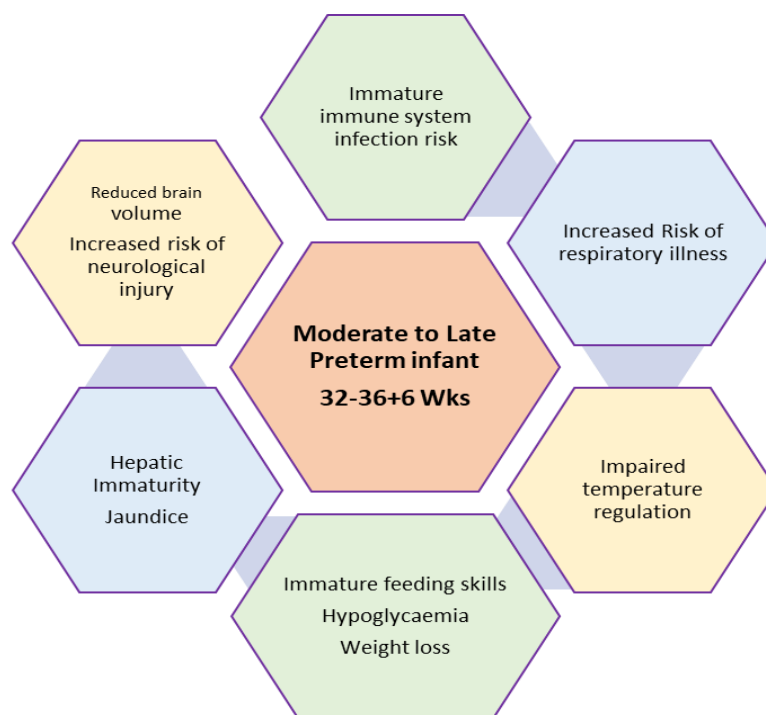


Figure 1.8 Common physiological difficulties associated with mid to late preterm birth
RDS: respiratory distress syndrome, TTN: Transient tachypnoea of the newborn.

The immature brain and lung are especially susceptible to the consequences of preterm birth leading to high rates of short (and long-term) neurological and respiratory health problems.(64, 70, 106) Almost 29% of infants born between 35-36 weeks gestation are noted to exhibit some form of respiratory distress as opposed to 5.3% of

term infants.(107, 108) Respiratory distress syndrome occurred in 14% of neonates born at 33 weeks and 3% of those born at 34 weeks.(109) In a population-based study of 150,426 live-born singleton infants, respiratory failure decreased from 19.8% at 34 weeks' GA to 0.28% at 39 - 41 weeks GA.(110)

In a study conducted by Boyle et al, late to moderate preterm (LMPT)(n=1146) required a higher level of resuscitation, respiratory support, nutritional support with overall higher clinical care needs in comparison to a term control group (n=1258).(111) When subdivided into a MLP group (32-33+6 weeks GA) (n=124) and a LPT group (34-36+6 weeks GA) (n=799), all MLP infants required admission to the neonatal unit whilst 64.3% of the LPT group were cared for on the post-natal ward with mother.

From a physiological perspective, autoregulation and autonomic control are immature in the MLP infant. Pain and stress-related events may potentially impact breathing (e.g. may cause apnoea), blood pressure and overall psychological wellbeing. (112, 113) Approximately 7.1% of late preterm infants have short term issues with hypoglycaemia in comparison to 0.69% of term infants.(107) Developmentally immature feeding skills, poor suck swallow coordination may challenge the MLP infant to meet his/her nutrient requirement orally.(114) MLP infants may tire easily which can be easily mistaken for intake sufficiency.(115) This increases the infant's susceptibility to slow weight gain, the potential need for supplementary tube feeding/intravenous fluids and prolonged maternal separation.

MLP infants are also at risk of hypothermia requiring an intervention in comparison to term-born infants with the incidence highest amongst infants of lower birth weight and

GA (107, 111). Hypothermia and sepsis that may contribute to hypoglycaemia must also be considered and ruled out in the care of a preterm infant (107, 116). A systematic review conducted by Teune et al. identified 22 studies that focused on the LPT group including a total of (n=2,368, 471) and a total of term born infants (n=27,007,204).(64) LPT infants are more likely to require: ventilation (2.5% versus 1.2%), nasal CPAP (5.5% v 0.14%), and have apnoeic episodes (0.87% versus 0.05%), intra ventricular haemorrhage of any grade (0.41% versus 0.09%) or have seizure activity (0.2% versus 0.12%). LPT had higher metabolic morbidity also including hypoglycaemia (7.1% versus 0.69%) and feeding problems (34% versus 6.7%). Rates of hyper-bilirubinaemia in the late preterm group (34-36 weeks gestation) were identified as being over fourfold that of term infants, with infants of 35-37 gestation over twice as likely to require phototherapy.(108) As the immaturity immune system increases so too does the infant's vulnerability to infection, hence sepsis evaluation was performed more frequently amongst MLP infants in comparison to the term born infant (36.7%in MLP vs 12.6% in term infant).(107)

In the absence of medical concerns, healthy uncomplicated MLP infants in the neonatal unit have been considered to have a prognosis similar to full-term infants. (117).The effect of being born between 32-36+6 weeks GA on brain development and potential long-term neurological consequences is underappreciated. Evidence is growing that the MLP infant group are at increased risk for neurodevelopmental delay, cognitive deficits, and learning difficulties compared with term-born peers.(118)

1.3.5 Prematurity and developmental outcome

Despite increasing survival rates of preterm infants, improvements in survival have not collectively reduced adverse developmental outcomes. Prematurity impacts brain development with findings supporting a decrease in cerebral volumes, cortical grey matter, cortical white matter, basal ganglia, with smaller cerebellums in comparison to term born infants. (119) Challenges preterm infants may experience for the rest of their lives include learning challenges, vision and hearing impairments, as well as other long-term developmental issues.

There is an inverse relationship between birth GA and risk of adverse neurodevelopmental outcome.(87, 120, 121) Therefore, the time frame of interruption to the normal in utero maturation continuum impacts the sequela of potential physiological and neurological adversities that may occur. (22, 105) In reviewing the literature pertaining to outcome of the healthy uncomplicated MLP infant group, defining uncomplicated may prove problematic as preterm birth itself is not normal and its' aetiology may involve deteriorating fetal or maternal health resulting in confounding by indication.

1.3.5.1 Developmental assessment tools

Developmental assessments have been conducted using multiple methodologies amongst multiple different birth GA groups and under variable conditions. The age at which developmental assessments are carried out and the precise domains that are assessed must be considered in determining its predictive value.(122) Accuracy in determining presence and degree of adverse development is also dependant on the skill of the examiner. An awareness that variation in performance across testing sessions may not necessarily imply an evolving developmental disability but rather the child's status (tired/ unwell) at time of assessment is important. (123)

Developmental assessments generally focus on multiple factors including but not limited to cognitive development, school/academic performance, working memory, processing speed, vocabulary and mathematical ability, co-ordination, sensory perception, gross motor skills, personal /social and emotional skills and relating these skills to age and developmental status at time of assessment .(124, 125, 126) Whilst assessment of an infant or toddler may be an important predictor of outcome, it is not always predictive of school-age cognitive functioning, long term learning disabilities or academic achievement.(127) Many behavioural problems do not become evident until the preschool and school age years.(87, 92, 93, 117, 128)

1.3.5.2 An overview of developmental outcome of the MLP infant

The range and frequency of sub-optimal developmental outcomes is lower in the MLP group in comparison to the very preterm infant, but greater in comparison to the term-born infant. (64, 117, 120, 129) Despite this incidence decreasing with advancing GA, a spectrum of developmental deficits remains evident in the MLP infant group. (Fig 1.9). Birthweight is closely associated, but does not exactly correspond with gestation.(130) Low birth weight (LBW) further increases risk factors for adverse developmental outcome. (131, 132) The MLP infant has an overall 1.4% increased risk of intellectual disability which increases to 2.3% if birth weight < 2.499kg.(133)

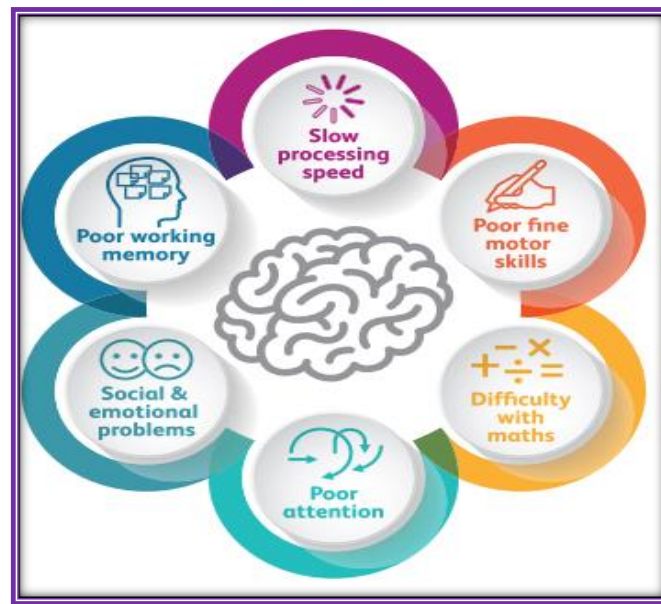


Figure 1.9 Spectrum of developmental deficits that may become apparent in the MLP infant group. Available from: www.pretermbirth.info (134)

Cognitive deficits with or without major motor disabilities are now the dominant benchmark for a neurodevelopmental outcome.(24) In an Australian study Cheong et al. compared outcome of a MLP infant group (n=198) to a term control group (n=183) at 2 yrs corrected age.(135) Development was assessed by means of clinical exam (assessed for motor, visual hearing impairments and cerebral palsy), the Bayley Scales of Infant Development–Third Edition, (Cognitive assessed language, and motor development) and a parent reported questionnaire (Infant Toddler Social and Emotional assessment) assessed social-emotional and behavioural development. (136) Developmental delay was defined as more than 1 SD below the mean i.e. relative to the mean in a term control group in any domain of the Bayley’s scales. In comparison to the term control group, MLP infants were delayed in cognitive, language and motor development i.e. 0.5-0.7SD (-6.3 to -11.8, points) with the greatest difference noted in the area of language development (-11.4 (95% CI, -15.3 to -7.5).

Parent reported questionnaires are valuable sources of information about school-going children. Johnson et al's analysis was based on the validated Parent Report of Children's Abilities-Revised (PARCA) questionnaire completed by parents of MLP infants (n=638) and the term control group (n=765) at 24 months.(137) This study concluded the majority of impairments identified were in the cognitive domain, evident in 6.3% of MLP infants versus 2.4% (RR 2.09, 95% CI 1.19 to 3.64) of the term control group.

The EPIPAGE 2 study, focused on developmental outcome of preterm infants with a birth GA of 22-34 weeks(n=3083) and a term control group (n=592).(138)Assessment of outcome at 5 years included parental input (questionnaire and interview), a clinical exam and psychological evaluation. 1187 of the participating infants were moderately preterm (MPT) defined as infants with a birth GA between 32-34 weeks (Table1. 2).12% of this group MPT infants had severe or moderate developmental difficulties and 34% had mild disabilities. Only 54.6% of MPT infants had no disability in comparison to 71.3% of term born infants. Impairments present after five years of age have a greater association with challenges which persist into adult life.(105, 139)

Table 1.2 Percentage of infants born moderately preterm (32-34 weeks GA) and outcome (developmental delay) at 5yrs

	32-34 weeks GA (n=1187) % (Median (IQR))	Term control (n=592) % (Median (IQR))
Severe delay	3.0(1.7-4.4)	1.1(0.2-3.6)
Moderate delay	8.6(6.5-10.7)	3.9(1.5-7.9)
Mild delay	33.8(30.2-37.4)	23.7(18.6-29.4)
None	54.6(50.6-58.7)	71.3(63.3-76.9)

IQR: Interquartile range. Data extracted from the Pierrat et al (138)

Kerstjens et al. explored the percentage of developmental delay amongst preterm infants (including MLP infants defined as infants born between 32-35+6 weeks GA) and term born infants at 4 years using the Ages and Stages Questionnaire (ASQ).(140)(Fig 1.10) This developmental and social-emotional parental completed screening tool focuses on five domains of development including communication, fine motor function, gross motor function, personal-social functioning, and problem solving. (141) MLP infants were reported as having more difficulties within all domains of development in comparison to term born infants. The rate of developmental delay amongst the MLP (n=927) infant group was 8% with an odds ratio (OR) of abnormal ASQ total scores calculated as 2.1 (95% CI, 1.3-3.4) for the MLP infant group in comparison to term born infant group.(140)

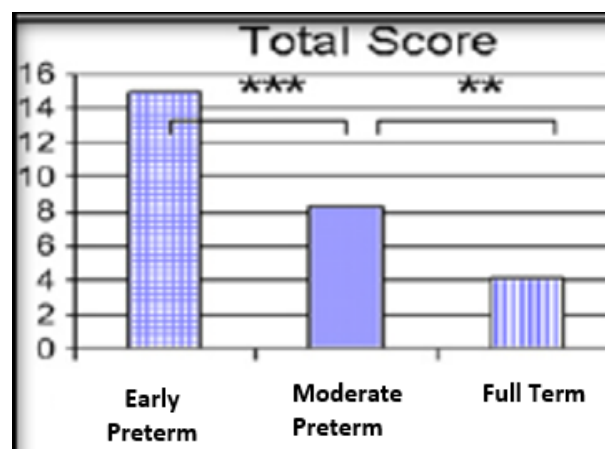


Figure 1.10 Percentages of children with abnormal scores on ASQ at 4 years who were born early preterm moderately preterm or at term. **P < .01; ***P<0.001. Extracted from Kerstjens et al (140)

In a systematic review and a meta-analysis of the literature (n = 74 studies; 64 061 children), Allotey et al aimed to quantify the effect of preterm birth on cognitive, motor, behavioural and academic performance of children born of various degrees of prematurity versus a term control group. (126) Overall cognitive performance of children in the moderately preterm group (defined as infants born between 28-33+6

weeks GA) was consistently lower in comparison to term born group in terms of working memory and processing speed in all the assessment age groups. This review serves as a reminder of the lack of consistency in defining the MLP group.

Of the domains/academic areas studied, maths is the subject where preterm infants frequently experience difficulties.(142) Working memory, counting, processing speed, and flexible thinking are just a few of the specialised abilities needed to solve mathematical issues. Any area of weakness could potentially have a negative impact on math ability and exam performance.(143)(Figure 1.11).

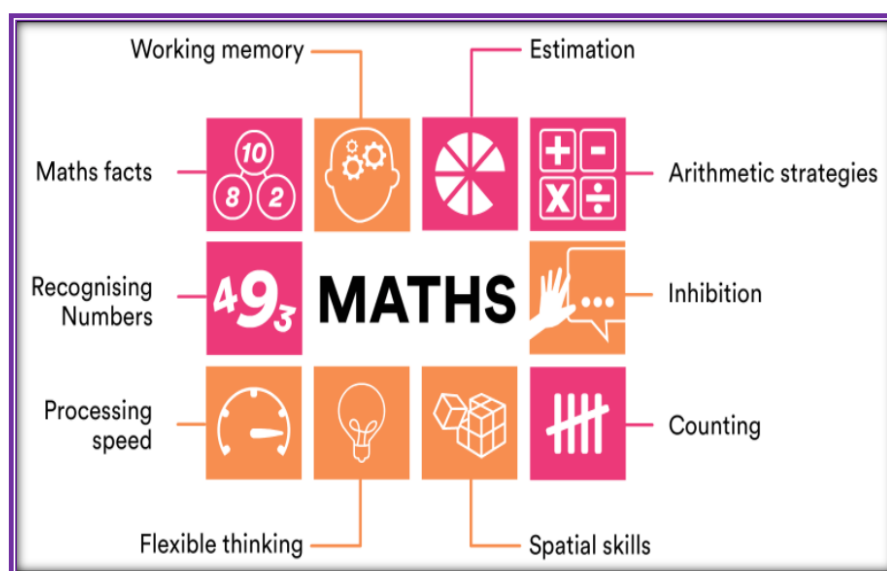


Figure 1.11 Domains of development active when solving mathematical problems Source: *e-learning resource Preterm Birth Information for Educational Professionals* www.pretermbirth.info (accessed March 21st 2022)

Scores achieved in general conceptual ability assessments are similar to intelligence quotient (IQ).(144) In a meta-analysis (27 eligible studies) of general conceptual ability (GCA) of preterm (n=3504) and term infants (n=3540) at 4 years, preterm infants scored 11.94 points less in IQ score (95% CI: 10.47-13.42, $p<0.001$) in comparison to term born infants.(145) Allotey et al considered the degree of prematurity (grouped as

very preterm, MLP and LPT infants) had an intermediate effect on full scale IQ (SMD: 0.70; 95% CI: 0.73 to 0.66; $I^2 = 70.8\%$), performance IQ (SMD: 0.67; 95% CI: 0.73 to 0.60), and verbal IQ (SMD: 0.53; 95% CI: 0.60 to 0.47) (126). As IQ has been correlated with problem solving and educational performance hence children with a high IQ generally excel academically (146).

However conflicting results were reported by Odd et al from the Avon Longitudinal Study of Parents and Children (ALSPAC) (n=14000 participants) which included MLP infants (n=741 or 5.4% of total eligible population).(147) Cognitive functions was investigated in 6957 infants (randomly chosen 50% of participants) at 11 yrs. Findings reported no variation in summary IQ measures between MLP and term born infants ($p=0.059$). When the MLP infant group was split into a MPT and LPT infant group, there was still no difference found between IQ scores between both groups ($p=0.430$). It is also worth considering that Odd et al's findings were based on one study, at one timepoint with assessment carried using an identical tool and participants from the same location at 11 years. By comparison Allotey et al's conclusion was based on a review which included multiple studies (n = 74; 64 061 children) of children at 4 years, using multiple methodologies from multiple geographical locations across a prolonged time frame.

1.3.5.3 Special education needs (SEN)

Special education needs (SEN) are defined as learning difficulties that require special education provision, including children with a physical disability that prevents them using school educational facilities.(148) There is an elevated incidence of SEN amongst children born preterm based on assessments carried in younger children or young adults up to 19 years.(149, 150, 151, 152) This is not unique to preterm infants

alone but a higher incidence has also been identified amongst infants born 37-38 weeks GA versus infants of 39-40 weeks birth GA.(121, 153)

Van Baar investigated the SEN of a MLP infant group (n=377) and term control infants (n=182) after 3 years in school (approx. 8 years of age) (151) using the Child Behaviour Checklist, a screening tool completed by parents and teachers. Overall the MLP infant group had a 7.7% SEN requirement in comparison to 2.8% of the term control group. When GA was further subdivided into moderately preterm (MPT) (32-33 weeks GA) and late preterm infants (LPT) (34-36 weeks birth GA) results indicated that SEN requirement was 9.7% and 7.3% respectively. Based on data obtained through the Avon longitudinal study of parents and children (ALSPAC) (154, 155), Odd et al identified MLP infants(n=885) as almost 50% more likely to have SEN requiring support in comparison to the healthy term born infant (n=13102).(156). Reports linking birth GA to school census data at a median age of 12 years (n=362,688) concluded that 7.6% children born between 33-36 weeks GA (n=18035) had SEN. (121)

1.3.5.4 Behavioural Issues

Problems are not limited to cognitive functioning for the MLP infant group. Developmental concerns include social and emotional difficulties with behavioural difficulties. Johnson et al investigated the social competence of MLP infants at 2 years PMA (n=1130) and a term control group of infants (n=1255) using two parental completed questionnaires, namely the Brief Infant and Toddler Social Emotional Assessment (BITSEA) and Parent report of Children's Abilities-Revised (PARCA-R) questionnaires.(118) MLP infants were at an increased risk of delayed social competence (26.4% in MLP infant group versus 18.4% of term control group).

Similarly, Cheong et al described MLP infants as having a higher prevalence of social behavioural issues scoring four times higher than the term control group. (4) Higher scores imply MLP infants have a predisposition to more challenging behaviour which increases with decreasing birth GA.(64, 157) At 7 years Huddy assessed hyperactivity scores of children with a birth GA of 32-35 weeks(n=117) using the Strengths and Difficulties Questionnaire.(158, 159) This study described 19% of participants having an abnormal hyperactivity score (10% is the population norm), 25 % were in receipt of non-teaching assistance with a higher incidence of emotional, conduct, hyperactivity and peer related problems (94). In Allotey's analysis, MLP infants were identified as being three times more likely to have a diagnosis of ADHD. (126) However, the strength of association of prematurity to hyperactivity was not shown to be inversely related to GA at birth, with 21.7% of infants with a birth GA of 35 weeks were reported as hyperactive in comparison to 8.8% of infants with a birth GA of 32 weeks GA. (94) Odd et al found no differences in scores achieved in attention screening (taken from the Everyday Attention for Children) between MLP infants and term born infants at 8yrs (n=6132) ($p=0.099$). (147) In a smaller study Wang et al also supported these findings with no difference found in attention/working memory between infants born 34-36 weeks gestation in comparison to term born infants.(107) Difficulties in sustaining attention will generally have a knock-on effect on learning and educational outcomes and an increased need for teaching and non-teaching assisted help.

1.3.5.5 Neuromotor dysfunction

Reduced mortality has also led to a higher prevalence of motor abnormalities, poor coordination and conditions such as cerebral palsy (CP) amongst surviving preterm

infants.(160) A strong correlation is evident between prematurity and a diagnosis of CP with the absolute risk of CP decreasing with advancing birth GA.(161, 162, 163, 164). Pierrat et al. reported 1.0% of infants of 32 to 34 weeks birth GA having a diagnosis of CP at 2 years of age. (165) Although more mature infants usually experience better outcomes, MLP infants have shown a six times higher prevalence of CP than children born at term (7 per 1000 vs. 1.1 per 1000 live births).(166) Marrett et al's findings that 4% of children born at 33 weeks GA and 1% of children born at 34 weeks GA are diagnosed with cerebral palsy at 5 years which is 10 times higher than that expected based on the general population norms.(92) Han et al's study of preterm infants <1500g (n=448) described a prevalence of 14.5% of CP, which decreased to 3.1% amongst infants weighing > 1500 g at birth.

Based on parental completion of the Ages and Stages questionnaire coupled with neurodevelopmental outcome at 2 years corrected age (n=3321), Leroux et al indicated an overall suboptimal neuromotor status in 10.7%(n=355) of participants which when subdivided by birth GA, 3% (1.9-4.8) of infants born between 33 and 34 weeks had a diagnosis of CP.(167) Williams et al identified non-CP motor skill impairment such as developmental coordination disorder (DCD) as a common negative outcome of pre-term birth and impaired motor skills such as those observed in children with DCD are commonly associated with deficits in educational, behavioural, and social domains.¹⁷⁸

1.3.5.6 The impact of Socio-economic disadvantage

Evidence of developmental delay is more prevalent amongst MLP infants born within disadvantaged groups. Lower parental education, and socio-economic status have

been associated with poorer developmental outcomes.(137, 149, 168, 169). Through linear regression, the additional variable of poverty was tested amongst preterm and term born children based on data generated through the Millennium Cohort study in the UK.(170) Children born preterm and living in poverty had poorer outcome and scored 0.3 to 0.4 SD worse than children not living in poverty on all assessments.(169) Overall, there is a paucity of research published on developmental outcome of the MLP infant in low to middle income countries (LMIC) countries. This may in part be attributed to lack of reliable data and the capacity to invest/prioritise research with clinical follow up. In such environments socio-economic disadvantage and lack of education further contribute to the potential adverse outcome the MLP infant group.

In summary, MLP infants have double the risk of neurodevelopmental disability (almost exclusively cognitive deficits) compared with term-born infants.(137) Although survival without neurodisability in the preterm infant increases with advancing GA, a higher degree of poor cognitive development and academic performance, language and mathematics scores, and the need to be enrolled in special education programs is evident amongst children born between 32-36+6 weeks GA. Whilst developmental deficits of MLP infants may be more subtle and varied in comparison to the very preterm infant there appears to be less evidence of MLP exhibiting 'no' deficits in comparison to their term counterparts.

1.3.5.7 Opportunities to identify and improve outcome for MLP

infants

The causes of adverse outcome for the MLP infant must be considered multifactorial and incorporate several etiologic pathways not limited to birth GA, nature of neonatal

course and duration of neonatal unit stay. Maternal age, level of education and socioeconomic background are factors that also warrant consideration in determining a child's risk profile and the degree of surveillance warranted. (109, 169, 171, 172, 173).

The weight of evidence supporting suboptimal developmental outcome in the MLP infant group has implications for obstetric practice in providing a case for delivery before 37 weeks or increasing the degree of monitoring and surveillance. Pre-eclampsia toxemia (PET) and interventional delivery (174) and male sex (175) are identified as antenatal/perinatal risk factors for poor school performance which Johnson et al. also identified as independent risk factors for cognitive impairment.(137) Parental input into all decisions relating to provider-initiated delivery should be informed with regards to preterm infant care and rates of mortality and morbidity.

Pre-discharge assessment tools may provide early identification of infants who warrant close surveillance and targeted interventions. Large longitudinal studies with consideration for birth demographics, data obtained through school-age testing using validated assessment tools (incorporating parental and teacher input) must be considered gold standard in determining developmental outcome to specific GA groups. Robust neurodevelopmental research studies will have implications for resource allocation to assist in the provision of a supporting pathway of multidisciplinary surveillance for all preterm infant groups.

Alteration in a phenotype as a result of experience is particularly relevant to the preterm infant who may be exposed to high levels of stress at a sensitive period of development. This epigenetic imprint may potentially alter the normal trajectory of

development. (22, 78, 105, 176) Reducing stress and negative environmental exposure may positively impact the trajectory of network development.

Recent trends have shown a small decrease in adverse developmental outcomes in all preterm infant groups than what has previously been recorded. It is likely that education pertaining to neuroprotective measures, optimal environmental measures and advances in neonatal technology have contributed to improved outcomes. Environmental factors will be discussed in further detail in section 1.3.6.1.

Improving the preterm infant's neurodevelopment outcome is a key aim of neonatal research. A Cochrane neonatal review supported a positive influence of early intervention programmes on cognitive and motor outcomes which persisted into school age years. (177) Follow up strategies must also be available for the 'at-risk' infant which will further assist in ensuring early interventions (such as the need for physiotherapy, speech and language therapy) having the greatest positive impact on outcome of the infant's overall quality of life.(177) Early intervention must be coupled with an enriched supportive home environment with high cognitive stimulation.

MLP infants meeting developmental milestones in the early years require ongoing surveillance as deficits may not develop until early childhood years. In order to improve the lives of children affected by impaired learning, educational professionals must have appropriate training to ensure challenges and the early signs of learning difficulties acknowledged. Active engagement with parents will contribute to identification, diagnosis, intervention and provision of support to the child with learning difficulties (178)

1.4. Neonatal sleep

Sleep is the predominant behavioural state of the newborn.(179) Preterm infants may sleep up to 90% of the day whilst this may fall to ~70% for the term born infant. (180) There is no one all-encompassing definition of sleep, but rather variable definitions exist with common threads. The literature describes sleep through behavioural observations, utilising terms such as reversible diminished responsiveness, inactive nervous system, suspension of consciousness, suspension of muscle activity or muscle relaxation, eyes closed, hypotonic and for the most part motionless +/- noisy nasal breathing.(13, 126) Much of the scientific literature has focused on disorders of sleep and conditions that impact sleep with less on the positive aspects of sleep.(181)

1.4.1 The functions of sleep

Infant sleep is a regulated neurological function and a prerequisite for normal neural network development, synaptogenesis, synaptic plasticity and for overall health and wellbeing.(6) Structural and functional connectivity in the brain coincides with the ontogeny of sleep. Therefore, sleep may be used as a framework in understanding the early organisation of brain networks and the implications of a neurological insult (e.g. hypoxic ischaemic encephalopathy) on later development.

Sleep is a prerequisite for neural reorganization and growth of the brain's structure and function. During sleep, synapses are forming, connections are established and there is a conservation in energy.(182) Sleep is of particular importance during the critical period of development and enhanced neurological activity, that occurs during the MLP period.

Evidence suggests that the brain drives sleep for its own maturational needs(183) requiring the interplay of multiple neural circuits and cellular factors.(184) The states that constitute the main sleep states are evident from 30-32 weeks GA and identified as active sleep (AS) (see section 1.4.2.1), quiet sleep (QS) (see section 1.4.2.2) and transitional periods of indeterminate sleep (IS) (see section 1.4.2.3).(185) Whilst AS and QS may be considered a precursors to rapid eye movement (REM) sleep and non-rapid eye movement(NREM) sleep respectively they are not synonymous.

Both AS and QS are prerequisites for neuronal development, but the contribution and role of each are likely to be different and unique to gestational age.(186) During sleep memories are stored and its restorative nature is associated with tissue repair(187), thermoregulation and metabolic regulation (188) and psychological wellbeing.(112) Sleep also has a role in brain housekeeping and the clearance of toxins and metabolic waste products that accumulate during wakefulness.(189, 190) By downregulating specific biological processes during sleep, there is an overall energy conservation of up to 10% in the newborn potentially enhancing weight gain.(191)

Theories of sleep explored include:

- The Inactivity theory (animals sleep at night as less die from injury in darkness),
- The Energy Conservation theory (decrease in body metabolism by up to 10%),
- The Restorative theory (allows body an opportunity for tissue repair, muscle repair and the production of hormones for growth which are increased at night)
- The Brain Plasticity theory suggesting sleep is necessary for neural reorganisation, growth of brain structure and function.(182)

1.4.2 Sleep regulation

States of sleep and wakefulness are regulated, co-ordinated and promoted by the hypothalamus, thalamus, brainstem, pineal gland and basal forebrain. The preoptic area (POA) plays an important role in the initiation and maintenance of sleep.(192) This area contains clusters of sleep activating neurones that increase firing during sleep and inhibit the arousal centres of the brain. (193, 194)

Due to immaturity of the brain at birth, newborn infants have an unprogrammed and poorly developed circadian rhythm. A sleep-wake ultradian rhythm is functional in the early postnatal period with multiple short bouts of sleep and wakefulness throughout a 24hour period. Sleep may be dictated in part by a need for feeding or by the homeostatic regulation of sleep pressure. (195, 196) The drive for sleep gets stronger the longer the infant is awake and decreases during sleep, being at its lowest after a prolonged sleep period.(197)

Cortisol, melatonin, body temperature, blood pressure, and digestion consolidate sleep and are all part of the circadian rhythm.(198) Fetal adrenal steroidogenesis and the secretion of cortisol commences as early as the eight week of gestation. By nine weeks postnatal age, the suprachiasmatic nucleus signals the pineal gland, to increase its production of melatonin promoting the coordination of sleep and darkness solidifying sleep by night. Body temperature rhythm and that of circadian genes develop at 11 weeks.

Within the hypothalamus, the suprachiasmatic nucleus (SCN) contains light-sensitive cells that trigger the circadian rhythm. By producing an alerting signal during daylight, the SCN or biological clock offsets sleep drive whilst it promotes sleep by turning off the alerting signal. In addition, the SCN actively maintains sleep throughout the night even after the sleep drive has decreased in the second half of the night. The activity of SCN neurones also changes in parallel to cycling between sleep states as evidenced by an increase in SCN activity during AS and waking and a decrease during QS.(199)(Fig 1.12)

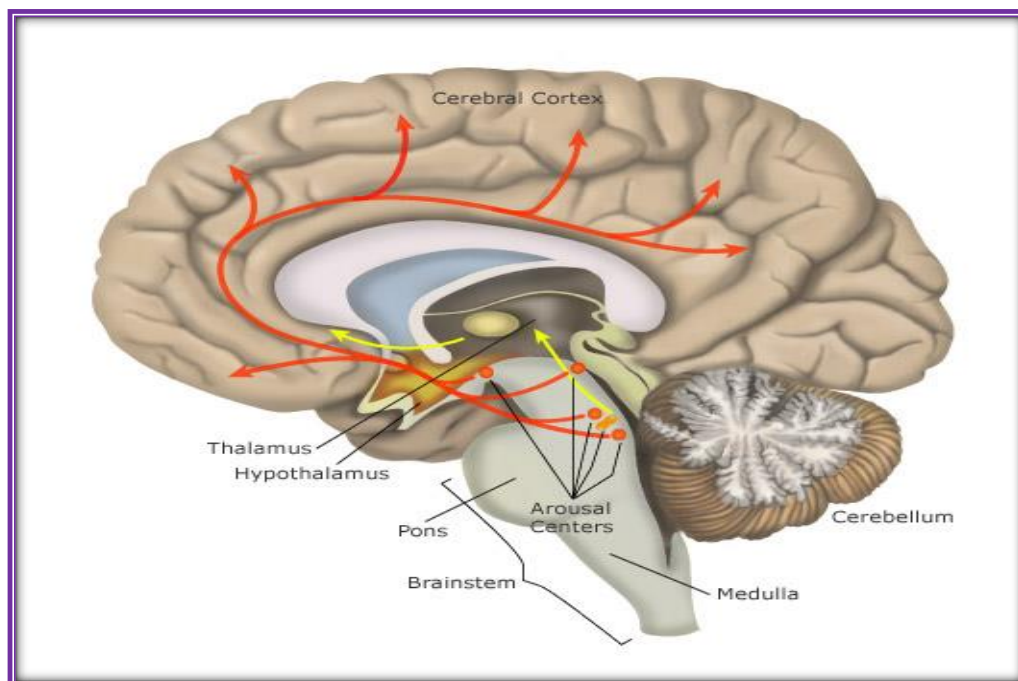


Figure 1.12 Anatomy of the brain indicating the arousal centres
downloaded from <https://sleep.hms.harvard.edu/education-training/public-education/sleep-and-health-education-program/sleep-health-education-48> (200)

The accumulation of metabolites such as adenosine (a chemical by-product of cellular energy consumption) during wakefulness has a signalling function which shifts the balance toward sleep. GABAergic neurons (located in the hippocampus, thalamus, basal ganglia, hypothalamus and brainstem) produce Gamma-aminobutyric acid (GABA), an inhibitory neurotransmitter reducing neuronal excitability by inhibiting nerve transmission and histaminergic cells (required for regulating arousal and

maintain wakefulness) and promoting AS.(193) GABA is more active in QS than AS. The reticular activating system (RAS) in the brain stem facilitates alteration in brain wave activity that occurs between slow sleep rhythms and fast sleep rhythms and for general cortical activation. (201)

1.4.3 Sleep in the neonatal unit environment

The uterine environment is controlled with minimal sensory input providing an optimal environment for development and maturation of the fetal brain. Infants born too soon lose this regulated naturally supportive environment in exchange for the extrauterine environment of the neonatal unit in which the developmental process must continue.

The growing brain of the preterm infant is especially vulnerable to environmental influences for which preterm brains are poorly prepared. Sensory overload caused environmental noxious factors (including visual, auditory, tactile and painful stimuli) within the neonatal unit during a critical period of development may disrupt normal neuronal rhythms and synchronized oscillatory activity that may be essential for the formation of neuronal cortical circuits. (202) The protective nature of neuroplasticity may not be sufficient to overcome prolonged and continuous exposure physiological stressors, with tolerable stress evolving to toxic stress which may weaken brain circuits and impact development.(203, 204, 205, 206, 207) Alteration in the pathway of development may lead to subtle or profound long-term effects.

1.4.3.1 The neuroprotective nature of sleep

Sleep is a naturally protective prerequisite for normal cortical organisation (208, 209, 210, 211) and a buffer in protecting infants from the negative impact of the

environmental stressors of the neonatal unit. (208, 212) Disturbance to the normal ontogeny, quality or quantity of sleep and the multiple neurological processes that are actively taking place, may lead to a reorganisation of neural networks, with a positive or negative effect on development.(213)

High disability rates remain evident amongst surviving low birth weight very preterm infants. Exposure to stress and interference with sleep may underlie many of the soft and subtle deficits experienced by the preterm infant with downstream effects causing generative changes with lifelong effects. (177, 214) The challenge for the clinical team is to identify specific risk factors for impaired sleep and to support interventions that will improve the quality and quantity of sleep for the infant in the neonatal unit. (6)

1.4.3.2 Sensory exposure

Sleep may be disturbed in an environment with high levels of noise (209, 215, 216), light (113, 217, 218), frequent handling and painful procedures (204, 205, 219) overloading the immature sensory system and negatively impacting physiological responses. (220) Identifying optimal light and sound thresholds of (which alter with advancing GA and clinical condition) in the neonatal unit continue to pose a challenge to the clinicians and managers who play a critical role in early regulation of stress and the preservation of the normal neurodevelopment trajectory of the preterm infant. (221, 222, 223) Our knowledge pertaining to the timing and exposure to environmental stressors and the impact it may have on maintaining neural, neuroendocrine, and immune systems integrity remains incomplete.

1.4.3.3 Environmental modifications to improve sleep

Several components of care in the NICU can contribute to sleep disruption. As part of this thesis we investigated factors related to the number of overnight disruptions to sleep cycles of healthy MLP for the purpose of feeding which will be discussed in Chapter 3.(224) Levy et al. described their observations concluding that only 50% of neonates in the NICU completed a complete sleep cycle before being disturbed for care. (212)

The recommended limit for noise in the neonatal unit is 45 decibels, as per the American Academy of Paediatrics Committee on Environmental Health.(225) Despite this recommendation, noise levels frequently rise above this acceptable limit and fall on the sensitive ears of the immature preterm infant. Modifying the environment may require reducing telephone ring tones, bleeps carried by personnel, removing printers to an area outside the neonatal unit and lowering and limiting cotside conversation. Sound may also be modified through the use of earmuffs and reducing volume on alarms. (209, 226) Acoustic detectors such as visible ears on the wall of the neonatal unit serve as traffic light systems for noise levels and as a reminder to clinicians and parents when sound is at an acceptable level, escalating when noise levels have exceeded the set recommended limits (Figure 1.13)

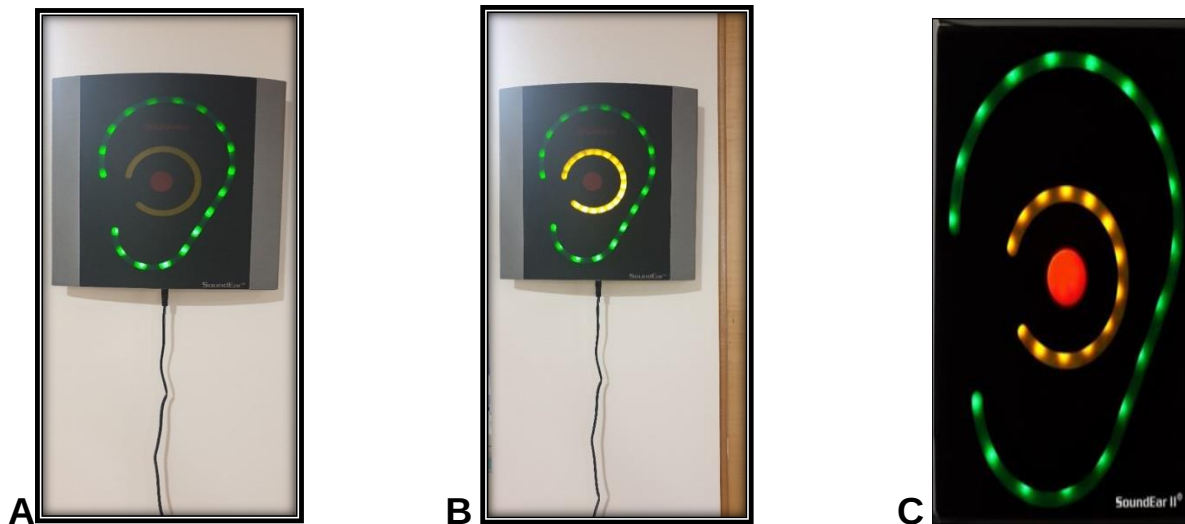


Figure 1.13 Acoustic sensors on the walls in CUMH. A visible ear which serves as a traffic light system measures noise levels in the neonatal unit. The outer Green area in **Ear A**- informs staff that the noise level is within normal limits. **Ear B**- the noise level is increasing. **Ear C**- the noise level has exceeded the manual set upper limit in decibels.

Daily specific time periods or ‘quiet periods’ in NICU are observed, during which lights are dimmed and no unnecessary procedures are carried out. Cycled lighting which mimics day and night helps to develop normal transition to night-time sleeping patterns. Supporting dimmed or cycled lighting and adapting lighting between “cares” will support sleep and reduce disturbance.(227) Lights should be dimmed to a safe level where the neonate can still be seen with all neonates assessed on a regular basis with additional lighting.

The clinical demands of the nursing team for feeding preterm infants in the NICU are generally time-based and carried out two hourly, three hourly or four hourly depending on weight, clinical need, previous feed intake and parental presence. This comes at a cost in terms of disturbing and fragmenting sleep to ensure adequate nutritional intake. An emphasis on routine caregiving based on infant cues whenever possible, rather than scheduled “care times”, is preferable. Clustering nursing care activities together and clinical interventions allowing more sleep between interventions have also been

considered as a protocol for developmentally appropriate care. (212) Developmental care emphasizes protecting sleep and uninterrupted sleep cycles and allowing rest periods of at least 60 minutes to complete a normal sleep cycle.

There is a need for continuous investment in staff education pertaining to sleep and neurodevelopmental practices that support optimal neurodevelopmental in the NICU. Staff and parent education about the benefits of sleep, kangaroo care supporting skin to skin, clustering care, in a safe environment will positively impact developmental outcomes. (228, 229, 230)

1.4.4 Neonatal individualised developmental care and assessment programme (NIDCAP)

With an increased emphasis placed on developmental outcome in preterm infants, many neonatal units have structured neonatal individualised and developmental care and assessment programs (NIDCAP) for preterm infant care. All infants differ in the continuum of development, a factor which in itself supports a need for an ongoing behavioural assessment which will support the provision of a developmentally appropriate, brain sensitive neuroprotective care to all high-risk neonates.(42, 231) An individualised developmental support plan is based on observation of the infant and his/her ability to regulate their physiological or neurological status within a specific environment or following a clinical or nursing intervention.

The NIDCAP strategy is based on a set of general principles which incorporates environmental modifications whilst recognising the importance of emotional

development in infancy. Based on the synaptic theory of development, emphasis is placed on the interaction of various subsystems of development unique to GA and is dependent on the environment to positively impact development. Environmental modifications aim to optimise sleep, reduce stress and protect neurodevelopment whilst maintaining parent infant interaction in the neonatal environs.(232) NIDCAP principles also promotes and supports family centred care (FCC) with parental involvement in making decisions about their infant(s).(Fig 1.14) FCC warrants consideration for cultural differences and evidence-based interventions to each family. Parental involvement as a prerequisite for physical well-being as well as for the child's cognitive, motor and social affective interactive development.(233, 234)

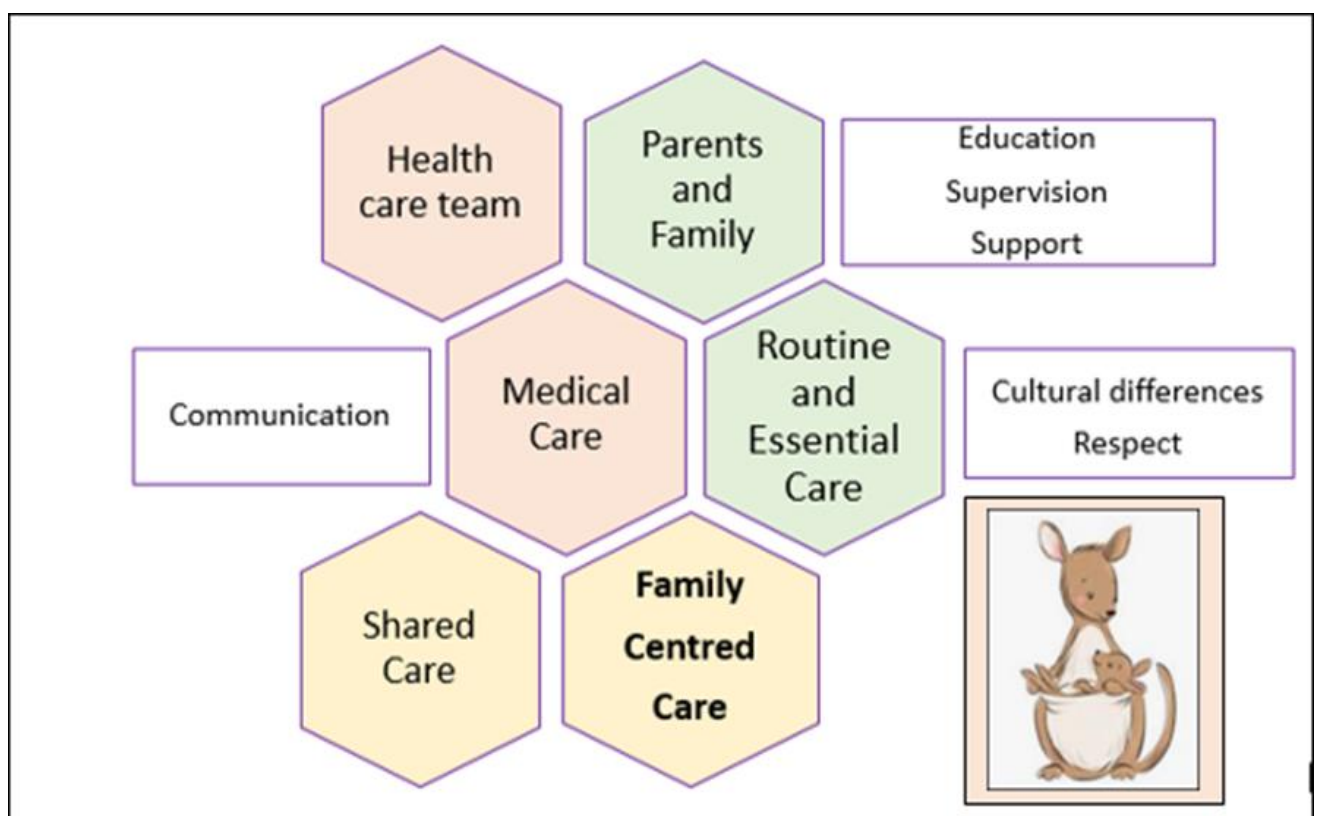


Figure 1.14 A model of Family Centred Care (FCC)

Als et al. have produced a large body of literature reporting on the positive affect of NIDCAP on the care of low birth weight preterm infants to include fewer days on mechanical ventilation, less time to establish feeding and hence reduced duration of enteral feeding, more rapid weight gain, shorter inpatient stay, lower age at discharge and an overall lower cost of care.(233, 235, 236, 237, 238)

In a randomized controlled trial of 38 preterm infants (≤ 29 weeks postmenstrual age) NIDCAP intervention demonstrated lasting effects into school age.(239) EEG coherence and developmental assessment in the NIDCAP intervention group (n=11) demonstrated better right hemisphere and frontal lobe function in comparison to a control group (n=11) at 8 years. The effect of NIDCAP on two groups of IUGR preterm infants (with comparable in health and demographics) was described based on EEG, MRI, growth and neurobehavior at 42 weeks and 9 months post menstrual age(PMA).(240) The follow up intervention group were healthier with improved brain development concluding that NIDCAP improved participants neurobehavior, electrophysical activity and brain structure.

However, not all researchers supported the findings of Als et al. In a randomised control trial Westrup et al. sought to identify the impact of NIDCAP on sleep maturation (identified as the duration of QS) (n=22) of very low birth weight preterm infant < 32 weeks birth GA. Infants were randomised to receive NIDCAP care (n=11) or conventional care within NICU (n=11). For the most part infants in the intervention group were cared for in single rooms and based on observation, an individualised care plan was devised. A twenty-four period of monitoring with aEEG was used as an objective measure to quantify the duration QS at 32 weeks and 36 weeks respectively.

Whilst the percentage of QS decreased with advancing GA on both intervention (NIDCAP) group and controlled group, there was no significant differences between the intervention versus control group on QS duration at 32 weeks (33.5% versus 33.3%) or at 36 weeks (24.5% versus 25.7%) PMA.

Als et al. also reported higher Bayley's scores at 9 months amongst low-birth-weight preterm infants in the NIDCAP intervention group. (241) Peters et al reported less disability amongst intrauterine growth restricted preterm infants especially in the area of mental delay at 18 months (NIDCAP: 10%; control: 30%; odds ratio: 0.25 [95%CI:: 0.08–0.82]; $P = .017$). Contrary to these findings, based on Bayley assessments carried out at 4,12 and 24-months PMA Ariagno showed 64% of the NIDCAP intervention group had low Bayley's scores versus 33% of the control group. Despite further investigation neither medical condition or socioeconomic demographics could explain this negative finding. (242).

Ohlsson and Jacobs conducted a systematic review and meta-analysis to determine impact of NIDCAP on developmental outcome. (232) Criteria for inclusion required a NIDCAP intervention to have been applied to inpatient low-birth-weight preterm infants. Research had to be of a randomized controlled trial design with a non-interventional control group. The primary outcomes were death or major sensorineural disability at 18 months corrected gestational age (CGA) or later in childhood (3 trials, 302 children; relative risk 0.89 [95% confidence interval 0.61 to 1.29]) and survival free of disability at 18 months CGA or later in childhood (2 trials, 192 infants; relative risk 0.97 [95% confidence interval 0.69 to 1.35]). The authors concluded that there is no clear benefits of NIDCAP for long term neurodevelopmental outcome or short-term medical outcomes. The duration of the intervention was not associated with neurologic

and developmental outcome. Outcome of this review was echoed in a study carried out by Wallin and Erickson in 2009. (243) These authors went one step further concluding that based on findings they could not recommend NIDCAP in its current form. However, Jacobs et al. (pg.705) acknowledged that *Modification of the extrauterine NICU environment and care giving according to each infant's current physiologic and neurobehavioral functioning is a rational and intuitive approach to caring for preterm infants and their families and to supporting infant development.* (244)

There does seem to be an issue with the research design amongst NIDCAP studies with multiple hypothesis and outcome variables measured. Differences between a control group and an intervention group may not have been captured and for the most part studies were small with minimal follow up. However, this does not imply that the impact of a NIDCAP approach to care does not have its benefits. Firstly, parental involvement in care and closeness to the infant will support parental wellbeing and will assist in embedding a parental bond. All positive experiences in early life support the establishment of a firm foundation for optimal brain development.(245)

The concept of minimal sensory exposure may be counter-productive, depending on the postnatal age of the infant, as areas such as the visual cortex are genetically programmed to use exposure to light to proceed along the normal trajectory of development.(246) A greater understanding of this process may help prevent developmental disorders that result from disturbances to neural activity.(214) More research is required in this area particularly with regard to the healthy MLP infant during a critical phase of development.(247) With the exception of the study by Westrup et al study there was little evidence of research quantifying the effect of

NIDCAP on infant's sleep.(248) Whilst it is important to provide a balanced viewpoint there is less evidence of negative consequences of a developmental approach to care in comparison to positive effects.

1.4.5 Kangaroo Mother Care

Kangaroo mother care (KMC) with skin-to-skin contact is a developmental approach to newborn care with that ensures a physical and emotional connection between mother/ father and infant.(Fig 1.15) KMC is associated with physiological stability, temperature regulation, improves growth and development, is associated with less arousals and enhances neurological and neuro-behavioural outcomes of preterm infants (13, 234, 249), whilst also reducing parental stress, anxiety and depression.(135, 250) Supporting parental wellbeing can only have a positive effect on an infant's long term physical and psychosocial development.

Skin to skin contact (SSC) has also been shown to accelerate brain maturation in healthy preterm infants at 40 weeks PMA.(249) Scher carried out sleep EEGs on preterm infants (31-32 weeks, n=8) who had SSC for 8 weeks and two non-SSC cohorts at term (n=126) one of which was preterm group at term PMA the other born at term GA. Preterm infants who had SSC showed increased EEG connectivity/ maturation closer to that of a term born infant in comparison to the preterm infant without SSC. This study supports the neuroprotective benefits of SSC in preterm infants.



Figure 1.15 Kangaroo mother care- skin to skin care

Family nurture intervention (FNI) is standard care but incorporates skin to skin care (minimum of 1 hr per session) in conjunction with mother and infant scent cloth exchange, gently sustained touch, maternal eye contact and maternal voice soothing. Walsh et al. conducted a randomised control trial to investigate if EEG activity was altered between standard of care alone (n=63) versus FNI (n=71). This study found that EEG power increased in both AS and QS in FNI group suggesting more stable sleep organisation, but the impact of these findings and the relationship to developmental outcome remains unknown.

Feldman et al. also sought to identify the effect of skin-to-skin care on sleep state organisation of preterm infants (n=146) with two groups (n=73 in each group) matched for birth weight, GA, medical risk, single/multiple pregnancy and family demographics (251). The intervention group received skin to skin care (n=73) and a control group not in receipt of skin to skin (n=72) were observed at 10 second intervals over a 4 hour period by trained observers to determine sleep state organisation. At term, cyclicity

had increased for both groups of infants but a higher rhythmicity in sleep and wakefulness was evident in the intervention group. Hence, infants who had skin to skin care showed more mature sleep. This study however did not control for interrater subjectivity.

A comparison study explored the effects of KMC versus in-arms-cradling on the sleep and wake states of stable MLP infants in a NICU in Tehran (n=72). (252) After a 20 minute pre-intervention period during which infants were placed in an incubator in a diaper only, infants were randomised to either 70 minutes of arm cradling by mother or Kangaroo care (KC) (skin to skin contact). Based on behavioural cues such as breathing patterns, facial expressions, body/eye/mouth movements, sounds and skin colour sleep state observations were carried out at 2 minute intervals. Sleep states were identified as one of six levels of sleep (including deep sleep, light sleep, drowsy, quietly awake and/or alert, actively awake and aroused, and highly aroused and agitated/crying). There was no difference in AS duration between groups however, the kangaroo care group had longer periods of QS ($p < .001$). The in arm holding group spent significant more time in light sleep, the drowsy state ($p < .001$), and the actively awake state ($p = .02$) than the KC group.

1.5 Electroencephalography and behavioural observation

Richard Caton (1842–1926), was the first to detect electrical activity in both hemispheres in animals, noting fluctuations and oscillations in activity. However, it was

Hans Berger (1873–1941) who conducted the first recording of electrical activity in humans in 1924, calling the device an electroencephalogram (EEG).(253). This recording was carried out on his 15 year old son from which he observed an 8–12 Hz rhythm while his son sat passively with his eyes closed and called it the alpha rhythm. (254) Through further EEG observation he commenced identifying age related features. Many scientists thereafter confirmed Berger's findings with scientific publications relating to specific features of activity in healthy and pathological conditions.

The EEG has made a major contribution to neuroscientific discovery. In clinical practice, EEG technology continues to have a high level of importance in the diagnosis of neurological conditions. It is a safe and non-invasive neuromonitoring tool providing a measure of electrical activity and information on the functional status of the cerebral cortex. In neonatology, EEG is especially useful as an unobtrusive cot-side tool in the NICU providing continuous real-time monitoring of brain activity of the critically ill infant with minimal burden to the infant (255). As EEG measures differences in electrical voltage it plays a key role in seizure detection and in monitoring the efficacy of treatment.(256, 257) .

EEG can be used to differentiate between levels of consciousness and provides a unique overview of sleep state organisation and an opportunity to gain further insight into the complex nature of sleep and the presence of sleep-wake cycling - a marker of neurological wellbeing.(258) EEG can be used to accurately identify disorganised and dysmature sleep state organisation of newborn infants.(10) However, the prognostic

value of electrographic monitoring in the healthy MLP infant remains unclear.(259, 260)

1.5.1 Physiological principles of EEG activity

An understanding of the neurophysiological processes that underpin EEG is a prerequisite to understanding its use. Neurons are the functional units of the central nervous system.(261) Synapse formation between neurons facilitates the transmission of electrochemical signals to a target neuron. Millions of neurons form synapses with other neurons, leading to trillions of connections between cells creating an information processing network through which information is transmitted within the central nervous system.

For the nervous system to function, neurons must be able to send and receive signals. Electrical potentials are generated across the membranes of neurons because there are differences in the concentration of specific ions across the membrane, mediated by ATP-dependent ion exchange pumps and the fact that the membrane has selective permeability to ion flow. This results in polarisation of the membrane (positive outside, negative inside) and a resting membrane potential of $-70\mu\text{V}$. For a given target neuron, inputs from other upstream neurons onto its cell body or dendrites result in the influx of either positive Na^+ ions (excitatory post synaptic potentials-EPSP) or negative Cl^- ions (inhibitory post synaptic potentials -IPSPs). If a number of EPSPs summate at the same time to depolarise the membrane beyond a certain threshold (-55mV), an all or nothing event, known as the action potential (Fig 1.16) occurs. First voltage gated Na^+ channels are activated, resulting in a rapid further depolarisation of the membrane to $\sim +30\text{mV}$. Following this rise, sodium (Na^+) channels inactivate and potassium(K^+)

channels activate and K^+ leaks out of the cell, leading to a decrease in the potential. Finally, all channels attain steady states and the membrane reverts back to the resting membrane potential. Ion flow is not only determined by ionic concentration gradients, but also is dependent on the selective permeability of ion channels as well as electrical forces that arise from the membrane potential.(22)

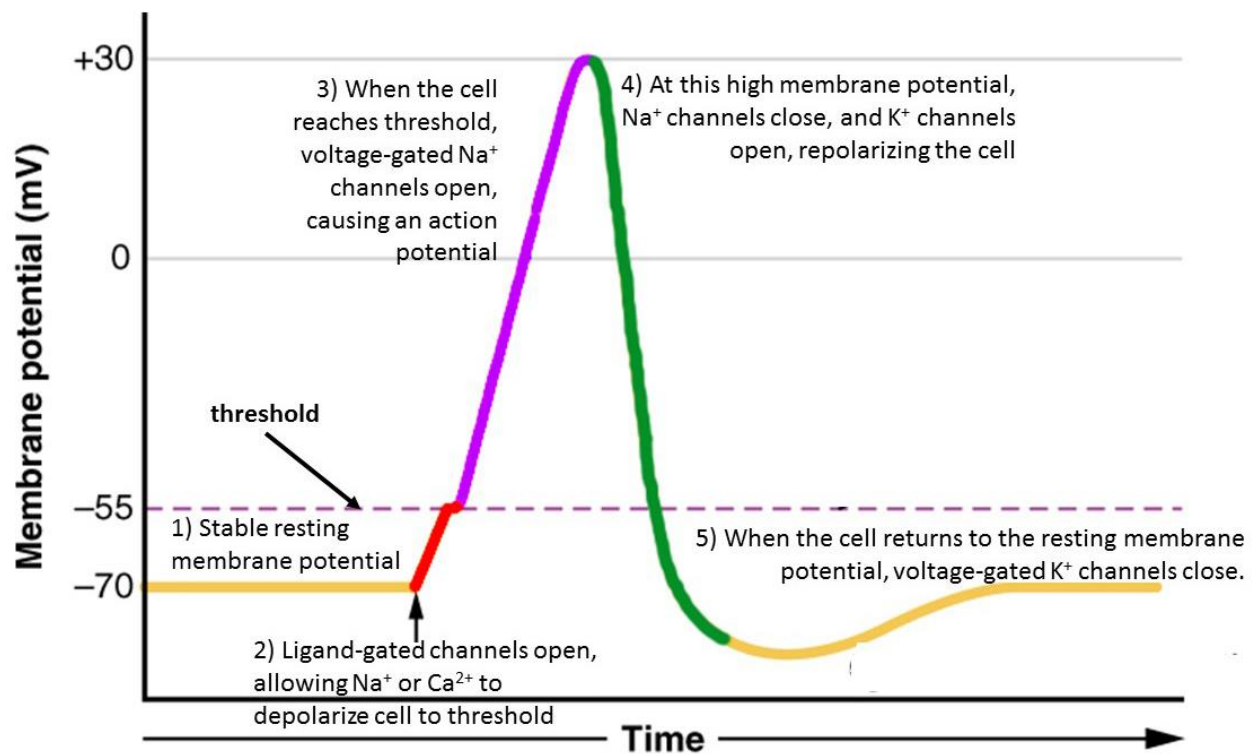


Figure 1.16 A schematic representation of an action potential.
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The action potential is rapidly conducted down the length of the axon. When an action potential, or nerve impulse, arrives at the axon terminal, it activates voltage-gated calcium channels in the cell membrane. Ca^{+} which is present at a much higher concentration outside the neuron than inside, passes into the cell depolarising the terminal and releasing neurotransmitter into the synaptic cleft. The molecules of neurotransmitter diffuse across the synaptic cleft and bind to receptor proteins on the postsynaptic cell. The excitatory post synaptic potentials require neurotransmitters

glutamate and acetylcholine, depolarizing the membrane and moving closer to the threshold for an action potential. An Inhibitory post synaptic potential involves neurotransmitters gamma-aminobutyric acid (GABA) and glycine which open chloride channels, hyperpolarising the membrane and decreases the likelihood a neuron will be able to fire an action potential. Inhibitory neurotransmitters such as GABA open chloride channels allowing negative Chloride ions (CL-) in, and hyperpolarising the cell. The normal function of neural networks is determined by a balance between excitatory and inhibitory synaptic input (262).

Scalp electrodes record potential differences that are caused by postsynaptic potentials in the cell membrane of cortical neurons. Hence activity displayed on EEG is generated by post synaptic potentials (263) and reflects the summed synchronous electrical activity generated by populations of neurons (pyramidal cells) in the more superficial layers of the cortex. (264, 265) The site of the synapse (dendritic vs cell body) may affect the polarity detected on the surface of the skull.

1.5.2 Visual analysis of sleep on EEG

Activity generated and displayed on EEG varies with degree of brain maturation and functional integrity of the brain. EEG activity is influenced by the interplay between genetics and the environment. Observed sleep behaviours of the neonate become more pronounced and integrated with recognizable patterns on EEG from 30 weeks GA.(13, 260) When reviewing EEG activity, consideration must be given to GA and any underlying pathology or medications given that may alter background activity. The most frequently used terms describing the architecture of sleep on EEG include frequency, amplitude, and continuity.(266, 267)

- 1.5.2.1 Frequency:

Frequency is a key descriptor of the EEG and simply describes the number of waves per second expressed in hertz (Hz). Neonatal EEG shows a mixture of frequencies including delta activity (1–3 Hz), theta activity (4–7 Hz), alpha activity (8–13 Hz) and beta activity (14–30 Hz).(268, 269) (Fig 1.17)

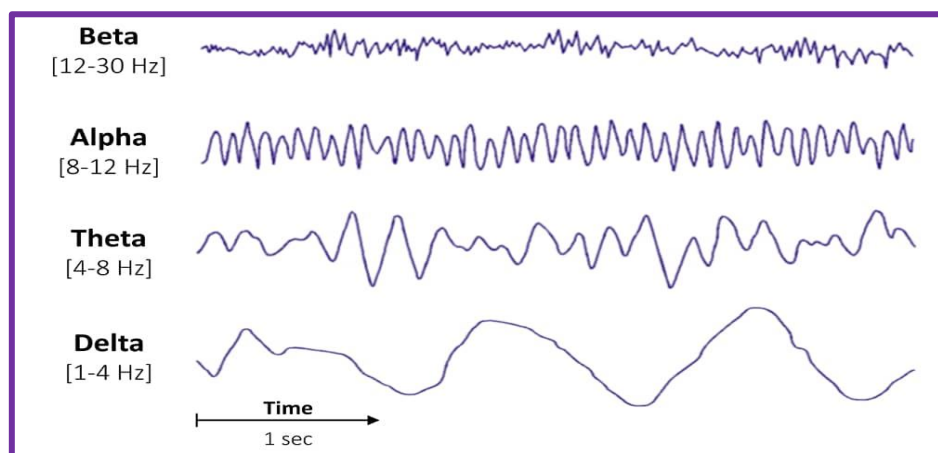


Figure 1.17 Frequency of brain wave activity through sleep and wakefulness.
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- 1.5.2.2 Amplitude

The magnitude of the brain wave activity detected is referred to as the amplitude of the EEG and is measured from the upper to lower peak in microvolts (μV). (14) Amplitude is noted to generally decrease with advancing GA. A decrease in amplitude of both delta and theta occurs with maturation. (270) (Table 1.3)

Table 1.3 Summary of amplitudes during sleep states of moderate to late preterm infants

Sleep State	Voltage value
Quiet sleep	50-300 μ V bursts of activity with IBI periods of $<25\mu$ V
Active sleep	30-70 μ V, may be <2 secs voltage attenuation of $<25 \mu$ V
Indeterminate sleep	100-200 μ V
Awake	70-100 μ V

Table devised based on literature by Tsuchida (271), Scher (272), Britton²⁷⁰ and Andre(273)

1.5.2.3 Continuity

Continuity on neonatal EEG refers to the relatively constant and uninterrupted presence of activity above a certain amplitude. This thesis defines continuity as predominantly over 25 μ V while periods of < 1 second of below 25 μ V are permitted. Periods > 1 second at $<25 \mu$ V are described as discontinuous. In the very preterm infant the natural occurring background activity is predominantly discontinuous becoming more continuous with advancing GA with a lengthening of burst activity and a shortening of inter-burst activity (Figure1.18-1.20).

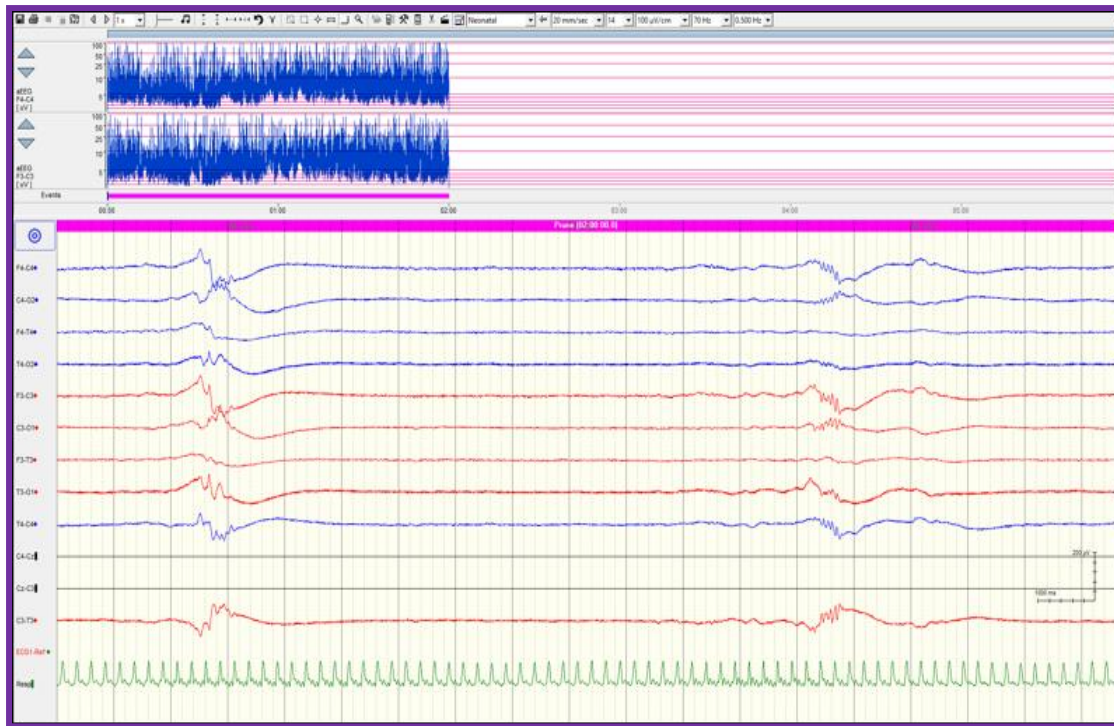


Figure 1.18 EEG monitoring of infant at 24 weeks birth GA, predominately discontinuous activity with bursts of activity every 30-60 seconds

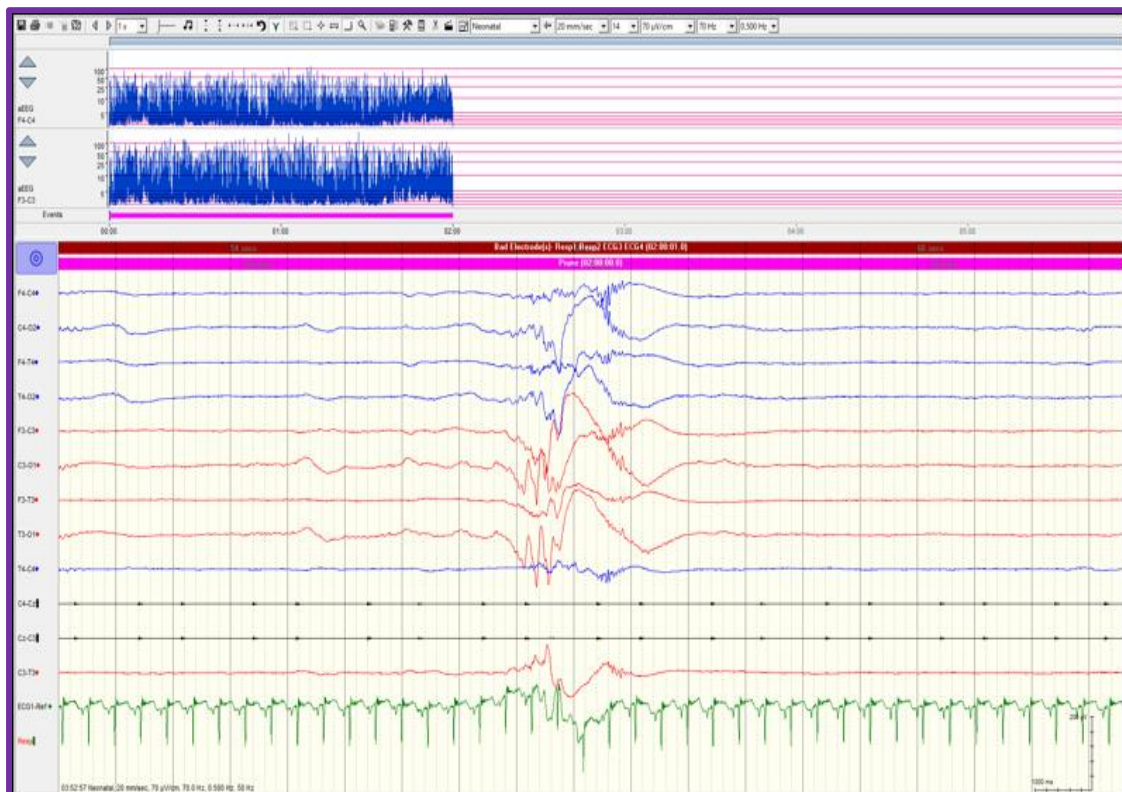


Figure 1.19 EEG monitoring of infant at 28 weeks birth GA- predominately discontinuous activity with 2-3 second bursts of mixed frequency high amplitude activity

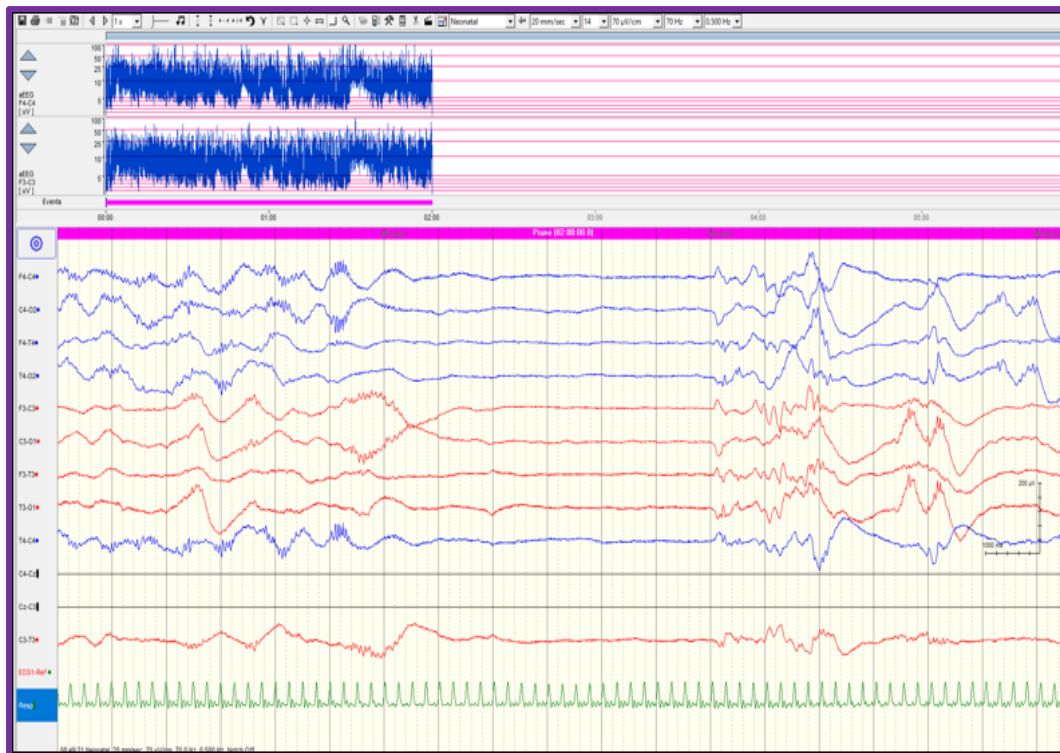


Figure 1.20 EEG monitoring of infant at 30 weeks birth GA – increasing continuity and decreasing discontinuity. AS is pre- dominantly continuous at this GA with short periods of discontinuity. QS remains discontinuous.

1.5.3 Sleep states of the MLP infant group

Three sleep states dominate MLP infant sleep. These are Active sleep, Quiet sleep and Indeterminate sleep

1.5.3.1 Quiet sleep

Quiet sleep (QS) is precursor of non-rapid eye movement (NREM) sleep has been identified from 25-27 weeks GA.(274) The function of QS is thought to relate to supporting the development and preservation of brain plasticity, consolidation of learning, memory and cognitive functioning (195, 275) and the maintenance of physiological homeostasis The synaptic homeostasis hypothesis proposes that at the end of a day of wakefulness and learning there is an increase in widespread synaptic strength and a subsequent increased energy requirement. While some circuits

containing pertinent information are strengthened during sleep, net synaptic strength is downregulated during the down-time provided during quiet sleep when there is a disconnection from the environment. This resetting of synaptic strength facilitates an environment for subsequent new learning (276) Developmentally, sleep onset changes from AS to QS by 4-5 weeks post-term. (274)

Discontinuity is described as relatively higher amplitude bursts of activity that alternate with intermittent periods of discontinuous activity or inter-burst intervals ie. $<25 \mu\text{V}$ on EEG channels. Discontinuity may be pathological i.e. associated with hypoxia ischaemia encephalopathy in the term born infant or as a normal pattern in the preterm infant EEG known as *tracé discontinu*. An increase in burst duration and a decrease in the inter-burst interval (IBI) occurs with advancing GA and is a measure of neuronal maturation. An alternating pattern of high voltage (50-150 μV) mixed frequency activity ≥ 3 seconds separated by an inter-burst interval or period of hypoactivity (lower-voltage activity which may be greater than or equal to 25 μV) on all EEG channels is termed *trace alternant*. (271, 277) (Figure 1.21, A and B).

Duration of IBI's appropriate to GA include up to 60 seconds amongst preterm infants 23-27 weeks GA, ≤ 30 seconds @ 28–29 weeks GA, ≤ 20 seconds @ 30–31 weeks GA, ≤ 10 –15 seconds @ 32–34 weeks GA and < 10 seconds @ 35–36 weeks GA. (13, 273) This decrease in IBI duration with advanced GA is in itself an indicator of neurodevelopment and has a positive association with cortical folding and normal cortical function. (278) Prolonged periods of quiescence or longer IBI were associated with reduced total brain volume at term. (279) IBI's will be discussed in greater detail in Chapter 5.

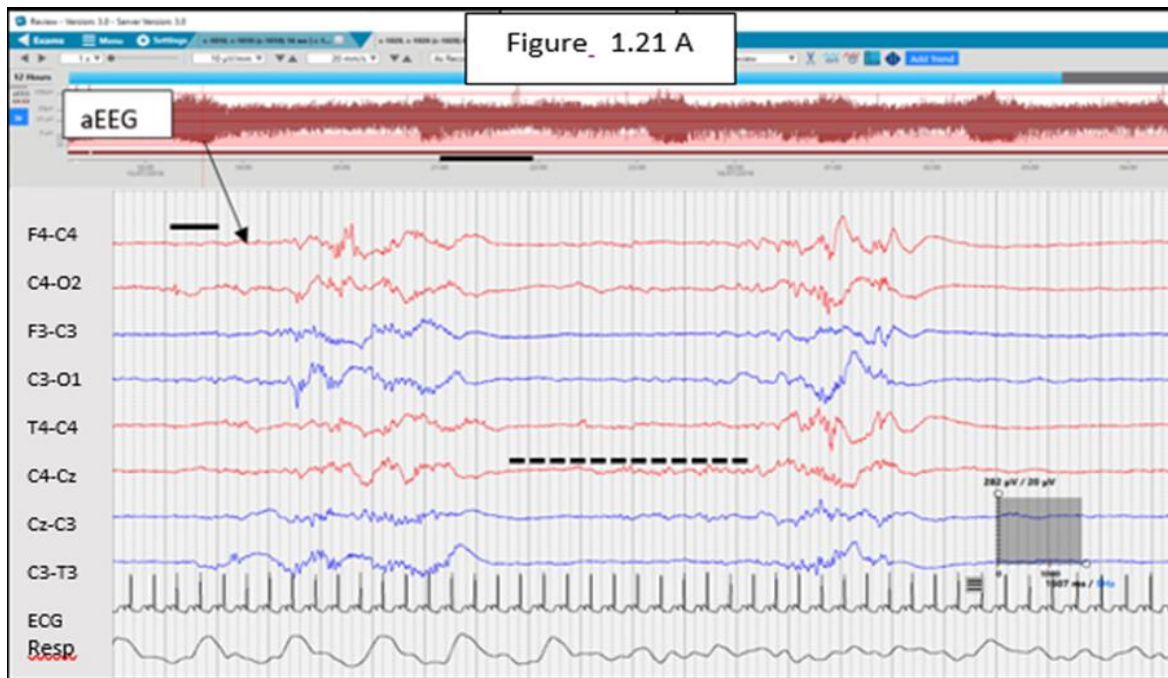
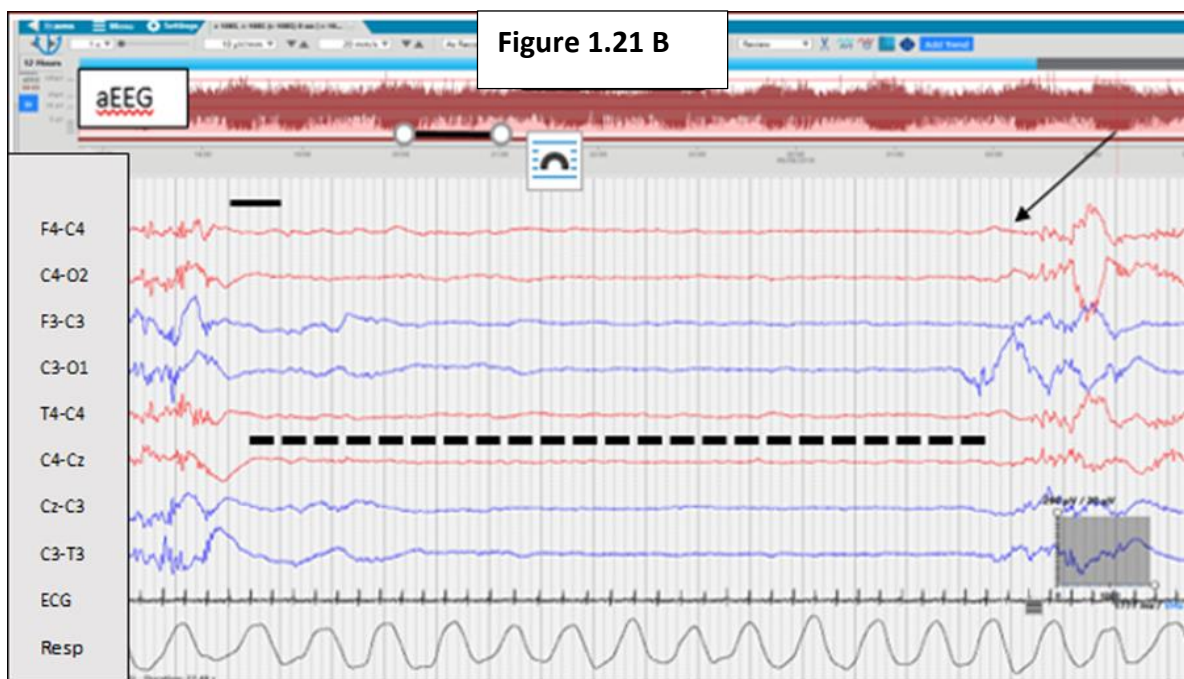


Figure 1.21 EEG activity during quiet sleep. The black horizontal line on raw EEG equates to 1 second of recording, the black line on aEEG equates to 1 hour of recording. **Fig 1.21a** Infant 36+3 GA at birth and recorded at -36+5 weeks PMA (Day 3 of life). EEG recording during QS – trace Discontinuity pattern. The IBI of <5 seconds is within the normal range. **Fig 1.21b** Infant 33+6 weeks GA at birth and recorded at 35+5 weeks PMA (Day 13 of life). EEG recording during QS – trace discontinuity pattern. IBI of >10 seconds (13 secs indicated by a broken line)..

1.5.3.2 Active sleep

Active sleep (AS) is a precursor of rapid eye movement (REM) sleep.(Fig 1.22) REM sleep has been identified in the fetus at 23 weeks GA but becomes more frequent between 24-40 weeks GA with variability noted amongst infants.(280) Sleep onset commences with AS in the newborn infant. As the dominant sleep state of the preterm infant during early brain development, AS with REM provides an endogenous source of stimulation i.e. neuronal discharges unrelated to the environment, facilitating axon growth, neural network maturation, establishing new connections/synapse formation and facilitating synaptic plasticity and overall normal healthy development of the central nervous system.(281, 282) This specifically relates to emotional, motor, psychosocial development (283) and sensory system development including visual, auditory, somaesthetic, olfactory, and limbic systems, as well as development of the hippocampus, pons, brainstem, and midbrain. A reduction in AS and reduced synaptic activity in the preterm infant may reduce the efficiency of information transmission, potentially leading to sensory motor issues and decrease overall brain volume.(284, 285) One such example includes the impact of AS deprivation on the connection between retinal ganglion cells and the lateral geniculate nucleus. Hence REM sleep deprivation, may result in underdevelopment of the central visual system.(286).

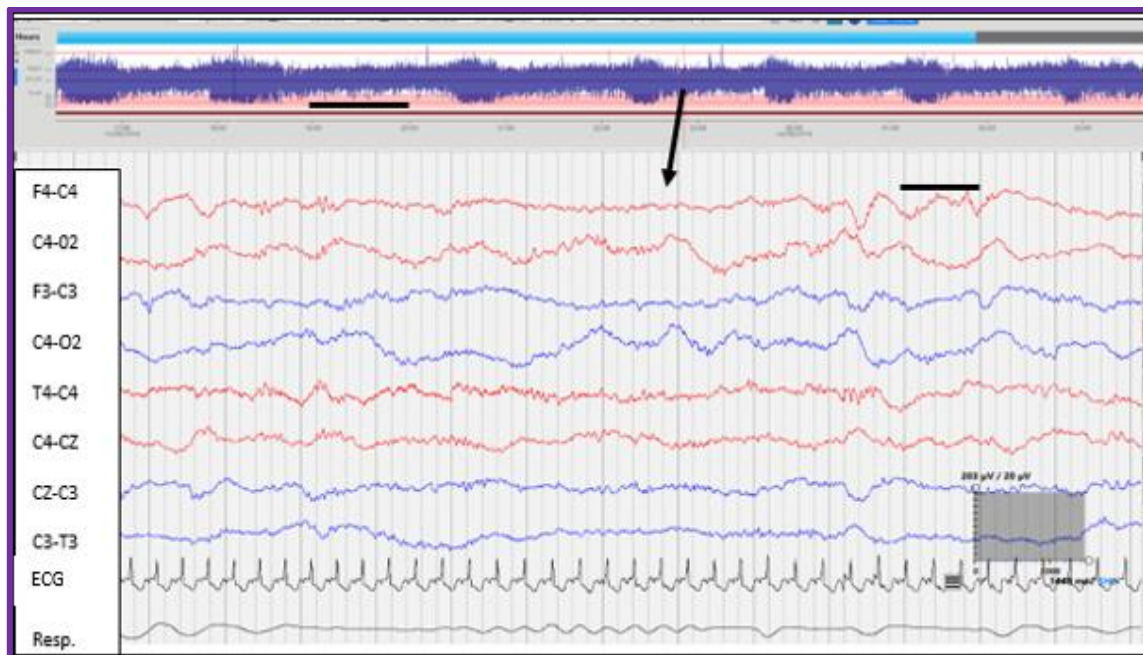


Figure 1.22 EEG recorded during AS. Infant 35+4 weeks GA at birth and recorded at 35+6 weeks PMA (Day 9 of life). Continuous mixed frequency activity is evident with low to medium amplitude and irregular respirations may be observed. The black horizontal line on raw EEG equates to 1 second, black line on aEEG equates to 1 hour of recording.

AS phase is the dominant sleep state in MLP infants with its duration gradually decreasing with advancing gestational age. (260) During AS the EEG displays a mixed frequency low to medium amplitude activity with a predominance of delta and theta waveforms. (273)(Figure 1.22). REM activity associated with AS will be described in section 1.5.3.2 (206, 287, 288) A higher degree of importance is placed on AS and REM activity in comparison to wakefulness with regard to supporting neural activity-dependent sensorimotor development of the preterm infant during early cortical development (289) .

1.5.3.3. Indeterminate sleep

Indeterminate sleep (IS)(Fig 1.23), a gradual transitional state where an infant demonstrates behavioural and electrographic features associated with both AS and

QS and wakefulness as he/she transitions between states. (185, 290) The functions of IS are non-specific in the literature.

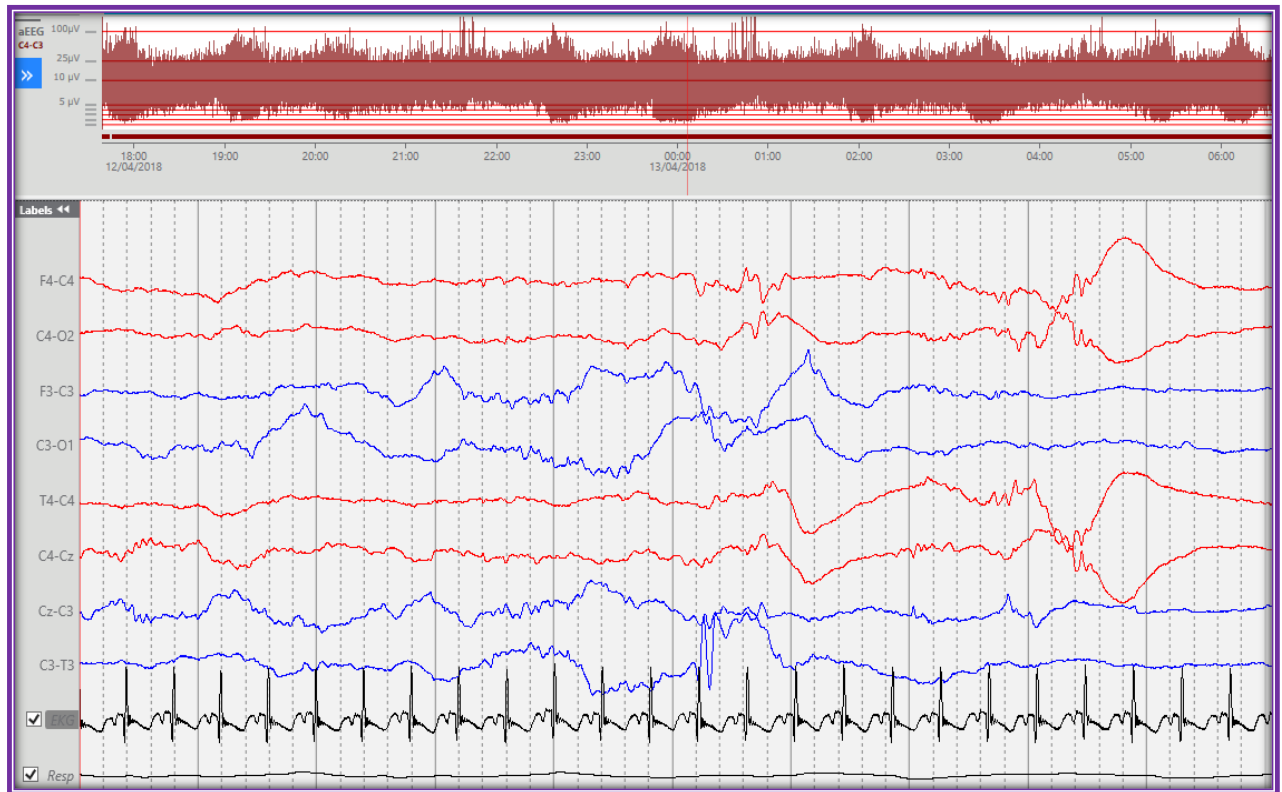


Figure 1.23 EEG activity during indeterminate sleep - incorporating low to mixed amplitude high frequency activity seen in AS and periods of hypoactivity <25µV seen in QS

IS does not fit into either sleep states with an activity referred to as semi-discontinuous (273) or undifferentiated (291)

1.5.4 Behaviour classification of sleep

We cannot physically observe fetal sleep. However, fetal movements (observed with ultrasound) and fetal heart rate (recorded on cardiotocograph) may be considered surrogate features of sleep states.(Table 1.4) A change in both indices may be associated with change in sleep state. From 30-31 weeks GA both eye and body movement along with respiratory rate become increasing concordant with changes in

EEG state.(13) Behavioural features observed during AS, QS, IS and wakefulness include:

In AS there is a range of motor activity of both upper and lower limbs and occasional sucking movements with abrupt rapid eye movements (REM)/eye rolling (Table 1.5). Infants may grimace or smile and exhibit periodic groaning or crying. Respirations become shallow and irregular with an increase in heart rate variability.(206)

In QS there is no REM activity seen. An atonic level of tone is evident, and the infant is more difficult to rouse. Motor activity is restricted to occasional startles and breathing and heart rate becomes more regular and slower than AS. In IS behaviours and respirations of each sleep state may occur which may include brief periods of eye opening as the infant transitions between states. Wakefulness may be expressed as quiet wakefulness, infant's eyes may be open but remains motionless, regular respirations and heart rate with EEG activity similar to that during AS or alternatively as actively awake with eyes open, gross motor movement of upper and lower limbs, unsettled, crying, irregular respirations and an increase in heart rate.

Table 1.4 Behaviour observations and background EEG and aEEG activity during each sleep state

	Active Sleep (AS)	Quiet Sleep (QS)	Indeterminate Sleep (IS)	Wakefulness
Behaviour	<p>Eyes closed, may open slightly, +/- REM</p> <p>Range of intermittent sporadic motor activity</p> <ul style="list-style-type: none"> - Low motor activity +/- Myoclonic twitching - Upper and lower limb movement <p>Occasional sucking movements</p> <p>Quiet and low muscle tone between movements</p> <p>Irregular shallow respiration, variable heart rate</p>	<p>Eyes closed-no eye movement</p> <p>Infant lying still / atonia</p> <p>May have some mouth/ sucking motion</p> <p>May have slight startle, jerk</p> <p>More difficult to rouse</p> <p>Respirations slower, more regular than AS</p> <p>Decreased heart rate variability than in AS</p>	<p>Periods of opening/closing eyes</p> <p>Slow eye movements</p> <p>Slow startles</p> <p>Intermittent sucking</p> <p>Regular respirations</p>	<p>Eyes open</p> <p>May be quiet, still or active</p> <p>Fussing or crying</p> <p>High intensity gross motor movements</p> <p>Irregular respirations with movement</p> <p>Variable irregular heart rate</p>
EEG activity	<p>Continuous activity above a threshold of 25μV</p> <p>Less than 3 seconds voltage attenuation <25 μV</p> <p>mainly high frequency >8 Hz, low/mixed amplitude (30-70 μV)</p> <p>Artefact with movement</p>	<p>Periods of discontinuity evident in QS</p> <p>High amplitude bursts 50-300 μV, minimum 3 secs</p> <p>Inter-burst intervals decrease with advancing PMA</p> <p>Artefact with movement</p>	<p>Continuous activity high voltage (100-200 μV)</p>	<p>Similar to AS in quiet wakefulness</p> <p>Mixed continuous activity</p> <p>Increased artefact with movement</p> <p>Duration increasing with advancing age</p>
aEEG activity	<p>Lower amplitude represented by narrower portion of band</p> <p>Normal: a lower margin > 5 μV and upper margin > 10 μV</p>	<p>High amplitude represented by wider portion of band</p> <p>Lower margin <5 μV in QS</p>	<p>Variable bandwidth</p> <p>Not discriminated on aEEG</p>	<p>Quiet wakefulness displayed similar to AS</p> <p>High amplitude activity with movement</p>

The behaviours identified above are based on the similar themes generated by Curzi-Dascalova(288), Prechtl (287) and Brazelton(292).

It is difficult to identify the exact timing of onset of AS and QS sleep states through behavioural observation alone in a preterm infant, due to frequent body movements associated with prematurity and rapid alterations between states.(293) Visual observation of respiratory state is a valuable characteristic for scoring sleep stage in the newborn.(185) In a study by Stefanski et al which designed a system for identifying sleep states in term and preterm infants there was a lower level of agreement of 77.6% in identifying sleep states based on behaviour pattern in comparison to an 87.4% agreement of sleep state based on EEG patterns.(294)

1.5.5 Sleep maturation

The normal ontogeny of sleep follows a specific, pre-programmed process that begins in utero. As the brain matures changes occur during identified time windows throughout infancy with more subtle changes occurring throughout childhood and into adulthood. Hence the background of the EEG evolves with advancing GA.

Sleep-wake cycling (SWC) refers to transitions between the neurophysiologic states of AS, QS, IS and wakefulness(W). Sleep changes developmentally undergoing quantitative and qualitative changes with advancing age.

1.5.5.1 Sleep wake cycling

Sleep cycling is influenced by GA, becoming well defined on EEG from 30-32 weeks GA (295)(Figures 1.18 -1.20). Varied definitions of a sleep cycle are evident in the literature. Bourel-Ponchel et al. define a sleep cycle as a period of sleep that commences from the end of post-waking AS to the end of the next AS period without awakening,(179) whilst Korotchikova et al. identified a sleep cycle as complete if it lasted from the onset of QS/AS through a required period of AS/QS (and IS if present)

to the onset of the next QS/AS phase(296). However, as the MLP infant commences sleep in the AS phase, for the purposes of uniformity in the onset of monitoring, we defined newborn sleep cycling as commencing with an AS phase which transitions to and completion of a QS phase without interruption. A complete sleep cycle may end in wakefulness or the infant may enter another phase of AS. Sleep cycling is readily identified on aEEG as fluctuations in the width of the trace but AS and waking are difficult to differentiate due to similar appearance (Described in section 1.3.5.6.)

Kidokora et al presented a simple view of a SWC in the term infant by identifying four different patterns of EEG background activity in one sleep cycle which are seen at term in healthy infants.(297) AS is divided into AS1 and AS2. AS1 is a mixed frequency activity and precedes QS. AS2 is a low-voltage irregular (LVI) activity following QS. Transitioning into QS there is evidence of a brief period of high-voltage slow wave (HVS)(QS1) followed by diffuse high-voltage bursts of 100 to 150 μ V alternating with attenuated activity of 25-50 μ V termed trace alternant (QS2)(Figure 1.29). However, the course of cycling from AS to QS and wakefulness is also impacted by physiological and environmental factors which may interrupt and fragment sleep (230, 298)

An environment that supports the preservation of sleep cycling will strengthen sleep architecture, support optimal physiology, neuronal growth, connectivity and provide the scaffolding for future higher-order functions and normal neurodevelopment as the infant grows toward adolescence and adulthood.(190, 267) Established SWC's are necessary for optimal growth, normal neurosensory and cortical development. (299, 300) Sleep cycling is a surrogate marker of neurological wellbeing. (301, 302) Delayed

onset or disrupted cycling is associated with neurological dysfunction. (5, 300, 303, 304, 305)

More mature cycling had been described amongst very preterm infants at the same corrected GA as infants born at a later GA. This suggests a more mature sleep organisation with advancing chronological age. (306, 307, 308) Sleep cycles are shorter in preterm infants which increase in duration with advancing GA but a wide range in cycle duration is evident. (179, 271) Based on a collaborative review Tsuchida et al. described the duration of a sleep cycle for infants <35 weeks PMA as 30-50 minutes, which increases to 50-65 minutes after 35 weeks PMA (271). Scher et al. described findings comparing preterm-infants (29-32 weeks GA at birth)(n=18) and found that preterm born infants had longer sleep cycles (70 mins versus 53 mins) and a higher percentage of QS suggesting more mature sleep state organisation.(309) This will be further discussed in Chapter 4.

1.5.5.2 Sleep state organisation

The preterm infant who fails to achieve mature sleep organisation appropriate to GA, have shown lower scores on neurodevelopmental scales at 9 months but this did not persist at 18 months. (303) A neurological condition and poor clinical wellbeing may affect the development of sleep cycling (e.g. HIE) whilst medications (morphine or phenobarbital) may suppress cycling.(310)

The architectural organization of sleep refers to the coordination of AS, QS, IS and wakefulness. Changes in sleep occur developmentally with advancing GA (Figure 1.24). Whilst the very preterm infant may sleep up to 90% of the day this decreases to

70% by term GA. Changes in sleep organisation include an increase in QS, a decrease in AS, a decrease in IS and an increase in wakefulness.(274, 311, 312, 313) Changes in the organisation of AS also occur overtime with an increase in REM activity.(206)

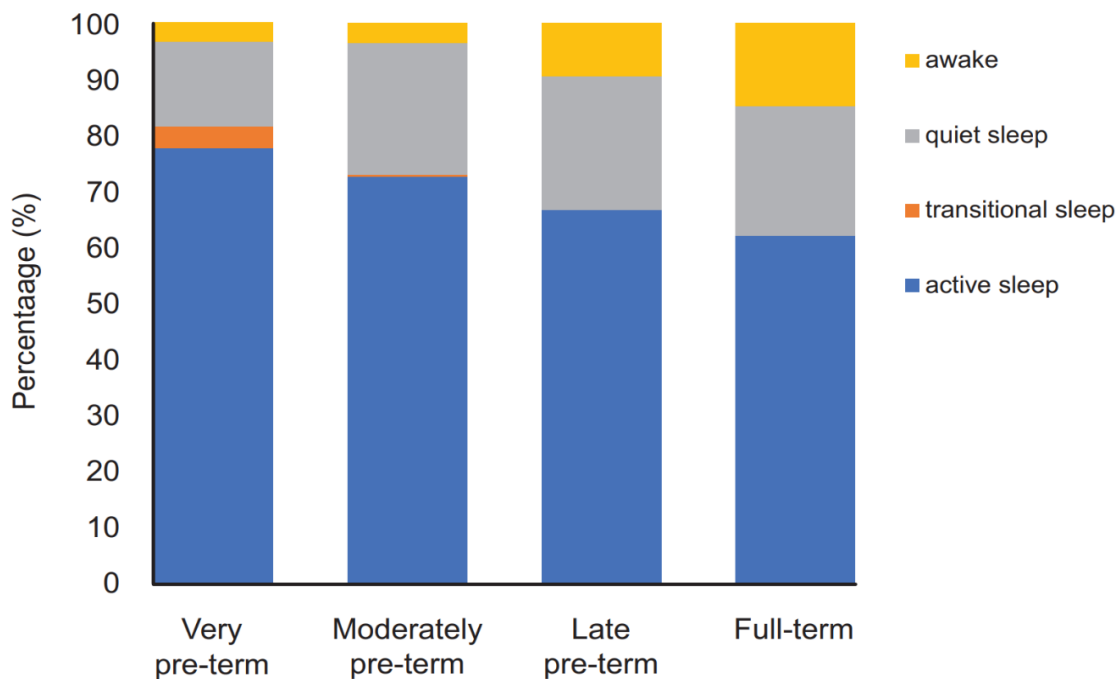


Figure 1.24 Changes in sleep organisation that occur with advancing GA. (197)(permission for use received)

Video EEG (vEEG) with polygraphy is currently considered the gold standard for identifying sleep states. This may be further facilitated through automated sleep detection algorithms which will be discussed in Chapter 5.

Overall sleep duration alters with GA (308) and correlates with measurable structural changes in the brain.(314) Similar sleep states are evident between and within similar GA groups. Infants with medical conditions that require oxygen for prolonged periods, or with inborn errors of metabolism and altered metabolism may have disrupted cycling, showing a decrease in AS and increase in QS. (315) Infants with neonatal

abstinence syndrome generally exhibit prolonged periods of wakefulness and increased sensitivity to noise and light which fragments sleep and disrupts sleep cycling.(274, 316, 317).

Mirmiram describes the normal progression in preterm sleep pattern to include an increase in the duration of wakefulness, decrease in IS, increase in QS whilst the duration of AS remains the same with advancing age.(195).In a review of unobtrusive sleep states in preterm infants, Werth et al. concluded that AS decreases from 28 to 30 weeks, plateau's for approximately 4 weeks until 34 weeks GA before decreasing again toward term GA, whilst QS increases until 34 weeks but then starts to decrease with advancing GA (Table 1.5).(258) Dereymaeker et al. also concluded that QS slowly increases with advancing GA. (13)

Table 1.5 Alteration in the percentage of active and quiet sleep unique to each GA group

GA in weeks	% Active sleep	% Quiet sleep
29-30	80	20
30-32	32	64
32-34	22	64
34-36	38	64
36-38	35	58
38-40	32	55

Data derived from Werth et al (258)

Scher et al. compared preterm infants (29-32 weeks GA at birth)(n=18) to that of term infants (n=18) at term corrected GA. Preterm infants had a higher percentage of QS suggesting more mature sleep state organisation. (309) However, not all research supported a notion of accelerated brain maturity in preterm neonates. At term PMA,

Hoppenbrouwers et al. described a higher percentage of AS amongst infants born preterm in comparison to term-born infants which may reflect the importance of AS in brain development and plasticity.(318) Sleep architecture will be further investigated and discussed in Chapter 4.

Information pertaining to medications, montage used and environmental factors that may cause undue artefact on EEG are necessary in determining if EEG is appropriate for GA.

1.5.5.3 Normal transient features seen on EEG in premature infants unique to GA

As well as changes in continuity and sleep cycling, there are also a number of unique transient waveforms seen during certain developmental periods from prematurity to term. Dysmaturity of the EEG of a preterm infant is difficult to ascertain as normal EEG ranges in the preterm infant are more variable than that of the term infant. Not all preterm EEG activity is unique to a specific age, nor do we know the relevance of some waveforms.(269) Normally features of the neonatal EEG evolve in parallel with brain maturation and are uniquely associated with GA.(14) The presence of rhythms and activity outside the normal range for GA may be associated with long-term adverse developmental outcomes.(319)

A normal MLP EEG may show occasional sharp waves originating in different regions of the brain (particular to the developing cortex) which are considered normal in the preterm infant.(14, 320) Transient waveforms of prematurity relevant to the MLP infant

period include; Delta brushes, Frontal Transients such as Encoche Frontales, Slow Anterior Dysrhythmia (SAD), Premature Temporal Theta (PTT) and Sharpened Theta on the Occipitals of Premature infants (STOPS), as described by Pavlidis et al and Andre et al, and will be discussed further below .(14, 321) (Figure 1.25).

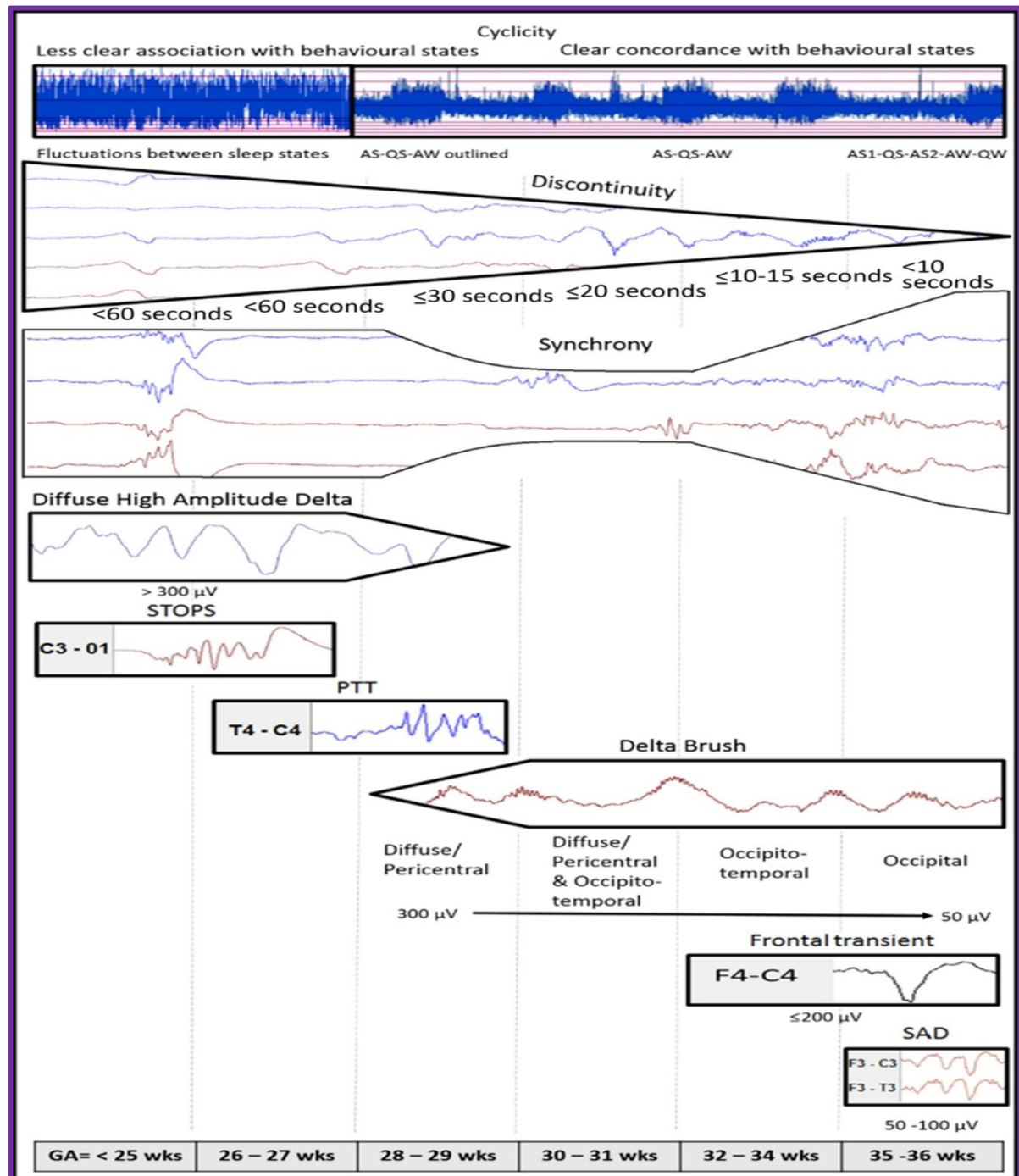


Figure 1.25 Summary of maturation of preterm EEG features by Pavlidis et al.(14) .

Key: AS, active sleep; QS, quiet sleep; AW, active wakefulness; QW, quiet wakefulness; STOPS, Sharp theta on the occipitals of premature infants; PTT, premature temporal theta; SAD, slow anterior dysrhythmia; GA, gestational age.

1.5.5.3.1 Delta brushes

The most recognisable transient activity and a hallmark of preterm EEG are delta brushes the incidence of which peaks at 34-35 weeks PMA(322). Delta brushes are slow delta waves (1-2 Hz) with an amplitude of 100-200 μ V with superimposed high-frequency rhythmic spindle like alpha or beta activity of 8-25 Hz (41, 273)(Figure 1.26).

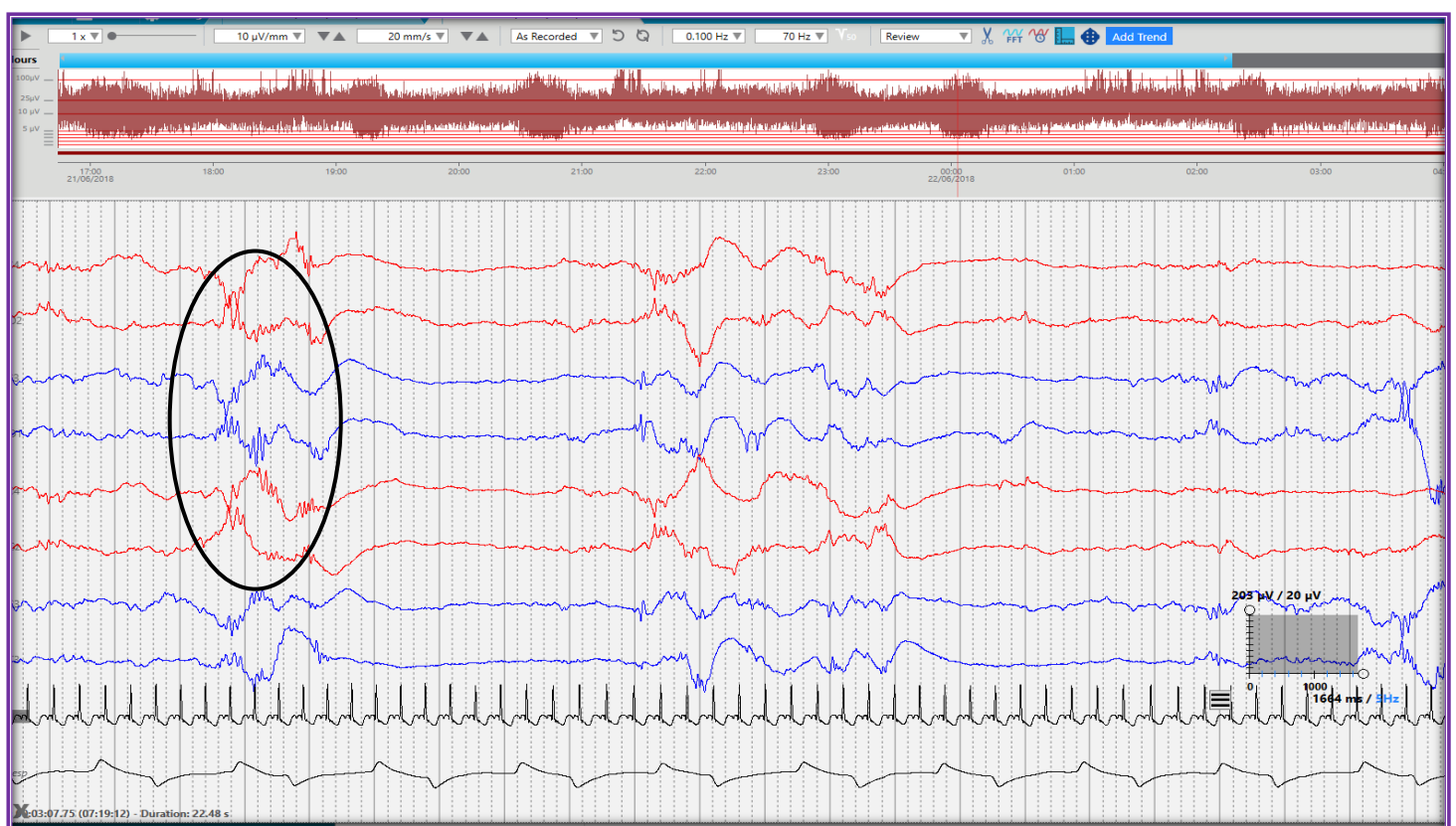


Figure 1.26 Delta brushes. A characteristic feature of the preterm EEG. Circled as seen by slow-wave delta activity superimposed high-frequency activity

Delta brushes can occur spontaneously or can be induced by sensory stimuli and are involved in fine tuning the topographic organisation of sensory areas.(323)The peak frequency of superimposed activity is 16-20 Hz between 32-35 weeks PMA falling to 10-15 Hz from 36 weeks PMA (322). Delta brush activity occurs in both AS and QS

(more dominant in QS of the MLP) with the fast frequencies most frequently appearing in the ascending arm of the delta wave (14). Whilst predominantly evident centrally from 28 weeks GA(202), and in the temporal region between 30-34 weeks, by 36 weeks they have decreased and more evident in the occipital areas until term equivalent age and generally disappear by 38-42 weeks PMA. (324) The bursts of activity have a significant effect on brain maturation of preterm infants who have limited periods of wakefulness.(314, 325) Holmes and Lambrosco propose that the absence of delta brushes is an indication of neurological compromise and poor prognosis (326)

1.5.5.3.2 Encoche Frontale

Encoche Frontale are distinct biphasic frontal sharp waves with an initial negative deflection followed by a positive deflection with amplitude varying from 50 and 200uV lasting 0.5 to 0.75 seconds(327, 328)(Figure 1.28). They can be seen bilaterally or unilaterally and more frequent in quiet sleep from 35-44 weeks GA. Andre identified this as a feature of normal healthy maturation (321).

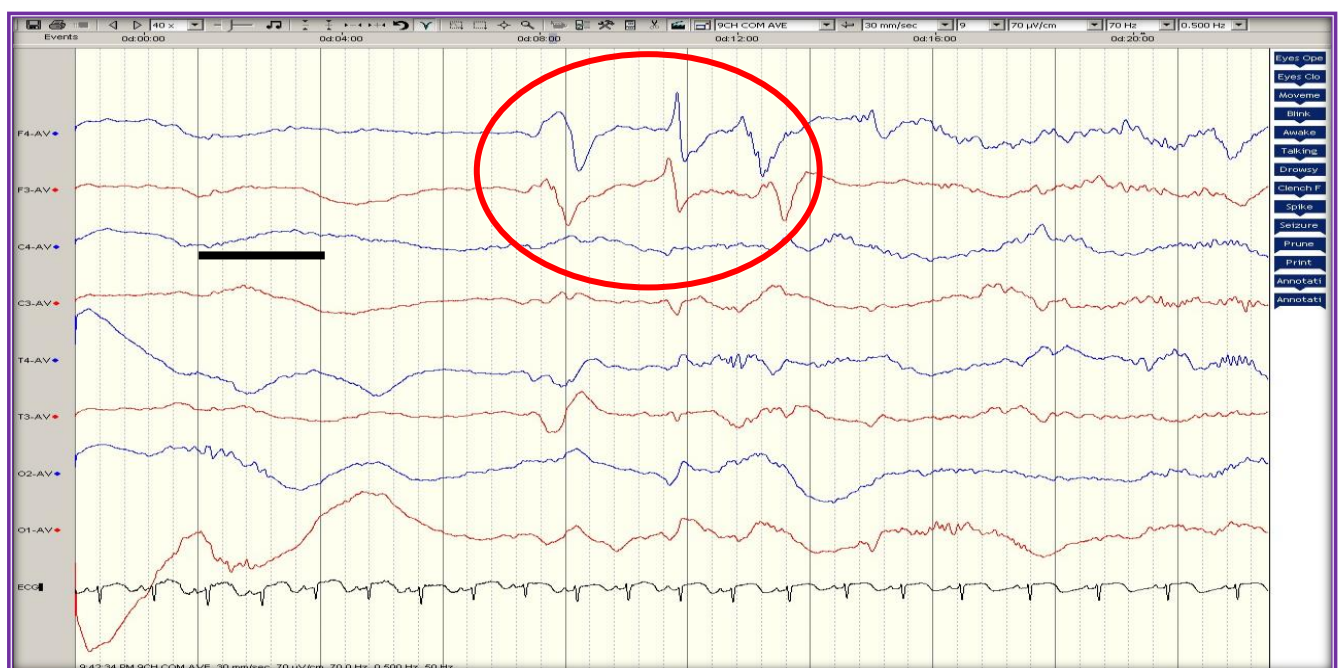


Figure 1.27 Encoche Frontale on preterm EEG showing symmetrical sharp activity in frontal region. Black horizontal line on EEG indicates 1 second of EEG activity

1.5.5.3.3 Slow anterior dysrhythmia

Slow anterior dysrhythmia(Fig 1.28) is a maturational pattern that may be seen in late preterm and near term infants (evident from 34-44 weeks GA but peak between 36-40 weeks GA).This pattern is characterized by short runs of rhythmic, high amplitude (50-100 μ V) slow delta activity of 1-3 Hz, over the frontal regions(329).

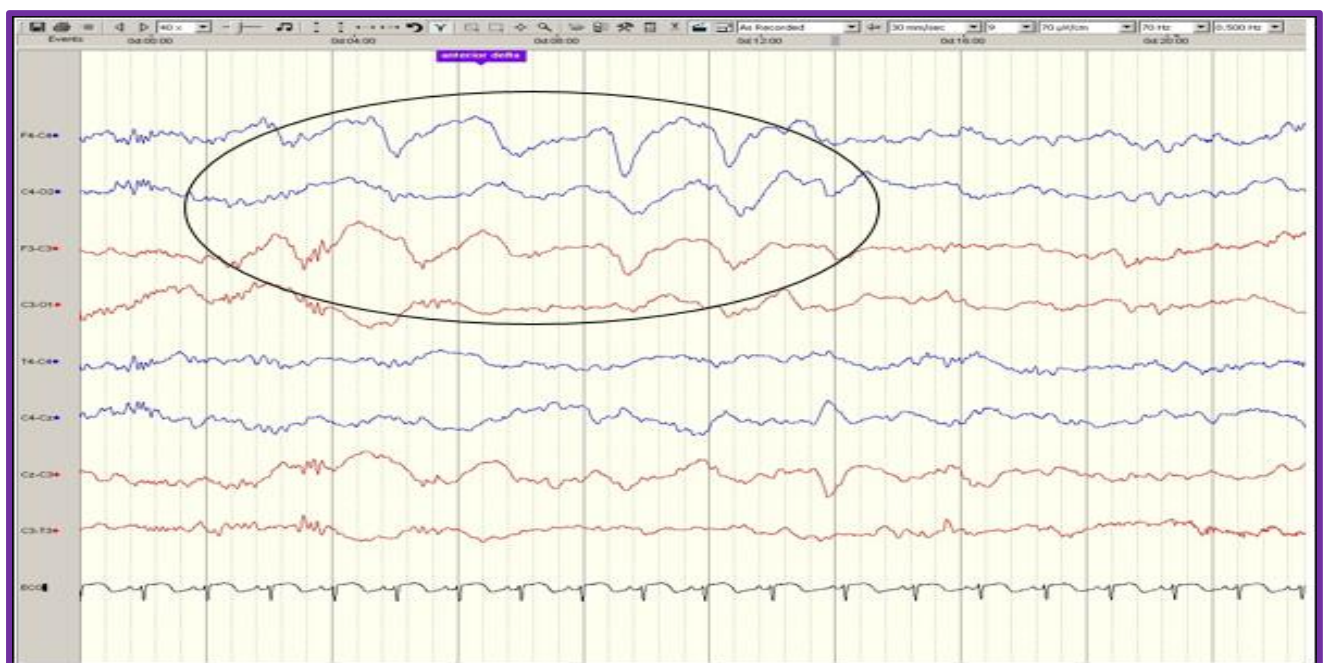
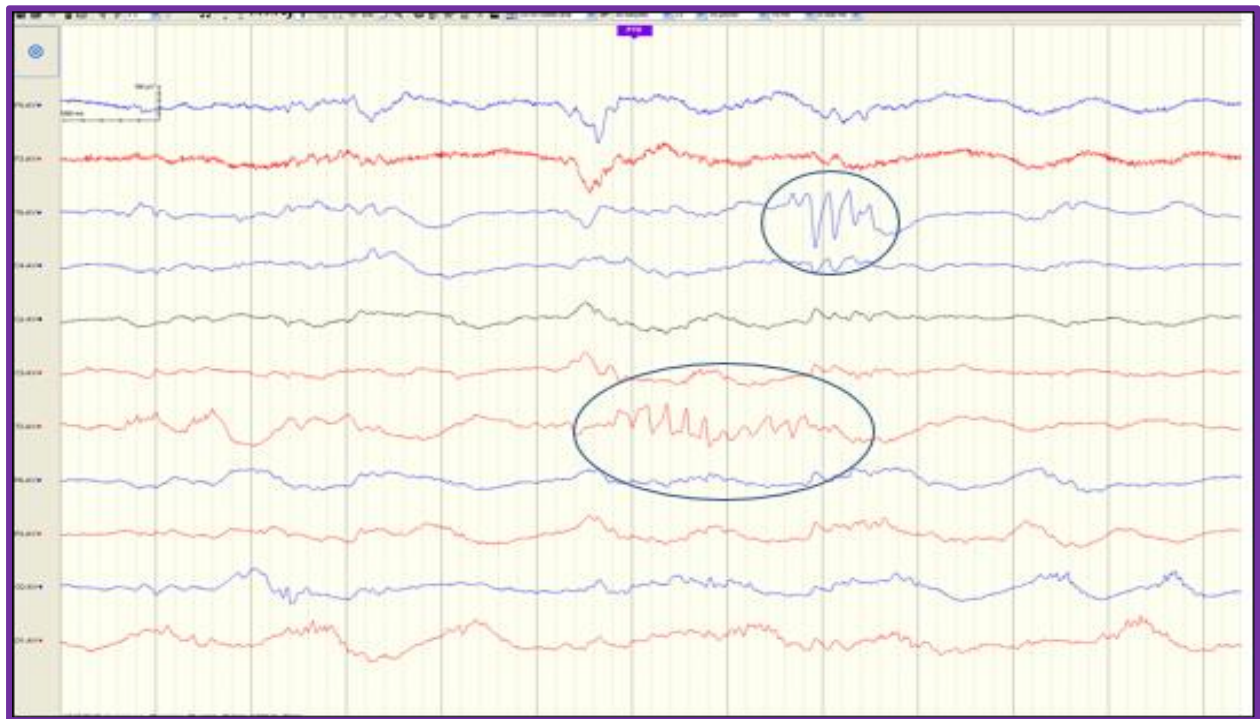


Figure 1.28 Slow anterior dysrhythmia over frontal regions

1.5.5.3.4 Premature temporal theta

Premature temporal theta (PTT)(Fig 1.29) is a feature of early prematurity, peaking at 29-31 weeks, disappearing from AS at 32 weeks and QS near term GA(327). Usually the pattern is found independently on both temporal areas, but with a right-sided preference. Pavlidis et al describe this activity as runs of rhythmic theta activity of 4.5–

6 Hz, usually bilateral, often asynchronous.(14) The absence of PTT or diminished PTT is associated with neurological or non-neurological (medical) conditions.(330)



. **Figure 1.29** Premature Temporal Theta

1.5.5.3.5 Sharpened Theta on the Occipitals of Premature infants (STOPS)

STOPS(Fig 1.30) are similar to PTT activity but faster in frequency, lower in amplitude and are predominately unilateral in the occipital area. (327, 331) The highest incidence of STOPS is evident in very preterm infants at 25 weeks GA (14) and are less prominent after 28 weeks. (331)

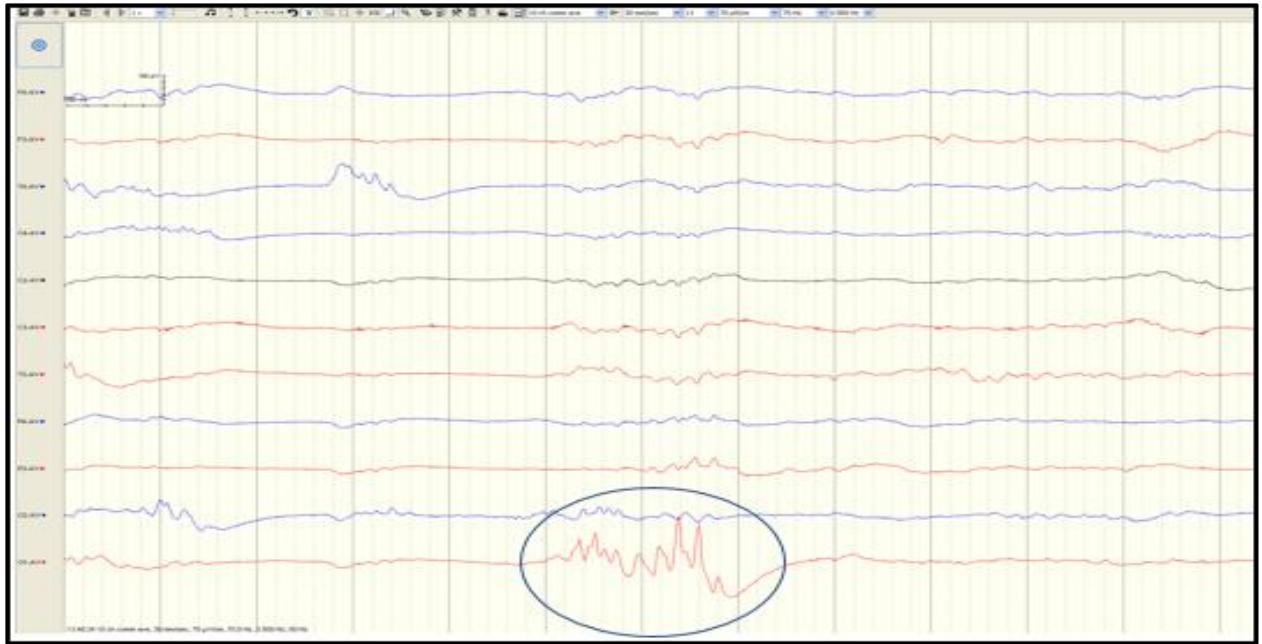


Figure 1.30 Sharpened Theta on the Occipitals of Prematures.

1.5.5.4 Synchrony

Synchrony of activity refers to the timing of appearance of waveforms between hemispheres, especially evident during the discontinuous periods of the neonatal EEG. (332) EEG bursts during quiet sleep are considered synchronous if less than 1.5 second separates the onset of the burst between the two hemispheres.(269) Location and synchrony of activity alter with maturity and advancing PMA. Before 30 weeks paradoxical hypersynchrony predominates as activity is primarily driven by the thalamus. From 30 weeks there is an increase in asynchrony until 34 weeks when synchronicity re-emerges with the development of the Corpus Callosum. After 36 weeks asynchrony decreases and it is not normal for this feature to be present at term age.(333)

1.5.5.5. EEG artefact

Physiological sources of artefact include, but are not limited to, eye movement, jaw movement, sucking, head movement, muscle activity, respiratory movement, sweat and ECG and pulse artefact. Handling/movement artefact is shown on Figure 1.31 as erratic high amplitude activity. Patting appears as rhythmic like activity which the video will confirm. Sources of artefact where possible should be removed or minimised to support accurate interpretation of EEG activity.

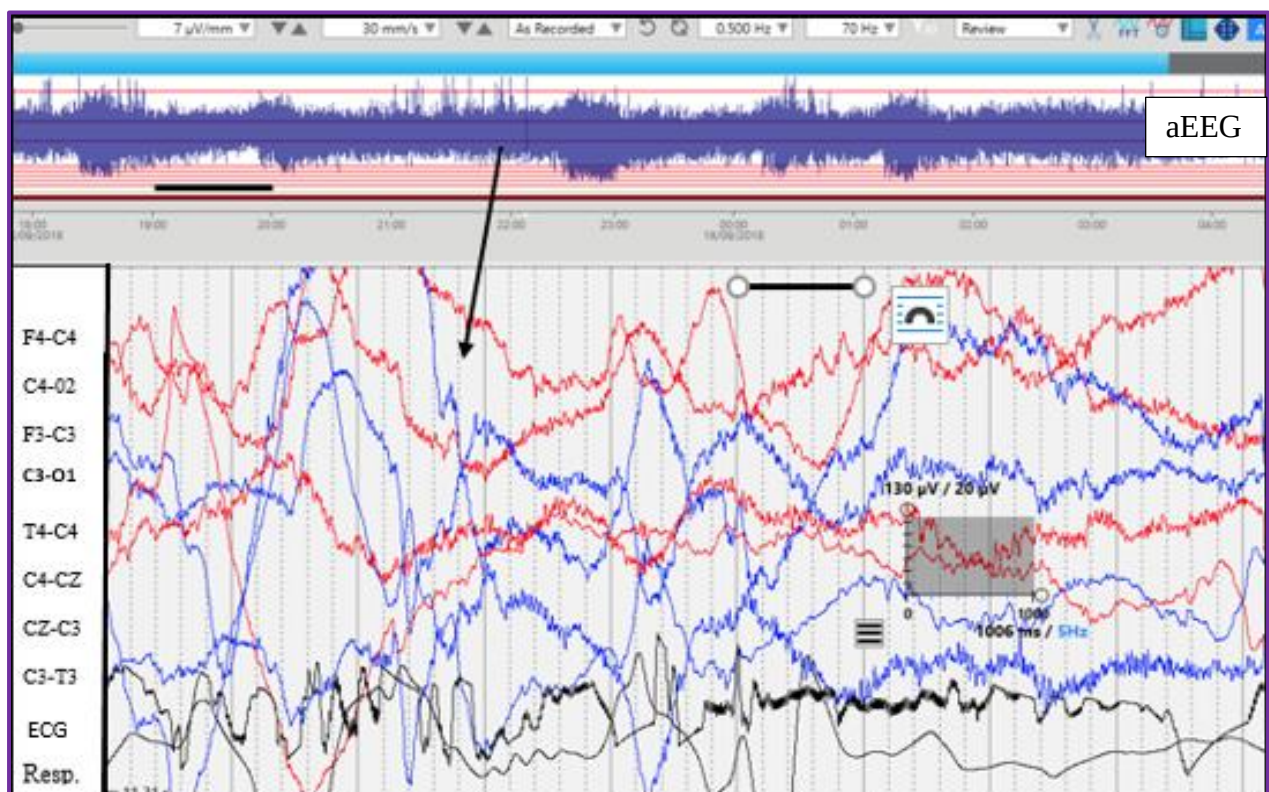


Figure 1.31 Movement artefact. The black horizontal line on raw EEG equates to 1 second of recording, the black line on aEEG equates to 1 hour of recording

1.5.6 Amplitude integrated EEG (aEEG)

Amplitude integrated EEG (aEEG)(Fig 1.32) is derived from conventional EEG and is a simplified compressed trend of EEG amplitude that uses a limited number of channels. The EEG signal is filtered with a bandpass of 2 Hz to 15 Hz, rectified, processed and displayed in a time-compressed semi-logarithmic scale. The band produced has values for upper and lower margins that represent the minimum and maximum EEG amplitude over time. The semi logarithmic scale is a linear form 0-10 μ V then logarithmic from 10-100 μ V (334) to attenuate the influence of high amplitude movement artefact. aEEG provides an easily interpretable visual trend of EEG activity providing broad information on sleep states(335, 336) and sleep-wake cycling (337).

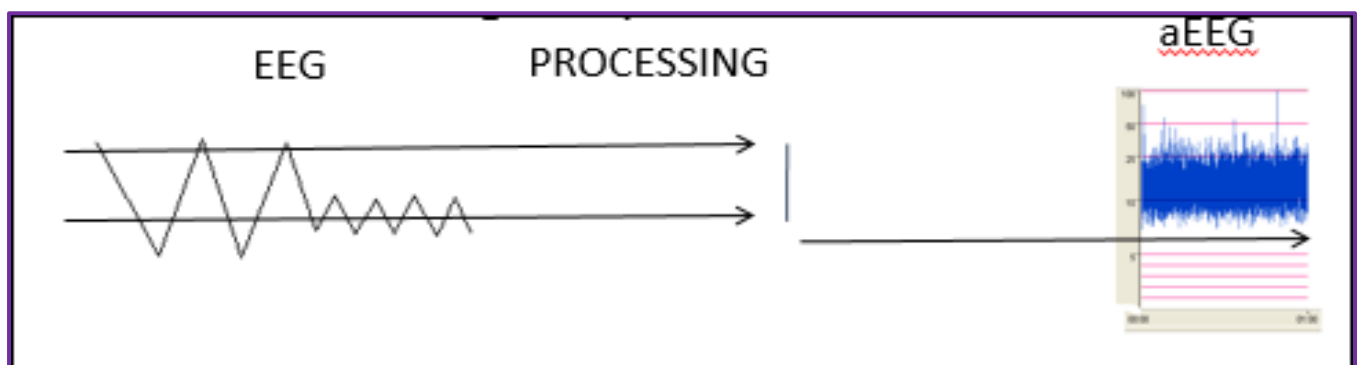


Figure 1.32 Amplitude integrated EEG (aEEG) derived from the raw EEG EEG signal that is filtered, rectified, processed, and displayed on a time-compressed scale

A single interhemispheric bipolar aEEG recording may include central (C3-C4) or parietal (P3-P4) electrodes, or one channel on each hemisphere i.e. centro-parietal electrodes (C3-P3, C4-P4) or front-central electrodes (F3-C3, F4-C4). Positioned as per the international 10/20 system of electrode placement.(319)

Cyclic variations or sleep state alterations are identified on aEEG from 30-32 weeks GA as sleep architecture emerges.(275, 307, 338) The narrow areas of the aEEG trace represent the more consistently lower amplitude activity that occurs in AS and wakefulness(W) whilst the wider sections of the aEEG represent the alternating higher voltage bursts of activity followed by periods of quiescence or discontinuity that occur in QS of the preterm infant (Figure1.33). Research findings support the prognostic value of aEEG in assessing general neurological wellbeing and in determining the long-term outcome of both the term and preterm infant (339, 340).

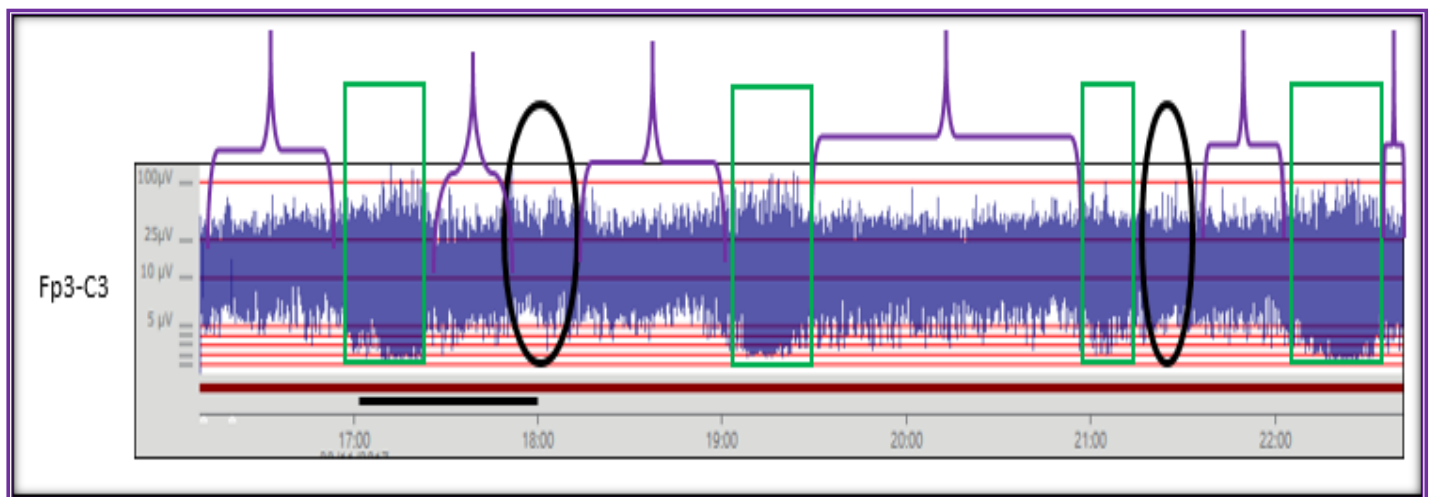


Figure 1.33 Over 6 hours of aEEG recording of a preterm infant of 34 weeks birth GA, recorded at 36 weeks PMA. Recording displays 4 QS periods (within green boxes) and periods of AS (identified by purple brackets) displayed on semi log-rhythmic scale. The narrower portion of aEEG represents the relatively low amplitude and higher frequency activity that occurs in AS and wakefulness (indicated by black circles). The wider band of aEEG represents the bursts of high amplitude activity that occur in QS.

Originally referred to as a cerebral function monitor (CFM), amplitude-integrated EEG (aEEG) has a greater presence than EEG in the neonatal unit due to ease of application with limited electrodes and a trend that is easily interpreted with minimal cot-side education. aEEG provides a general overview of EEG activity easily interpreted and especially valuable for the identification of sleep cycling.(297, 338, 341) Seizure activity on aEEG may be seen as an upward deflection of the trace. There is however a reduced

sensitivity of the aEEG to seizure detection, as seizures of short duration may not be seen, aEEG channels may not cover the area of a focal seizure and artefact such as movement may cause similar deflections and be misinterpreted as seizure, (342)

1.5.6.1 aEEG scoring and classification systems

aEEG classification and scoring systems have been developed to measure brain activity and maturation. A system based on pattern classification (voltage) was proposed by Toet et al(343) and by Al Naqeeb.(344) In the voltage method, a normal term and near term aEEG trace displays a lower margin $>5\ \mu\text{V}$ and an upper margin $>10\ \mu\text{V}$. A moderately abnormal aEEG displays a lower margin $<5\ \mu\text{V}$ and upper margin $>10\ \mu\text{V}$. A severely abnormal aEEG has a persistent low voltage with lower margin $<5\ \mu\text{V}$ and upper margin $<10\ \mu\text{V}$. Both systems are summarised in Fig 1.34.

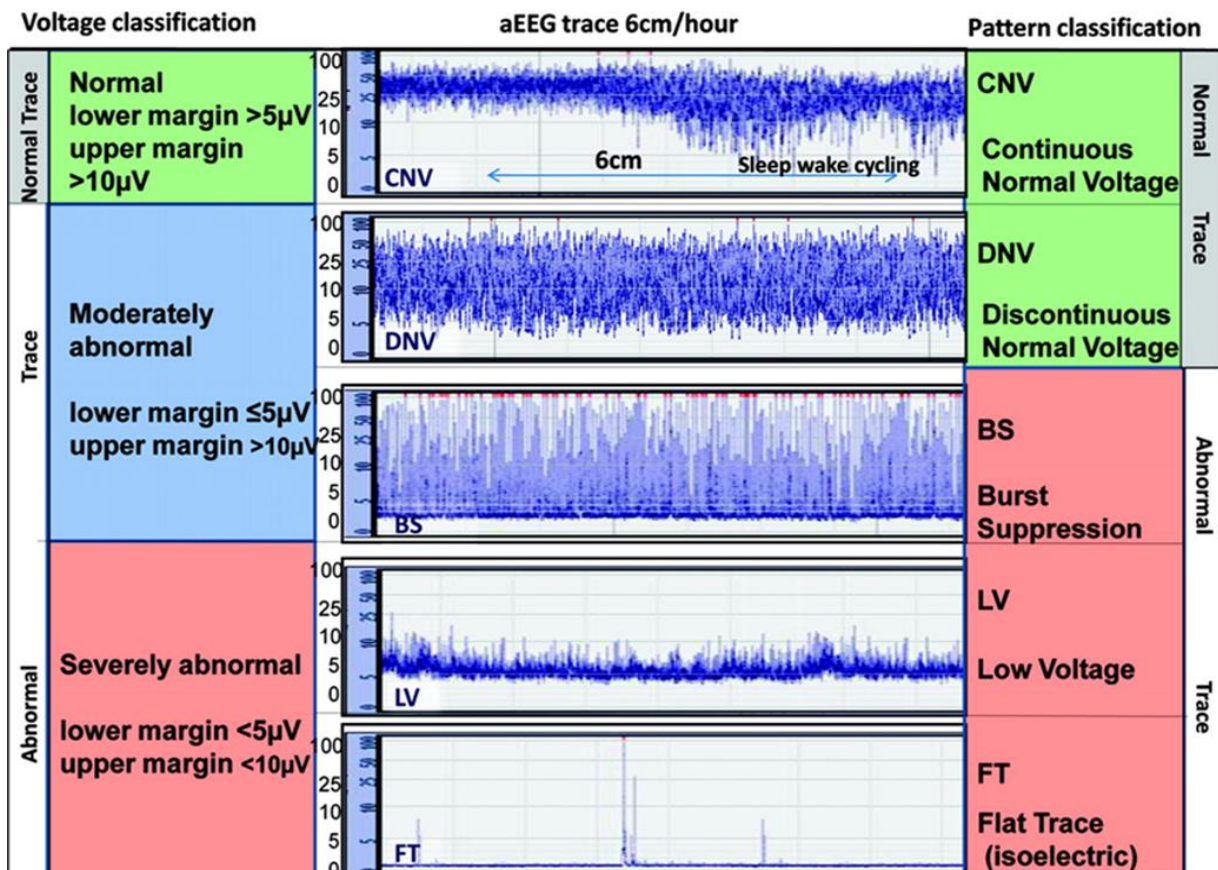


Figure 1.34 Combined representation of aEEG voltage and pattern recognition systems by Toet(343) and al Naqeeb(344) From Thoresen et al(345) (Permission for use received)

1.5.6.2 Continuous EEG (cEEG) versus amplitude integrated EEG (aEEG)

Although EEG has been recommended as the gold standard for identifying neonatal seizures by the World Health Organisation and the American Clinical Neurophysiology Society, it's use in neonatal units is limited.(346, 347) In a survey of neonatal units (n=25) in Canada, 92% of neonatologists reported using continuous EEG, however significantly more neonatologists favour the use of amplitude integrated EEG (aEEG) alone or in combination with cEEG.(348) A survey of neonatologists in China (n=251) found the only 64% used EEG and 78% used aEEG. A UK survey of NICU's (n=57) reported that only 4% did not have access to some form of EEG monitoring (aEEG or conventional EEG) with 46% having access to out of hours formal EEG monitoring.(349) An Irish national neonatal unit survey (n=18) reported that 5 units used aEEG which included all level 3 neonatal units and 75% of level 2 units (n=4)(350). Across all surveys, barriers to implementing EEG in neonatal units include cost, access to technology, lack of education and expertise in interpreting recordings.

1.5.7. Conclusion

Despite advances in neonatal care there is still a great deal we do not know about sleep. In the last few years there has been much research published about the importance of sleep, the environmental impact of the NICU along with measures we can take to support sleep in the NICU. However, much of the research has been based on behaviour observation alone which has some limitations.

The methods used to monitor sleep in this thesis combine subjective and objective measures using gold standard continuous EEG with video. EEG is a reliable bedside tool that has excellent diagnostic and prognostic properties. Unfortunately, the real time availability of expert neurophysiologists to interpret EEG is more the exception rather than the rule. A quantitative approach to analysis of EEG replaces the need for expert and subjective interpretation by a computer-based objective interpretation which will accurately identify, classify and interpret the background EEG activity. Methods used to record and interpret EEG during sleep will be described in Chapter 2.

Developmental assessments are used to identify infants with a developmental delay or at risk of adverse outcome. Despite the healthy MLP infant requiring minimal support in the neonatal unit they have at high risk of adverse development. The Griffiths III assessment may also be considered a gold standard method of scoring development. Further information and methods used in assessment will also be described in Chapter 2.

Chapter 2

Methodology

Methods used in this chapter for EEG monitoring have been published
(Appendix V)

2.0 Introduction

I conducted a prospective cohort observational study of moderate to late preterm (MLP) infants who had overnight continuous multichannel video-EEG monitoring performed at 36 weeks post menstrual age (PMA) and follow up developmental assessments conducted at 4 months and 18 months PMA. This chapter will describe the general methodology of this thesis and sub-studies contained within. The content includes research setting, participants, recruitment and informed consent, data

collection/management, education/training, procedures to which infants were subjected, EEG analysis, retention strategies and statistical analysis.

2.1 Research setting



Figure 2.1 Cork University Maternity Hospital

This was a single centre study in the neonatal unit of Cork University Maternity Hospital (CUMH)(Fig 2.1), and the Infant Centre research facility, UCC between 2017-2019. CUMH is the largest maternity hospital outside the country's capital with approximately 7500 births per annum.

The neonatal unit is a 36-bed facility close to the delivery suite and theatre.(Fig 2.2) This unit provides tertiary level care for infants born in CUMH and transfers (in utero or at birth) from smaller provincial hospitals in southern Ireland. As a designated therapeutic hypothermia centre, nursing and medical staff are familiar with caring for infants on continuous video EEG, whilst a research support team is responsible for the application and maintenance of recordings and all clinical recordings are reviewed by a consultant clinical neurophysiologist.



Figure 2.2 The neonatal unit incorporating Neonatal intensive care area (A), High dependency area (B) and a single room in special care are (C).

The INFANT centre is a research centre focused entirely on pregnancy, birth and early childhood. The centre is committed to improving maternal and child health, hence our research priorities lie in these areas. The centre team comprises primary Investigators, researchers, a research support team, postgraduate students and a multi-disciplinary team who implement and support our research. The centre is located in the Paediatric Academic Unit, Department of Paediatrics, Cork University Hospital. The facilities at INFANT include:

- INFANT Early life lab where children return for clinical follow up and neurodevelopmental follow up assessments with facilities that include EEG telemetry, high-resolution eye-tracking, heart rate variability assessments and near infra-red spectroscopy monitoring during multiple assessments of infants and children.(Fig 2.3)
- A data hub that provides the infrastructure to support physiological, cognitive and developmental assessments and data storage, curation and analysis for children participating in INFANT research.
- Infant Biobank which stores thousands of biological samples that have been collected for over a decade of research.

An INFANT research priority of particular relevance to this study includes sleep and exploring ways of enhancing the long-term wellbeing and development of children.



Figure 2.3 INFANT early life lab, INFANT centre, Cork University Hospital

2.2 Ethical Approval

Ethical approval was sought from the Cork Research Ethics Committee (CREC) prior to the commencement of the study. As per hospital policy, local approval of the South-South West hospital group was also required and obtained (Appendix 1).

2.3 Participants

This study focused on MLP infants (identified as those born between 32-36+6 weeks gestation) who required admission to the neonatal unit. Gestation was based on the first day of a mothers last menstrual period and confirmed scan in early pregnancy. In the case of a discrepancy, the expected date of delivery (EDD) was based on an ultrasound scan report.

A healthy term control (TC) group of infants who had a Griffiths assessment at 4 months and 18 months were used as a comparative group in this study (chapter 6). These infants were a part of the non-intervention group that participated in the BabySMART study which was an arm of a study called ENRICH (<https://clinicaltrials.gov/ct2/show/NCT03381027?term=enrich&cntry=IE&draw=2&rank=9>) a randomised controlled trial to investigate the effect of parent-led massage. The TC infant group were born in CUMH and cared for by mothers on post-natal floor before discharge home.

2.4 Inclusion/ Exclusion criteria

SLEEPi had very clear inclusion and exclusion criteria (Figure 2.4/ Fig 2.5). Infants were included if, according to the electronic health record (EHR) and following discussion with clinical team, there were no concerns regarding the neurological status of the infants, or congenital/chromosomal concerns. Visual inspection of the infant's head was performed to confirm the skin integrity of the scalp was intact. All parents of infants who satisfied inclusion criteria irrespective of infant's gender ethnicity or from a single/multiple pregnancy were invited to participate.

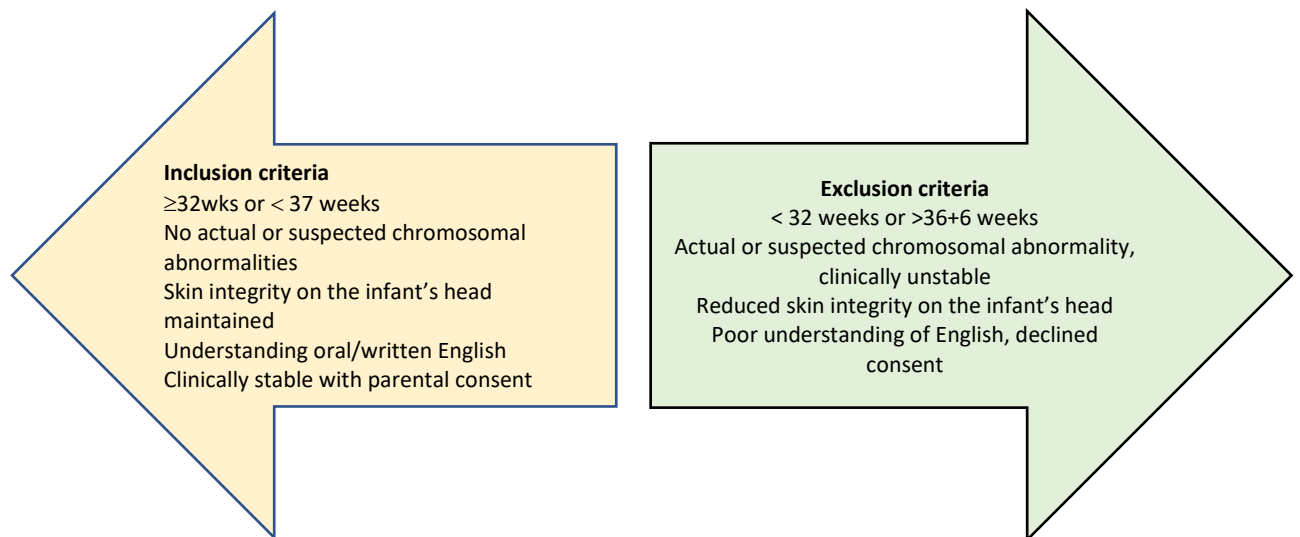


Figure 2.4 SLEEPi study Inclusion and exclusion criteria for the moderate to late preterm (MLP) infant group

Inclusion/exclusion criteria for the non-intervention TC group to which outcome was compared are presented in Figure 2.5 below.

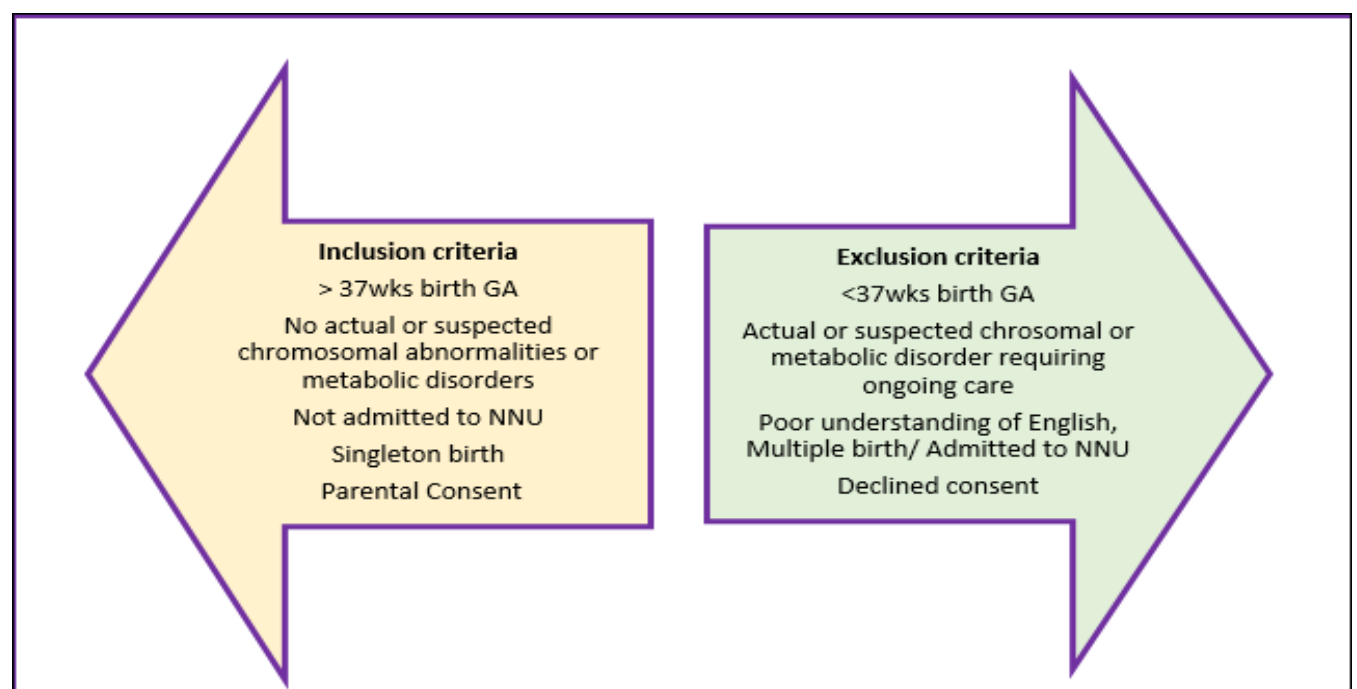


Figure 2.5 Inclusion /exclusion criteria for the non-intervention term control (TC) group

2.5 Informed Consent

Contact was made with the nurse caring for the infant to ensure appropriate timing of approach for consent. I also ensured that parents had a good understanding of English and there were no other extenuating medical/social circumstances that may delay approach or exclude the infant from potential participation. Consent was sought as per good clinical practice (GCP) ICH GCP E6 (R2) guidelines and data was recorded following General Data Protection and Regulations (GDPR 2016/679).

Parental consent for participation in SLEEPi also included consent for access to maternal and infant electronic health records (EHR) (electronic records were introduced to the hospital prior to commencing this study) (Appendix 2). A Patient information leaflet (PIL) was provided to parents detailing the study (Appendix 3). If parents agreed verbally, the researcher returned the following day, at which time parents were given an opportunity for further discussion and to ask questions they may have relating to procedures and follow up. If parents wished to participate a unique study ID number was assigned a consent form was provided and mother alone or both parents/guardians were asked to read, sign and date after which it was returned directly to the researcher.

Obtaining valid informed consent in the emotionally charged high tech environment of the neonatal unit can be challenging at times. Obtaining consent for this observational study that required EEG (which many parents had never encountered) further challenged recruitment. Identifying the timing of parental approach was key to obtaining consent for participation.

2.6 Data collection

Data collected including both demographic and clinical data which was pseudo anonymised with a protocol unique study ID number. A database was generated on a secure UCC server (CEREBRO) incorporating the following data for each participant:

Maternal demographic information recorded included: EHR number, date of birth, marital status, medical/ surgical history, recent and previous obstetrical history, twin/ multiple pregnancy gravida, parity, mode of delivery, smoking status, medications prescribed during pregnancy.

Infant data generated from EHR included: birth gestation, gender, weight, head circumference and mode of feeding (oral or via nasogastric tube), Apgar score, the reason for preterm delivery, medications given (including caffeine and antibiotics), respiratory support required (if any), requirement for phototherapy, and any complications throughout the stay in the neonatal unit. Additional data was also generated including date of EEG recording, postmenstrual age (PMA) (i.e. birth gestational age (GA) plus chronological age), mode of feeding, medications (if any), weight and location of monitoring (cot/incubator).

Data generated in follow up appointments included anthropometric measurements, any medical history to date including any illness/hospitalisation required, and any maternal concerns. Griffiths III assessment at 4 month and 18 months PMA also gave rise to a large volume of data relating to scores achieved in each subscale of development.

2.7 Data management

All data generated was stored and maintained as per Good Clinical Practice (GCP) - ICH GCP E6 (R2) and General Data Protection Rules (GDPR 2016/679). Digital data was stored on a secure UCC drive. A site file was maintained with each participant's paper records identified using a unique study ID number. Records included consent forms, records of follow up appointments, completed Griffiths III developmental assessment books and written reports by the assessor. The site file was stored in a folder, in a locked cabinet in a swipe accessed secure research room.

2.8 Education and training

- For the neonatal unit staff:

Brief informal information sessions were provided individually or small groups of staff about the study and its background. My contact details were posted on the neonatal unit notice board for any queries.

- For the researcher

Continuous group and individual presentations and informal cot side education sessions were provided by Dr Sean Mathieson, Professor Geraldine Boylan and the INFANT team. Monthly INFANT seminars and in-house weekly presentation/ journal club provided experience in public presentations. Education and training in the recognition of sleep specific states, sleep-wake cycling in the preterm versus term infant and identification of abnormal EEG/aEEG recordings took place before the commencement of this study. Training of the researcher in the clinical application of EEG as per a standardised protocol for the application of a reduced neonatal montage EEG was provided by Dr Sean Mathieson. This ensured minimal variability in the

technique used in EEG applications. This training has also paved the way for the researcher's involvement in future studies requiring EEG monitoring.

2.9 Procedures to which the infants were subjected

Participation of MLP infants in the SLEEPi study required infant participation at three specific time points including:

- Pre-discharge overnight EEG
- Follow up Griffiths III developmental assessment 4 months PMA and a full head sleep EEG (nap time)
- Follow up Griffiths III developmental assessment 18 months PMA.

Participation in the TC group required:

- Follow up Griffiths III developmental assessment 4 months PMA and a full head sleep EEG (nap time)
- Follow up Griffiths III developmental assessment 18 months PMA follow up

2.9.1 Pre-discharge EEG for the MLP infant group

All included infants had overnight video EEG (vEEG) monitoring performed by MAR before discharge from the neonatal unit or at 36+6 weeks gestation (whichever came first). All studies followed a standardised protocol. For the most part, recordings were made using Lifelines iEEG machines (Lifelines Neuro, Woking, United Kingdom) (Fig 2.6). When a lifelines machine was not available the NicoletOne EEG monitor was used (Natus, USA). Both systems could be accessed remotely and displayed both

conventional EEG and amplitude-integrated EEG (aEEG) on the screen. Standard operating procedures in keeping with hospital and manufacturers infection control policy were adhered to in cleaning and maintenance of machines.

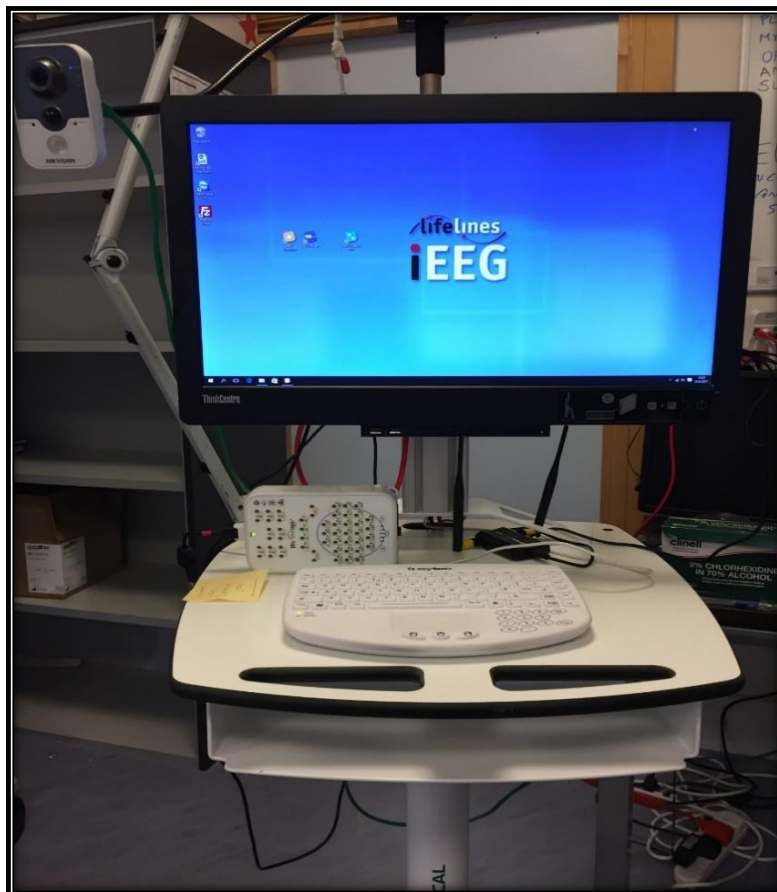


Figure 2.6 Lifelines machines used to record EEG at 36 weeks PMA and 4 months PMA

The timing of electrode application was agreed in coordination with the clinical team, parents and feeding regime, EEG recordings commenced between 17:00 hours and 20:00 hours and discontinued the following morning between 07.30 hours and 10.00 hours. All equipment required was prepared on a tray before arrival to the cotside of the infant (Figure 2.7). Rubbing the skin with a cotton tip stick and Nu-prep gel prepared the skin for electrode application. This served to remove residue or dead cells on the scalp, ensuring good electrode contact and low impedance, reducing

electrical noise on the recording. A water-soluble conductive fixative paste (Ten20™, Covidien) was used with the primary purpose of conducting an electrical signal. Mefix tape (2.5 cms) and a cotton adjustable hat were also used which aimed at reducing electrode displacement overnight. Single-use disposable surface gel electrodes (Ambu Neuroline 700) of 100cm or 150 cm in length were used in this study. The longer lead electrodes facilitated a greater range of movement without handling the amplifier and more appropriate for breastfeeding mothers. Length of the electrode did not alter the quality of the EEG recording.

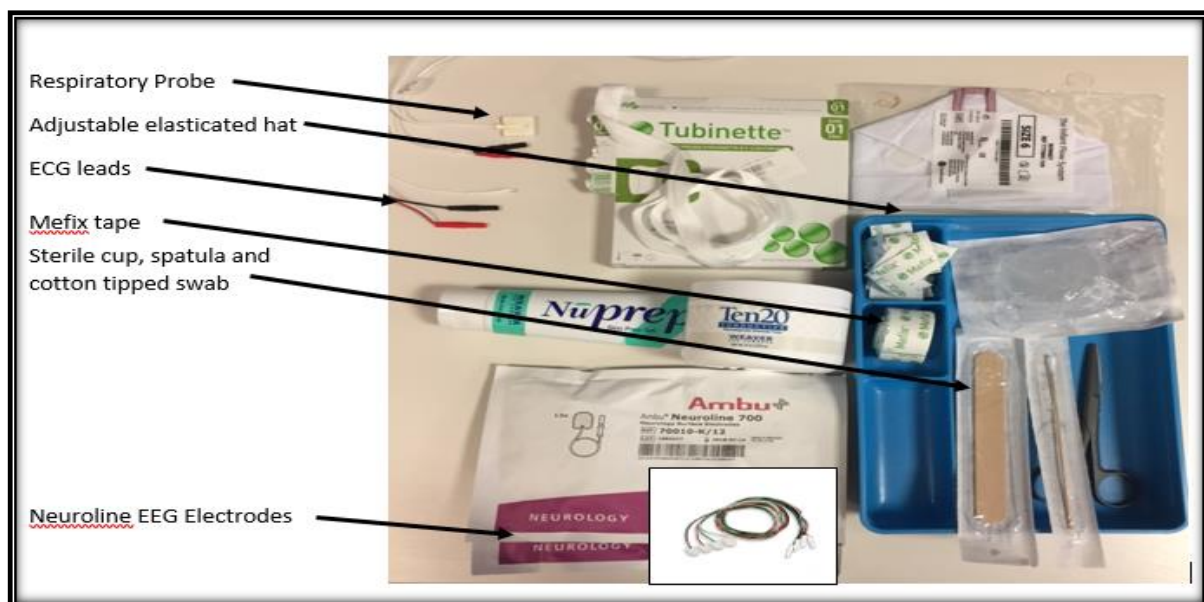


Figure 2.7 Preparation and requirements for EEG application

For appropriate assessment of preterm EEG, recordings used a standardised reduced neonatal montage or modified version of the standardised international 10/20 system of electrode placement (351) including electrode placement at positions F3, F4, C3, C4, Cz, T3, T4, O1, O2, plus a reference (R) applied to position Fz and a ground electrode (G) placed behind the left ear (Figure 2.8). O1 is applied to the back of the head 1 cm up from the inion (base of the skull) and 2cm to the left of the midline, O2

is applied in the same position on the right side. A bipolar montage displaying channels F4-C4, C4-O2, F3-C3, C3-O1, T4-C4, C4-CZ, CZ-C3, C3-T3 was used. Activity from two channels (F3-C3 and F4-C4) of aEEG were simultaneously displayed on the screen above the raw EEG recording

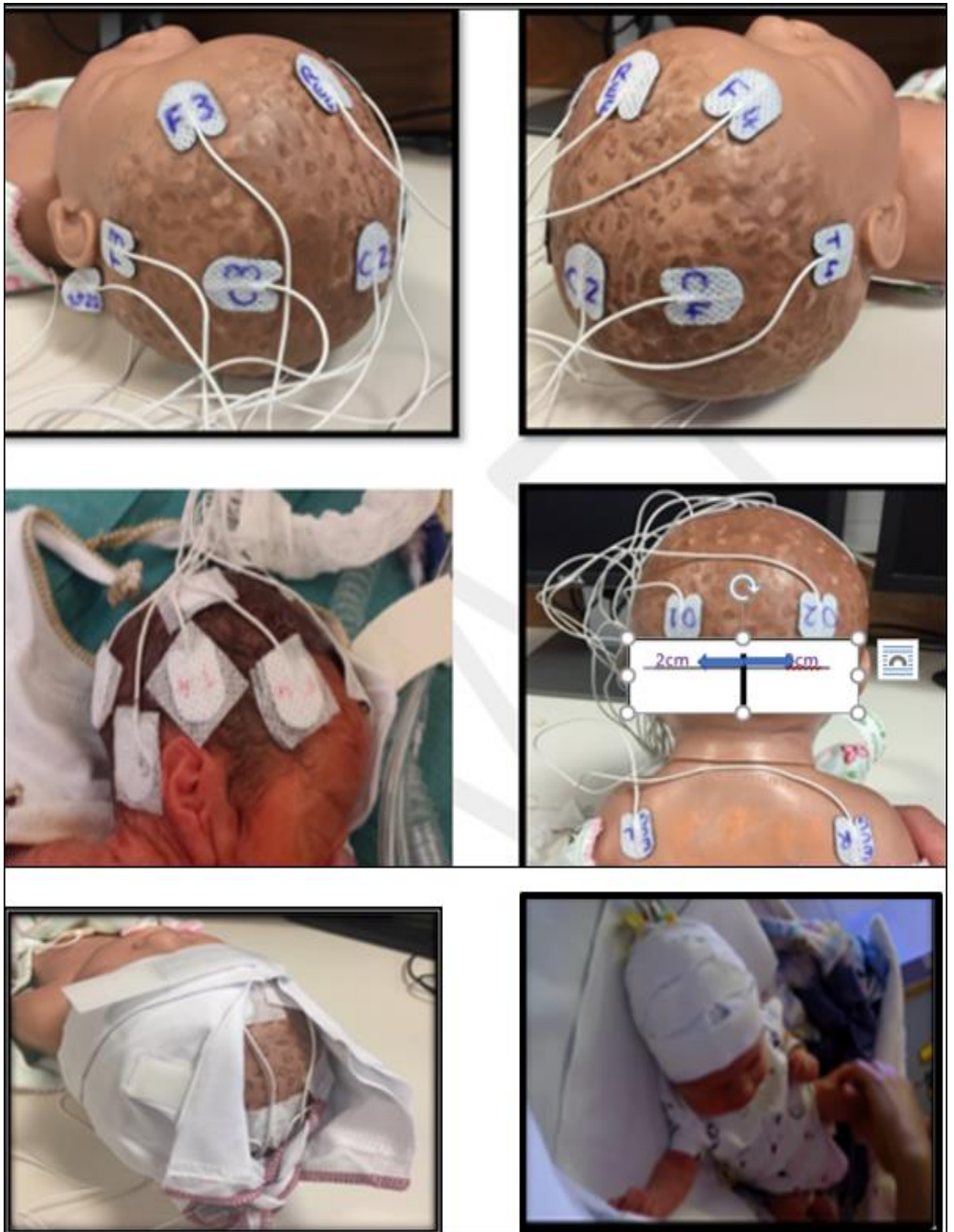


Figure 2.8 Reduced neonatal montage used for EEG monitoring.

Channels incorporated into a neonatal montage are displayed on EEG (Fig 2.9)

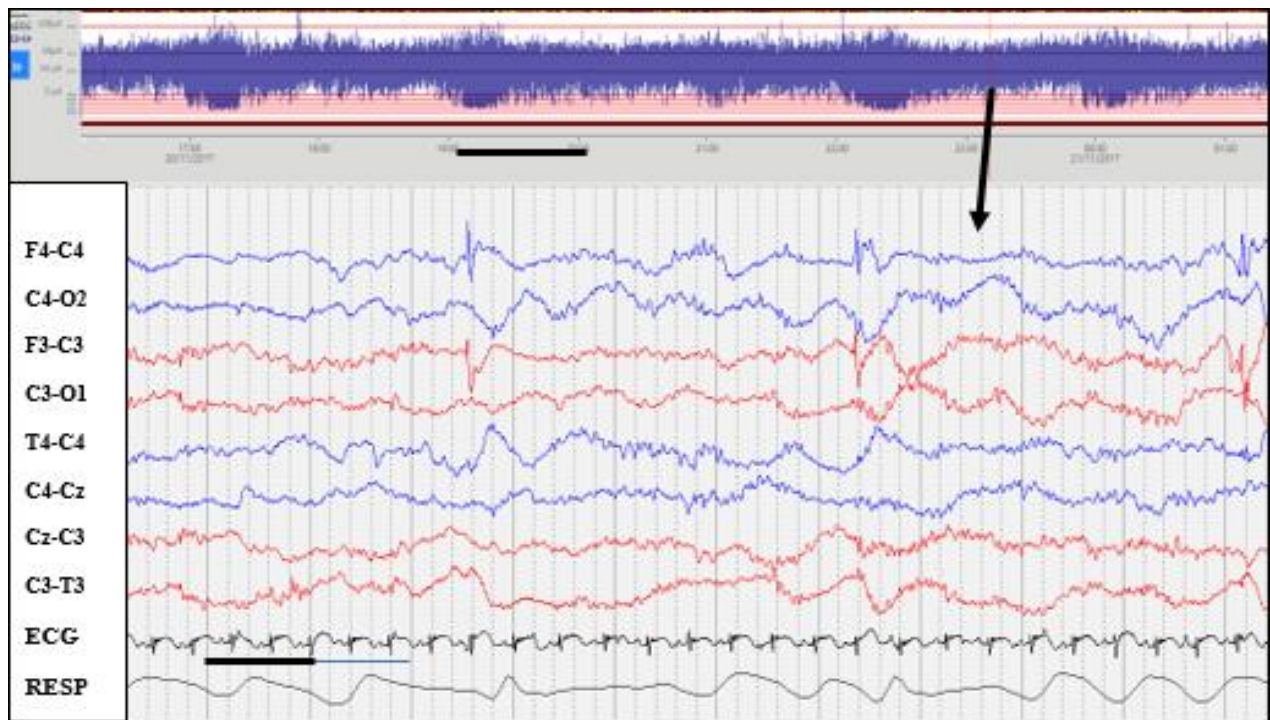


Figure 2.9 EEG screenshot with channels displayed including ECG and respiratory trace. aEEG displayed above raw EEG on channel F3-C3. Black line on aEEG indicates one hour of recording. Black line on EEG indicates one second of recording

ECG leads were applied to the left shoulder and right shoulder to monitor heart rate and to provide a reference channel to aid the identification of ECG artefact on the EEG. Respirations were recorded using a micro movement sensor (MMS/TP, Unimed, UK) secured to the abdomen which also provided a reference channel to aid the identification of respiration artefact on the EEG. All electrodes were connected to the amplifier (Figure 2.10).

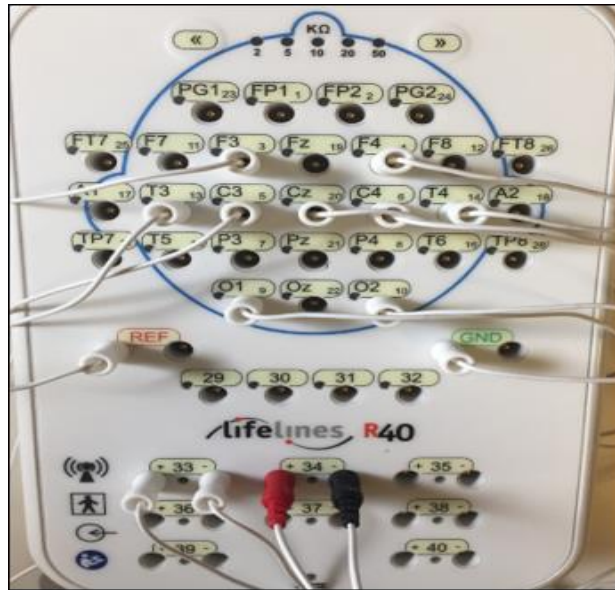


Figure 2.10 Lifelines amplifier with position of leads inserted corresponding to placements on infant's head

- Channel 33 + for left ECG and channel 33 – for right ECG.
- Channel 34 connected the respiration electrode into the + and – sockets
- The headbox was placed in a plastic bag before recording commenced

Time locked video recordings to EEG recordings facilitated visual observation of infant activity post EEG acquisition. Biological and non-biological artefact was identified and minimised where possible to ensure clarity of EEG recording and accurate interpretation. All EEGs were displayed on screen at 3 cm/s and $7\mu\text{V}/\text{mm}$ with a low pass filter set at 0.5 Hz and the high pass at 70Hz. EEG monitoring commenced when electrode impedances were $< 10 \text{ k}\Omega$'s on all electrodes.

All overnight EEGs were reviewed by Dr Sean Mathieson and the researcher for potential incidental findings.

2.9.2 Follow up visits

Developmental outcome was determined using the validated Griffiths III mental developmental scales (MDS) at 4 months and 18 months PMA.

2.9.2.1 Four months follow up visit

All infants from the MLP and TC groups returned to our dedicated sleep laboratories in the INFANT Research Centre, Department of Paediatrics, Cork University Hospital at 4 months for a full head nap time EEG and a follow-up Griffiths III Mental development assessment/ scales. All efforts were made to have infants return at the exact age required, however a +1month leeway for each assessment ensured parental time constraints, work commitments, and unforeseen circumstances could be accommodated.

Visits were coordinated by the researcher to suit routine feeding and nap times of the infants. Additional data generated at each of these visits included up to date weight, length and head circumference, mode of feeding, if infant had commenced solids, had any illnesses, hospitalisations or medications, if vaccinations have been given and any other concerns parents may have had were discussed.

A small group experienced ARCHID (Association for research in Infant and Child Development) assessors carried out developmental assessments following the same protocol throughout all assessments. This group was limited to two psychologists and 2 research fellows. Training in the use of Griffiths III was updated prior to commencement of the study which aimed to further improve the accuracy of findings and consistency in scoring. The 4month assessment was carried out whilst the infant

was positioned sitting facing forward on a parent's/carer's knees took approximately 30 minutes to complete.

A multichannel EEG accompanied by video with additional polygraphy (electrooculogram, submental EMG, ECG, respiration) to aid sleep state identification was set up when the infant was feeding (Lifelines machine used). All EEG's were carried out by the researcher (MAR) with the initial guidance of Dr Sean Mathieson. Electrode positioning was made as per the recommended international 10–20 system, disposable cup electrodes were applied in positions, FP2, FP1, F8, F7, F4, F3, Fz, A2, A1, T4, T3, C4, C3, Cz, T8, T7, P4, P3, Pz, with a reference and ground electrode also applied. A bipolar montage was used with channels displayed including F8-A2, A2-T6, F7-A1, A1-T5, Fp2-F8, F8-F4, T4-T6, T6-O2, Fp1-F7, F7-T3, T3-T5, T5-O1, Fp2-F4, F4-C4, C4-P4, P4-O2, Fp1-F3, F3-C3, C3-P3, P3-O1, Fz-CZ, Cz-Pz, C4-Cz, Cz-C3. Screen shot also displays EMG(Chin), ECG, Respirations, EOG (right), EOG (left) (Fig 2.11).



Figure 2.11 Multi channel EEG at 4 months

- Two submental electrodes were also applied to chin to monitor EMG tone
- ECG disposable gel electrodes were placed on the posterior aspect of shoulders
- An easily removeable sponge tape was used to secure a respiratory probe to abdomen

Bilateral gel electrooculogram electrodes were applied with the right electrode 1 cm above the outer canthus of the right eye and the left electrode 1 cm below the canthus of the left eye to acquire both lateral and horizontal eye movements. Infants slept in a parent/guardian's arms or in a cot provided until the infant woke spontaneously.

Analysis of data from the 4 month EEG was beyond the scope of this PhD. I did however partake in analysis and annotation of EEG spindle activity and co-authored two papers relating to same. (352, 353) (see appendices (viii) and (ix))

[2.9.2.2 18 months follow up visit here](#)

The infants returned again at 18 months PMA for another Griffiths III MDS assessment carried out by the same group of assessors. All infants sat on a chair in front of a table (parent alongside him/her). This assessment was complete within 60 minutes. Additional data generated at each of these visits included up to date weight, length and head circumference, mode of feeding, diet, illnesses or hospitalisations, vaccinations given and any other concerns parents may have had were noted.

2.9.2.3 Referral pathway

Within 2-3 weeks following each neurodevelopmental assessment all parents (MLP and TC group) received a written report by mail. In the event of any concerns or incidental findings at either time point, a pathway of care was in place (planned prior to commencement of this study). This included a rapid referral to the Professor of Neonatology (Professor Eugene Dempsey) for further investigation with further rapid referral to appropriate services/ department and ongoing surveillance if deemed necessary.

2.10 Post-acquisition visual analysis of EEG at 36 weeks PMA.

Periods of each sleep state were identified based on visual analysis of EEG activity (Chapter 1, section 1.5.2), heart rate and respiratory rate/regularity and behaviour observations (Chapter 1 section 1.5.4). Duration annotations were made for each phase of sleep by the researcher (MAR), identifying periods of active sleep (AS), quiet sleep (QS), indeterminate sleep (IS) and wakefulness (W) which supported the identification of sleep-wake cycles (SWC) and created a sleep profile for each infant. Periods of wakefulness (spontaneous or interrupted) were identified on EEG and the reason for same identified through video. A random sample of 50% of EEGs/- recorded were also reviewed by an experienced neurophysiologist Dr Sean Mathieson to ensure consistency in sleep state identification. Analysis conducted included those identified in Chapter 3, 4, 5 and 6.

2.11 IBI Detection algorithm development

Dr John O'Toole from the INFANT research centre adapted an algorithm previously developed to detect bursts and inter-burst intervals on EEG in QS of preterm infants < 30 weeks PMA for use in the MLP infant group. A random sample of QS epochs in MLP infants (n=12) were manually annotated by the researcher to train and adapt the algorithm's performance in recognising IBI's in the MLP infant group. The algorithm provided quantitative analysis of five IBI features of QS in the MLP infant group. These measures included IBI length maximum, IBI length median, IBI length IQR, IBI number and percentage of QS epoch that was an IBI. For each infant, each IBI feature was averaged across two uninterrupted QS epochs and summary measures were used in the analysis amongst infants with a normal developmental outcome at 18mths. This provided descriptive normative data unique to the MLP infant group.

2.12 Statistical analysis

All statistical analysis was conducted under the primary supervision of Dr Vicki Livingstone, INFANT centre biostatistician, who advised on the appropriate methods for analysis and reviewed analysis/results. Statistical analysis was performed using IBM SPSS Statistics (version 25.0, IBM Corp, Armonk, NY, USA). All tests were two-sided and a p -value<0.05 was considered statistically significant. Continuous variables were described using the median and interquartile range (IQR) /mean(SD) and categorical variables using frequency and percentage. Parametric testing was used for normally distributed data whilst non-parametric testing was used when data were not normally distributed. Statistical approaches made in data analysis depended on

the research question which will be described in further detail within each of the following chapters.

2.13 Strategies used for retention

Once consent was obtained a laminated SLEEPi logo was attached to the cot, identifying the infant as a study participant(Fig 2.12). In many cases, consent for participation in the SLEEPi study was obtained days or even weeks before overnight EEG monitoring. A conscious effort was made by the researcher to have ongoing engagement with parents in the neonatal unit which served to positively empower parental knowledge/interest in neonatal research and enhance the parent/researcher relationship.



Figure 2.12 SLEEPi logo

Consent to participation in this study also provided permission for the research team to contact parents by phone following discharge to arrange follow-up appointments. Calls were made to parents 2 weeks in advance of an appointment date. Text reminders were also sent the day before the appointment. To limit the burden on parents, SLEEPi follow-up appointments were carried out the same day as routine

follow clinical appointments if possible. Parents/guardians were also given a contact number for the researcher should they need to cancel/ reschedule an appointment. All infants were sent a specially designed birthday card from the SLEEPi team on their 1st birthday (Figure 2.13). This served as a thoughtful gesture to families and a reminder that contact would be made before 18 months corrected age for the final developmental assessment.



Figure 2.13 First Birthday card

Chapter 3

Overnight interruptions to sleep of the healthy Moderate to Late preterm infant in the Neonatal Unit

This chapter has been published – Appendix VI

Abstract

Introduction

Neonatal electroencephalography (EEG) is a useful cot-side tool providing detailed real-time information about brain activity of preterm infants and the changes that occur during sleep-wake cycling. Preserving and supporting optimal sleep organisation amongst infants will support a normal neural pathway of development.

Aim

The aim of this observational study is to describe the overnight sleep architecture of mid to late preterm (MLP) infants in a NICU setting. It will quantify the number of overnight uninterrupted sleep wake cycles. We will also quantify sleep interruptions and the sleep state from which they occur.

Methods

All participating infants had video EEG carried out overnight for a minimum of 12 hours. Duration annotations were made on all EEG's identifying sleep states, sleep interruptions, periods of wakefulness and feeding.

Results

98 infants had overnight EEG suitable for analysis. All infants displayed SWC with a median (IQR) of 6(5-7) per infant. 22.9% of the total SWC were interrupted for the purpose of feeding and <1% for clinical reasons. The majority of sleep interruptions occurred during active sleep (AS) (78.8%).

Conclusion

We have identified that disturbances to overnight sleep in MLP infants were frequent in the NICU, predominantly for the purpose of feeding. Reducing the number of interruptions and preserving sleep provides an opportunity to enhance brain development.

3.0 Introduction

Sleep is a prerequisite for neuronal maturation, and normal growth. It is a naturally neuroprotective activity providing a buffer in protecting infants from the negative impact of environmental stressors in the neonatal unit. The quality and quantity of infant sleep in the NICU is impacted by gene expression which may predispose a preterm infant to be more or less susceptible to adverse side effects of sleep loss. Sleep supports learning, the consolidation of memories, maintaining attention and assisting in regulating emotions and behaviours, whilst having a positive association with immune system wellbeing.(282, 354) It is likely therefore that repeated sleep interruptions will affect these processes. A careful balance between endogenous and exogenous stimulation is required in the last weeks of pregnancy during the peak period of cortical organisation.

This chapter presents an observational study of nocturnal sleep of healthy MLP infants at 36 weeks postmenstrual age in the neonatal unit. This study was designed to identify and quantify:

- The number of overnight sleep-wake cycles
- Through visual analysis of conventional EEG (cEEG), behaviour observations and alterations in rate and rhythm of respiration (and to a lesser extent heart rate) to identify sleep states, periods of wakefulness and feeding
- To describe awakenings as spontaneous or an intended interruption and identify the sleep state from which the interruptions occurred
- To determine the impact of spontaneous awakenings versus intended sleep interruptions on the duration of feeding

3.1 Monitoring sleep by Electroencephalography (EEG)

Electroencephalography (EEG) provides an opportunity to gain further insight into the complex nature of sleep and assists in achieving a high level of objectivity in identifying sleep states rather than base identification on behavioural sleep analysis alone. Gold standard EEG monitoring includes video recording time-locked to EEG activity.

Electroencephalography (EEG) is more frequently associated with monitoring the brain activity of the critically ill newborn at birth and to a much lesser extent as a cot side tool for monitoring background activity during sleep. Each sleep state is associated with unique EEG features.(13, 185, 275)(Chapter 1, section 1.3.3.3). Observed sleep behaviours of the neonate become more pronounced and integrated with recognizable patterns of activity on EEG from 30-31 weeks GA (260, 355)(Chapter 1, section 1.3.4). Sleep undergoes quantitative and qualitative changes with advancing GA (308) correlating with measurable structural changes in the brain.(314) Therefore, sleep at a micro-level reflects the degree of prematurity of the infant.

As previously described (Chapter 1, section 1.3.6), aEEG compliments cEEG providing an easily interpretable visual trend of activity.(337) Alteration in bandwidth on aEEG is representative of sleep-wake cycling (SWC) evident on aEEG from 30-32 weeks gestation as sleep-wake architecture emerges.(356) As described by Barbeau et al amplitude-integrated EEG (aEEG) may be used as a stand-alone mode of monitoring brain activity or in conjunction with cEEG (both frequently displayed on modern cEEG monitors).(274) aEEG has a greater presence in the neonatal unit which is due to ease of application with limited electrodes and a trend that is easily interpreted with minimal cot-side education.

EEG provides a blueprint for sleep-wake patterns and a signature for neurological well-being confirming a normal trajectory of development it also identifies abnormal or immature activity. However, the prognostic value of electrographic monitoring in uncomplicated medically stable MLP infants remains unclear (259, 260).

3.2 Method

Ethical approval was obtained prior to commencement of the study (ECM 4 (w) 09/05/2017). Clear inclusion /exclusion criteria identified (Chapter 2.0 Methodology, section 2.4). As described in Chapter 2, section 2.9.1 overnight EEG's were performed at 36 weeks postmenstrual age (PMA) in a quiet area of the neonatal unit, which commenced between 16.00.hrs and 18.00 hrs for a minimum of 12 hrs. As per unit policy, no routine examinations or routine neonatal procedures are carried out overnight.

Periods of each sleep state were identified and annotated based on visual analysis of EEG activity (Chapter 1, section 1.3.3.3), heart rate and respiratory rate/ regularity and behaviour observations (Chapter 1 section 1.3.4). Duration annotations were also placed on periods of feeding identified by video. All recordings were reviewed by a neurophysiologist for any abnormal incidental findings and thereafter pseudo-anonymised and transferred to a secure server for long-term storage.

3.3 Statistical analysis

Statistical analysis was performed using IBM SPSS Statistics (version 25.0, IBM Corp, Armonk, NY, U.S.A.). Demographic data is presented based on median and

interquartile ranges and frequencies are presented as percentages. For infants who fed orally with ≤ 1 NGT feed overnight, a random-effects model was used to investigate if the duration of oral feeding (feed given closest to midnight) differed by type of awakening (interrupted versus spontaneous). The type of awakening was a fixed effect and the subject a random effect in the model. The Mann-Whitney U test was used to investigate if the duration of oral feeding differed by sleep state from which the infant was woken (quiet vs active).

3.4 Results

Informed parental consent was obtained for 117 MLP infants admitted to the neonatal unit. Reason for admission to the neonatal unit included: low birth weight, respiratory concerns (e.g. tachypnoea, increased work of breathing, low saturations), low blood sugars, poor feeding and $>10\%$ weight loss, suspected sepsis, and for treatment of jaundice. Due to early discharge, transfers to provincial hospitals, later exclusion criteria or technical issues, 101 cEEG with video were carried out, 98 of which were suitable for analysis

In excess of 1,176 hrs of conventional EEG were reviewed and annotated in this study. All participants were clinically stable at the time of the recording. Demographic data and overnight SWC of infants are shown in Table 3.1. Similar numbers of male and female infants participated in this study, the majority of whom were nursed in a cot (83.9%).

SWC was identified on the EEG recording of all participants. The median (IQR) number of SWCs overnight was 6(5-7) (Table 3.1). On two occasions sleep was

interrupted for clinical reasons, (once to obtain a capillary sample of blood for bilirubin levels and a second for medical review following a desaturation with a vomit post feeding). The remaining interruptions were exclusively for feeding and occurred during both AS (77.8%) and QS (22.2%). Of the total overnight SWCs recorded (n=589), 23.3 % (n=137) were interrupted.

Table 3.1 Demographic data and overnight sleep wake cycling recorded over a 12 hr period (n=98)

	Median (IQR)
Birth gestational age (weeks)	34.1(33.5-35.1)
Gestation at time of EEG (weeks)	36.00 (35.6-36.6)
Birth weight (g)	2100 (1868-2378)
Weight at the time of EEG (g)	2270 (2123-2508)
Postnatal age at the time of video EEG (days)	11 (5-17)
Sleep-wake cycles per infant per night	6 (5-7)
Range	3 – 11
	<i>n</i> (%)
Sex	
Male	52 (53.1)
Female	46 (46.9)
Accommodation on EEG monitoring	
Cot	79 (80.6)
Incubator	19 (19.3)
Participants displaying SWC on EEG	98 (100)
Total number of sleep cycles recorded overnight	589 (100)
Total number of SWC interrupted	137 (23.2)
SWC interruptions clinical reasons	2 (<1)
SWC interruptions for feeding	135 (22.9)

Interquartile range, Wks: weeks, Kg: kilogram, vEEG: video electroencephalograph, hrs: hours, SWC: sleep-wake cycling

Feeds were given either orally (78.7%) or via a nasogastric tube (NGT) (21.3%) (Table 3.2). 68.8% of infants woke spontaneously before oral feeds whilst 31.2% were actively woken for one or more feeds. Some infants not actively feeding had sleep

disturbed before an NGT feed (61.8%) whilst 38.2% were undisturbed and slept throughout NGT feed.

Table 3.2 Overnight interruptions for feeding.

	<i>n (%)</i>
NG feeds (n=76)	
Uninterrupted sleep	29 (38.2)
Interrupted sleep	47 (61.8)
Oral Feeds (n=282)	
Woke Spontaneously	194 (68.8)
Sleep interrupted	88 (31.2)
Timing of interruptions (n=135)	
Active sleep	105 (77.8)
Quiet sleep	30 (22.2)

NGT: nasogastric tube

Further analysis compared the duration of feeding and sleep state from which the infant was interrupted (AS versus QS)). A total of n=44 babies are included in the analysis, n=34 in the AS group and n=10 in the QS group. Although the feeding duration was higher in the group woken from QS (median (IQR): 28.0 (15.8 to 40.8)) than the group woken from AS (median (IQR): 22.5 (14.0 to 26.3), the difference between groups was not statistically significant (p=0.102).

3.5 Discussion

All MLP infants in this study displayed periods of AS, QS, and wakefulness (W) with a wide range in the number of SWC seen. Interruption of overnight sleep was primarily for feeding purposes (both oral and NGT feeds), and equated to 23.3 % of all overnight

SWC. It is not unreasonable to consider that the number of interruptions would increase during daytime hours due to routine procedures and examinations carried out. Each sleep interruption will revert to a recommencement of a sleep cycle in AS which may lead to less time spent in QS. In a study carried out by Levy et al (212), 4-hour vEEG monitoring of clinically stable near-term at risk of neurological abnormalities and term infants in the NICU (n=25) showed that provider contact occurred for 27% (65 mins) of the recording time and only 50 % of infants engaged in a full SWC before interruption for care. Similar to our study, the majority of infant contact/disturbances occurred during AS, a state which has a greater association with the growth and development of complex neural networks. (357)

We investigated if there was a difference in feed duration based on spontaneous awakening from sleep versus sleep being interrupted. Considering the feed closest to midnight (n=83) the estimated marginal mean was 24.13 (95% confidence interval (CI): 20.55 to 27.71) minutes when the baby was interrupted from their sleep and 17.85 (95% CI: 15.38 to 20.31) minutes when the infant woke spontaneously (difference in means (95% CI): 6.28 (2.22 to 10.35) mins, $p=0.003$). Hence in the absence of clinical concern, spontaneous awakenings support's less time taken to feed. This significant difference has resource implications and perhaps policy implications when it comes to establishing oral feeding.

Preterm birth brings with it a sudden change in the quality and intensity of sensory information received which may lead to disrupted and fragmented sleep, impacting sleep organisation and developmental outcome.(212, 230) Prolonged and repeated sleep interruptions lead to continuous exposure to the multisensory environment of the

neonatal unit and may impact not only the physiological status but long-term neurodevelopmental outcome of the preterm infant including long-term sensory processing disorders. (203, 303, 358, 359) A priority to creating a culture of 'sleep protection' requires knowledge about SWC and recognition of sleep states which will assist in identifying the appropriate timing of handling for infant care (260, 288, 310). Despite being closest to the point of care of the preterm infant in the NICU, a low level of knowledge of behavioural features and sleep state identification has been found amongst neonatal nurses and this may lead to misinterpretation of sleep activity (i.e. active sleep) for wakening (360).

MLP infants frequently display disorganised feeding behaviours with difficulties evident in an ability to co-ordinate 'suck, swallow and breathe' when feeding (361). This factor is reflected in this study as 21% of feeds were given via NGT (n=76). Feeding ability may be further diluted by environmental factors leading to inadequate sleep in the NICU impacting clinical condition, growth and development, neuro-behaviour and the establishment of oral feeding.(361, 362) Hence in the absence of clinical need, disturbing sleep when the infant is not actively feeding does little to support brain development and is an area that warrants further research.(212) In the absence of a specific plan of care or local guidelines, the mode, frequency and schedule of feeds are very much guided by the bedside nurse which has led to 'ad hoc' practices used in establishing oral feeding. Longer feed duration, as reported in this study when sleep is interrupted, has a knock-on effect on human resources, duration of stay, and cost to the health care provider and warrants further consideration. In the absence of medical concerns allowing a healthy MLP preterm to wake spontaneously for feeding at 36 weeks will ensure little interruption to the natural

course of a SWC which may positively impact neurodevelopmental outcome.

The strengths of this study include the number of participants, similar clinical status, gestational age and the timing/duration of EEG monitoring. The use of time locked video supported visual observation of the infant with respiratory monitoring supporting sleep state identification i.e. rapid irregular respirations in AS (REM) with slower regular breathing in QS (NREM). Whilst a focus on sleep during night time hours only may be considered a limitation of this study, it did standardise care somewhat for all infants as no routine disturbances would occur, hence maximising sleep potential. Further research may incorporate a full 24 hr monitoring period providing insight into both day and night SWC and interruptions observed. The prognostic value of EEG in uncomplicated medically stable MLP infants remains unclear (259, 260). Hence the significance of the outcome of the number of SWC seen on EEG and the duration of each sleep state has yet to be determined and will constitute part of a future study.

3.6 Conclusion

We have confirmed overnight SWC is present in all participating MLP infants. Overnight disruptions to sleep were common, and accounted for 23.3% of the total SWC recorded. The primary reason was for routine cares/ feeding. Reducing this number of interruptions and protecting sleep provides an opportunity to enhance brain development but presents a real operational challenge in the NICU. Preserving optimal sleep organisation will support a normal neural pathway of development and positively support long- short-term outcomes and warrants further studies.

Chapter 4

Sleep state organisation of moderate to late preterm infants in the neonatal unit

This study has been published (Appendix VIII)

Abstract

Background

Sleep supports neurodevelopment and sleep architecture reflects brain maturation. This prospective observational study describes the nocturnal sleep architecture of healthy moderate to late preterm (MLP) infants in the neonatal unit at 36 weeks post menstrual age (PMA).

Method

MLP infants, in the neonatal unit of a tertiary hospital in Ireland from 2017 to 2018, had overnight continuous electroencephalography (cEEG) with video for a minimum 12 h at 36 weeks PMA. The total sleep time (TST) including periods of active sleep (AS), quiet sleep (QS), indeterminate sleep (IS), wakefulness and feeding were identified annotated and quantified.

Results

A total of 98 infants had cEEG with video monitoring suitable for analysis. The median (IQR) of TST in the 12 h period was 7.09 h (IQR 6.61–7.76), 4.58 h (3.69–5.09) in AS, 2.02 h (1.76–2.36 h) in QS and 0.65 h (0.48–0.89) in IS. The total duration of AS was significantly lower in infants born at lower GA ($p = 0.007$) whilst the duration of individual QS periods was significantly higher ($p = 0.001$).

Conclusion

Overnight cEEG with video at 36 weeks PMA showed that sleep state architecture is dependent on birth GA. Infants with a lower birth GA have less AS and more QS that may have implications for later neurodevelopment.

4.0 Introduction

One of the most important regulated processes in the development of newborn neural circuitry is sleep.(310) The activity of sleep supports optimal structural and functional integrity of the brain and a prerequisite for development of neural connections and myelination in all regions. (230, 281) As the primary activity of the developing brain, sleep plays a crucial role in brain development. As the dominant sleep state of the MLP group, AS provides endogenous stimulation facilitating neural network maturation, synapse formation and synaptic plasticity. AS supports memory, learning, emotional and motor development and has a strong correlation with brain growth.(357) It is also a prerequisite state for psychosocial and normal sensory system development.(363) A reduction in AS in the preterm infant may reduce the efficiency of information transmission and decrease overall brain volume. QS is a prerequisite for maturation, supporting connectivity between networks and the preservation of brain plasticity.(6) QS is also associated with cognitive function and the pre-consolidation phase of learning, memory and the maintenance of physiological homeostasis.

Sleep state organisation is an expression of emerging neural network development reflecting the underlying functioning of the brain and degree of CNS maturation and a marker of neurological wellbeing.(310, 364) Human sleep undergoes quantitative and qualitative changes from birth, hence organisation of sleep alters with increasing GA as the brain matures. (137, 365) Given the relationship between sleep and brain development, interference with sleep may underlie many of the neurodevelopmental

deficits experienced by the preterm infant with downstream effects on developmental processes and lifelong effects. (177, 214, 267)

Previous studies examining sleep architecture in the MLP group have analysed short periods of sleep alone which may not be fully representative of sleep at this age or classified sleep based on behaviour observation alone.^{209,334,396}

Maintaining the structural and functional integrity of the fragile preterm brain during in the extrauterine environment of the NICU is the primary challenge faced by the vulnerable preterm brain.⁽³¹⁴⁾ Preterm infants are less competent in coping with external environment and stressors within the neonatal unit may compromise the quality and quantity of sleep. (12, 230, 366) The genetically defined pre-programmed pathway of normal sleep ontogenesis may be altered or supported through physiological and sensory environmental factors. (293, 298) Supporting good quality sleep, of adequate duration and without disturbance will strengthen sleep architecture and support optimal neurodevelopment. (190, 267)

This study aims to describe the sleep state organisation of the healthy moderate to late preterm (MLP) infant at 36 weeks PMA. We will describe sleep by carrying out:

- An analysis of duration of sleep, feeding and wakefulness over a 12-h period (sleep-wake analysis).
- A total sleep time (TST) analysis, i.e., the total duration of all periods of AS, QS and IS in the 12-h recording period.
- A sleep cycle analysis described in terms of the median time in AS, QS

and IS across overnight uninterrupted complete sleep cycles.

We hypothesised that the sleep architecture of all healthy MLP infants at 36 weeks PMA would be the same, irrespective of birth GA, sex, mode of feeding or accommodation (cot or incubator) at time of monitoring.

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4.1 Methods

Sleep states are more robustly measured by EEG in combination with behavioural observations and physiological activity (respirations and heart rate). In this prospective observational study conventional EEG (neonatal montage) at 36 weeks PMA was used to record the electrical activity of the brain during sleep overnight in the neonatal unit of a group of healthy MLP infants (Described in chapter 2, section 2.9 and section 2.10). Defining terms of sleep cycling, AS, QS, and IS based on EEG activity and behaviours observations was a prerequisite to this study. As each sleep state is associated with a unique pattern of EEG, periods of each sleep state were identified based on visual analysis of EEG activity (Chapter 1, section 1.5.2 and 1.5.3), heart rate and respiratory rate/ regularity and behaviour observations (Chapter 1, section 1.5.4). Inclusion and exclusion criteria have been highlighted in Chapter 2 Methodology, section 2.4 with details pertaining to EEG monitoring in Chapter 2 Methodology Section 2.9.

4.2 Statistical Analysis

Statistical analysis was performed using IBM SPSS Statistics (version 25.0, IBM Corp, Armonk, NY). Continuous variables were described using the

median and interquartile range (IQR) and categorical variables using frequency and percentage. The Mann–Whitney U test was used for comparisons of sleep parameters between two groups and the Kruskal–Wallis test was used when there were more than two groups. If statistically significant differences were found between groups for the Kruskal–Wallis test, pairwise comparisons were performed using the Mann–Whitney U test, with Bonferroni correction. The groups investigated were GA at birth (32–32 + 6, 33–33 + 6, 34–34 + 6, 35–35 + 6 and 36–36 + 6 weeks), sex, accommodation at the time of monitoring (cot or incubator) and mode of feeding (oral only, mixed mode). The Mann-Whitney U test was also used to investigate the relationship between GA and mode of feeding. Relationships between number of interruptions and GA and active sleep duration were investigated using Spearman's correlation coefficient (r_s). All tests were two-sided and a p value <0.05 was statistically significant.

4.3 Results

The results of this study include an analysis of uninterrupted SWC's and an analysis of total sleep time (TST) overnight in the neonatal unit for the healthy MLP infants at 36 weeks PMA pre-discharge. All infants were healthy MLP infant's and transitioned through the neonatal unit without complications. Although 117 infants were recruited to this study, 98 infants had EEG's carried out that were suitable for analysis (Figure 4.1).

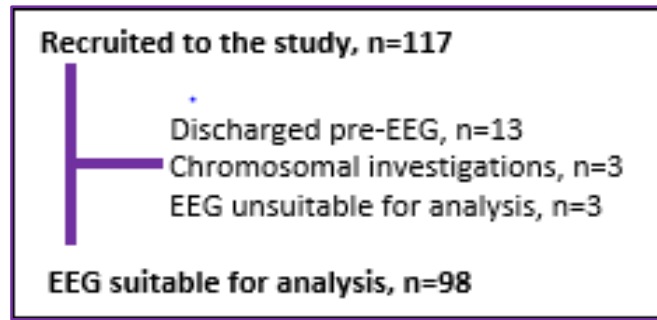


Figure 4.1 Recruitment

4.3.1 Sleep staging-Interrater agreement

Post-acquisition, each 12h cEEG with video were annotated by the same reviewer (M.A.R.) and a 2 h epoch of 50% of EEG's recorded were also annotated by a second reviewer (S.R.M.). A two-way random effects intraclass correlation was calculated between the two raters on a random sample of half the EEGs. Results showed the correlation between raters for AS was 0.910 (95% CI: 0.834–0.952), QS 0.901 (95% CI: 0.817–0.947) and IS was 0.668 (95% CI: 0.448–0.812). The total duration of each sleep state was recorded, rounded to the nearest minute, and exported to SPSS.

4.3.2 Demographics

Infants were from singleton (n=51) and multiple pregnancies (n=47) and nursed in a cot (n=80) or incubator (n=18) at time of monitoring. The majority of infants were exclusively fed orally (n=61) whilst the remainder had either a mixed-mode of feeding (n=36) or were fed exclusively via nasogastric tube (NGT) (n=1) (Table 4.1)

Table 4.1 Demographics of moderate to late preterm infant group(n=98)

Demographics(n=98)	
	Median (IQR)
Maternal age (years)	34.50(31.00-37.30)
Birth gestation (weeks)	34.00(33.00-35.00)
Gestation at time of EEG (weeks)	36.10(35.60-36.60)
Birth weight (kg)	2.10(1.87-2.40)
Weight at the time of EEG (kg)	2.26(2.02-2.47)
Postnatal age on EEG (days)	10.50(4.80-14.00)
	n(%)
Ethnicity – Irish	88(89.8)
Pregnancy	
Multiple	47(48.0)
Singleton	51(52.0)
Sex	
Male	52(53.1)
Female	46(46.9)
Accommodation at time of monitoring	
Cot	80(81.6)
Incubator	18(18.4)
Mode of overnight feeding	
All oral feeds	61(62.2)
All NGT feeds	1(1.0)
Mixed feeding (Oral and NGT)	36(36.7)

IQR: interquartile range, Kg: kilogram, vEEG: video EEG, NGT: nasogastric tube

4.3.3 Sleep-wakefulness analysis

The median (IQR) of TST over the 12 h monitoring period was 7.09 h (6.61–7.76) (Table 4.20). The remaining overnight hours were spent attending to the infant's care/feeding and periods of wakefulness. The median time spent attending to infant cares and feeding was 1.58 h (IQR: 1.18–2.13) and the median duration of wakefulness was 3.19 h (IQR: 2.38–3.85) (Table 4.2, Pie graph representation Figure 4.2). Wakefulness incorporated periods of quiet wakefulness, time spent unsettled/fussing and/or crying. As expected, infants who were fully feeding orally spent a significantly longer time feeding in comparison to infants who had a mixed mode of feeding ($p=0.004$) and had a significantly longer duration of wakefulness outside of feeding ($p=0.033$). Infants with a mixed mode of feeding had a significantly longer time spent sleeping ($p<0.001$), and shorter time feeding ($p=0.004$) and shorter time awake ($p=0.033$). There were no differences in TST, feeding or wakefulness based on GA, sex or accommodation at the time of monitoring.

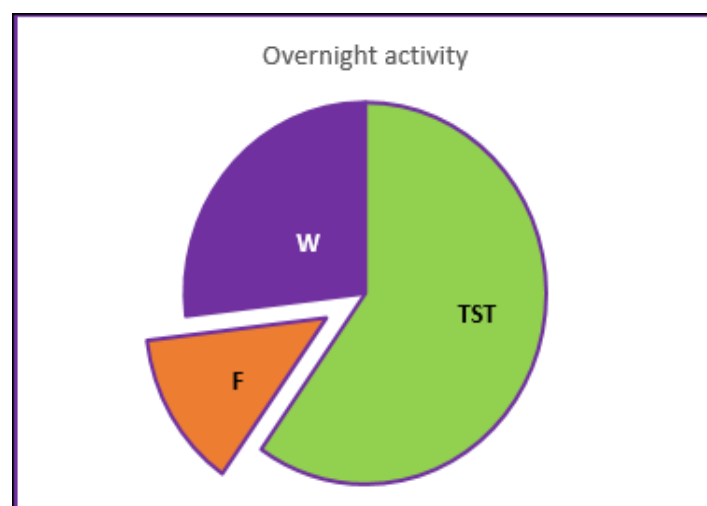


Figure 4.2 Pie graph representation of overnight duration of sleep, wakefulness and feeding. TST: total sleep time, W: wakefulness, F: Feeding .

Table 4.2 Overnight total sleep time(TST), duration of feeding/cares and duration of wakefulness at 36 weeks post menstrual age(n=98)

	TST (hours) P ¹ value	Feeding and cares duration (hours) P ¹ value Median (IQR)	Wakefulness duration (hours) P ¹ value Median (IQR)
Overall	7.09(6.61-7.76)	1.58(1.18-2.13)	3.19(2.38-3.85)
Birth Gestational Age group (weeks)	0.143	0.625	0.128
32(n=19)	6.96(6.06-7.34)	3.57(2.63-4.13)	1.60(1.34-2.06)
33(n=12)	7.09(6.84-8.01)	3.67(2.45-3.96)	1.34(1.03-1.89)
34(n=36)	6.96(6.30-7.84)	3.36(2.22-3.91)	1.47(1.12-2.36)
35(n=15)	7.50(6.91-7.82)	3.05(2.07-3.29)	1.52(1.13-2.09)
36(n=16)	7.17(6.83-8.16)	2.80(2.35-3.16)	1.87(1.13-2.20)
Sex	0.606	0.177	0.586
Male (n=52)	7.06(6.59-7.73)	1.75(1.26-2.17)	3.02(2.29-3.90)
Female(n=46)	7.21(6.70-7.83)	1.46(1.10-2.13)	3.32(2.43-3.84)
Accommodation	0.271	0.319	0.176
Cot(n=80)	7.03(6.52-7.73)	1.53(1.16-2.11)	3.27(2.39-3.90)
Incubator(n=18)	7.30(6.83-7.83)	1.87(1.18-2.28)	2.82(2.31-3.62)
Mode of feeding ²	<0.001	0.004	0.033
Oral(n=61)	6.91(6.30-7.35)	1.83(1.32-2.26)	3.37(2.43-3.99)
Mixed(n=36)	7.57 (6.94-8.20)	1.28(1.09-1.71)	2.87(2.09-3.69)

TST: total sleep time, IQR: interquartile range,

¹The Kruskal- Wallis test was used for gestational age and the Mann-Whitney U test was used for sex, accommodation and mode of feeding. ²One infant fed exclusively via NGT tube and eliminated from mode of feeding analysis (n=97). A p<0.05 is statistically significant.

4.3.4 Total sleep time analysis

Time and percentage of time (out of 12h) in each sleep state overall and by birth GA group, sex, accommodation at time of monitoring and mode of feeding are presented in Table 4.3 (Pie graph representation of time spent in each sleep state see Figure 4.3) AS was the dominant sleep state with a median (IQR) duration of 4.58 h(3.69-

5.09h) or 63% of TST. The median (IQR) for QS and IS were 2.02 h (1.76-2.36 h) or 28% and 0.65 h (0.48-0.89 h) or 9% respectively.

There was a statistically significant increase in AS noted with advancing GA ($p=0.007$). Using pairwise comparisons, we found this difference was primarily due to the significantly lower duration of AS in the youngest birth GA group (32 weeks) compared to older GA groups of 35 weeks (adjusted $p=0.019$) and 36 weeks (adjusted p -value=0.017).

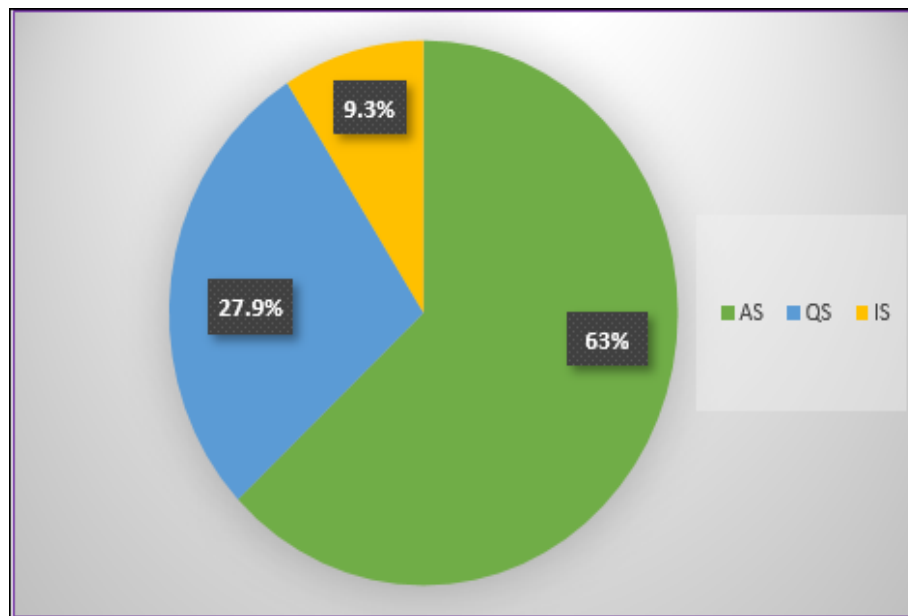


Figure 4.3 Pie graph representation of percentage of total sleep time spent in each sleep state
AS: active sleep, QS: quiet sleep, IS: indeterminate sleep

We investigated if the relationship between GA and AS was confounded by mode of feeding. We did not find a statistically significant relationship between mode of feeding and GA (weeks) ($p=0.107$) and therefore, the decreased time spent in AS amongst infants born at a younger GA cannot be explained by mode of feeding. Although there

was a trend for lower QS duration in older GA groups, the difference was not significant ($p=0.825$). There was no difference in duration of IS ($p = 0.544$) by GA group.

In a previous study of this MLP group ($n = 98$), we found that 23.3% of overnight sleep cycles in an MLP infant group were interrupted, almost exclusively for the purposes of feeding.(224) Therefore, we investigated if this increase in AS with increasing GA could have been due to a difference in the number of sleep interruptions that are followed by a period of wakefulness and then a recommencement of AS. The number of interruptions increased with increasing GA ($r_s = 0.20$, $p = 0.048$) but no association was found between number of interruptions and duration of AS ($r_s = 0.05$, $p = 0.593$). Thus, number of interruptions was not a confounder of the association between GA and AS. The total duration of AS did not significantly differ based on sex or accommodation ($p=0.457$ and 0.790 , respectively). There were no differences in duration of QS based on sex, accommodation or mode of feeding ($p=0.089$, 0.073 and 0.291 , respectively) or IS based on the same groupings ($p = 0.845$, 0.989 or 0.852 , respectively).

Table 4.3 Duration of each sleep state expressed in hours and as a percentage of total sleep time(n=98)

	AS median (hours) median (IQR)	p- value ¹	% median (IQR)	AS p- value ¹	QS median (hours) median (IQR)	p- value ¹	% QS median (IQR)	p- value ¹	IS median(IQR)	(hours) p- value ¹	% median (IQR)	IS p- value ¹
Overall	4.58(3.69-5.09)		63.07(56.25-67.43)		2.02(1.76-2.36)		27.88(24.26-34.24)		0.65(0.48-0.89)		9.13(6.30-12.24)	
Birth Gestational Age group (weeks)		0.007		0.097		0.825		0.122		0.544		0.298
32(n=19)	3.86(3.28-4.38)		56.22(49.43-64.79)		2.07(1.88-2.50)		33.33(26.63-36.00)		0.68(0.50-0.92)		9.51(8.36-13.59)	
33(n=12)	4.76(4.09-5.15)		63.01(58.49-68.29)		2.07(1.70-2.46)		29.14(24.68-32.77)		0.56(0.34-0.86)		7.23(5.31-12.92)	
34(n=36)	4.45(3.45-5.09)		64.11(51.20-68.33)		2.04(1.78-2.23)		27.65(23.39-34.84)		0.68(0.48-0.94)		9.86(6.96-12.61)	
35(n=15)	4.87(4.60-5.08)		64.94(60.49-66.86)		1.98(1.70-2.35)		26.19(22.43-30.65)		0.65(0.57-0.87)		8.87(6.51-11.73)	
36(n=16)	4.95(4.14-5.48)		65.91(57.62-69.35)		1.92(1.43-2.55)		26.15(21.53-34.21)		0.56(0.37-0.87)		7.16(5.44-12.26)	
Sex		0.457		0.200		0.089		0.129		0.845		0.825
Male (n=52)	4.59(3.70-5.24)		64.55(55.40-69.52)		1.95(1.71-2.28)		26.55(23.08-33.65)		0.64(0.48-0.88)		8.43(6.06-13.38)	
Female(n=46)	4.40(3.68-5.06)		61.76(56.25-65.73)		2.14(1.88-2.49)		29.59(25.84-35.09)		0.65(0.50-0.91)		9.23(6.78-11.69)	
Accommodation		0.790		0.335		0.073		0.263		0.989		0.869
Cot(n=80)	4.49(3.70-5.15)		63.78(56.23-68.22)		1.99(1.71-2.31)		27.88(23.62-34.09)		0.65(0.48-0.88)		9.23(6.38-12.26)	
Incubator(n=18)	4.75(3.60-4.98)		60.98(55.44-65.10)		2.24(1.93-2.51)		27.88(26.18-36.19)		0.60(0.49-0.93)		8.43(6.03-12.35)	
Mode of feeding ²		<0.001		0.433		0.291	0.473			0.852		0.229
Oral(n=61)	4.29(3.45-4.86)		63.25(52.19-68.05)		2.01(1.75-2.33)		27.90(24.62-34.66)		0.65(0.47-0.88)		9.68(6.42-12.81)	
Mixed(n=36)	4.94(4.47-5.32)		63.09(59.31-67.01)		2.04(1.73-2.52)		27.65(22.76-30.92)		0.59(0.51-0.93)		8.01(6.04-11.49)	

IQR: interquartile range. p-value¹ was calculated by Mann Whitney test for sex, accommodation, and mode of feeding and by the Kruskal-Wallis for GA group.

¹ a p-value<0.05 was significant. ²One infant fed exclusively via NGT tube and eliminated from mode of feeding analysis (n=97).

4.3.5 Uninterrupted sleep cycle analysis

All infants displayed sleep cycling on cEEG with a median (IQR) of 6.0 (6.0–7.3) uninterrupted sleep cycles throughout the night. Sleep cycle duration and the duration of AS, QS and IS within are described by GA group, sex, accommodation at time of monitoring and mode of feeding are presented in Table 4.4. The median (IQR) duration (min) of an overnight uninterrupted sleep cycle was 37.00 (32.00-45.13) min with median (IQR) of 16.50 (12.50-22.50) min for AS, 16.75 (14.00-19.50) min for QS and 2.75 (2.00-3.50) min for IS.

Within sleep cycles, QS duration differed by GA group ($p=0.001$). Pairwise comparisons revealed significant differences between the youngest GA group (32 weeks) and the older GA groups of 34 weeks (adjusted $p = 0.001$) and 36 weeks (adjusted $p = 0.010$). The duration of AS, IS or sleep cycle duration did not differ between GA groups ($p= 0.247$, 0.210 and 0.105 respectively). There were no differences in the duration of AS, QS, IS within an uninterrupted sleep cycle or overall cycle duration based on sex, accommodation, or mode of feeding.

Table 4.4 Sleep cycle duration overall by sleep state/ groups including feeding and wakefulness (n=98)

	Overnight sleep cycle duration (mins) median (IQR)	p-value ¹	AS duration(mins) median (IQR)	p-value ¹	QS duration(mins) median (IQR)	p-value ¹	IS duration(mins) median (IQR)	p-value ¹
Overall	37.00(32.00-45.13)		16.50(12.50-22.50)		16.75(14.00-19.50)		2.75(2.00-3.50)	
Gestational age per group(weeks)		0.105		0.247		0.001		0.210
32(n=19)	40.50(31.00-50.00)		15.00(9.00-23.00)		19.50(17.00-22.50)		3.00(2.00-4.00)	
33(n=12)	39.00(31.13-43.75)		16.50(13.00-22.00)		17.75(16.00-19.00)		2.00(1.63-3.00)	
34(n=36)	34.50(30.00-39.00)		14.25(12.00-21.38)		15.00(11.63-18.38)		2.75(2.00-3.38)	
35(n=15)	39.00(35.00-45.00)		19.00(15.00-23.00)		16.00(15.00-20.00)		2.00(1.50-3.00)	
36(n=16)	41.50(35.13-48.75)		20.00(15.00-25.88)		15.00(13.00-18.00)		2.75(1.63-3.38)	
Sex		0.706		0.591		0.151		0.294
Male (n=52)	37.00(32.00-44.50)		16.75(13.00-23.00)		16.00(13.00-19.38)		3.00(2.00-3.50)	
Female(n=46)	38.00(32.00-46.50)		16.25(9.88-22.13)		17.25(15.00-19.63)		2.50(2.00-3.13)	
Accommodation		0.151		0.451		0.666		0.386
Cot(n=80)	36.75(32.00-44.25)		15.50(12.63-22.00)		16.50(14.00-19.50)		2.50(2.00-3.00)	
Incubator(n=18)	43.00(33.50-48.25)		21.25(11.25-24.63)		18.00(14.63-19.63)		3.00(1.88-4.00)	
Mode of feeding ²		0.098		0.143		0.363		0.820
Oral(n=61)	37.00(31.50-44.00)		15.00(11.50-22.25)		17.00(13.25-20.00)		2.50(2.00-3.50)	
Mixed(n=36)	39.50(34.63-46.50)		19.50(13.25-23.75)		16.25(14.25-18.00)		2.75(2.00-3.00)	

AS : active sleep, QS: quiet sleep, IS::indeterminate sleep., IQR: interquartile range. p-value¹ was calculated by Mann Whitney test for sex, accommodation and mode of feeding and by the Kruskal-Wallis for GA group.¹ p-value<0.05 is significant. ²One infant fed exclusively via NGT tube and eliminated from mode of feeding analysis (n=97).

4.4 Discussion

In this study, we used cEEG with video to quantify the overnight sleep of a large cohort of healthy MLP infants in the neonatal unit. The duration of overnight sleep was shorter than expected whilst the duration of wakefulness was longer. Interestingly, at 36 weeks infants born in the lower GA groups had the longest duration of wakefulness however, these differences did not reach a level of significance ($p = 0.128$). We have shown that MLP infant group slept for a median (IQR) duration of 7.09 h (6.61–7.76 h) or 59.1%(55.1 -64.7%) of 12 hr monitoring period. It could be considered that 14.18 h extrapolated over a 24-h period falls short of the expected average of 16–18 h of sleep for newborn infants.(195, 367) Intuitively, this might be expected to be an overestimate of TST as an increase in clinical activity and sensory exposure by day may lead to infants sleeping less during daytime hours. However, Ardura et al. suggested the opposite to be true finding that preterm infants at 35 weeks GA($n=11$) slept for 8.88hrs during day time hours (08.00hrs-20.00hrs) and 8.22 hrs overnight (20.00hrs -08.00hrs) whilst at 36 weeks GA ($n=14$) infants slept for 8.96 hrs by day and 7.51 hrs overnight.(180)

When investigating the effects of handling on sleep in late preterm low birth weight infants nursed in an incubator($n=12$), Maki et al. found no significant difference in duration of AS, QS, IS or wakefulness between day and night time sleep ($p=0.83, 0.84, 0.73$ and 0.99 respectively).(368) These authors also observed that the infant group under study slept for approximately 13.7 hrs of the 24hour period i.e. 57.2% of 24 hours. As we found no significant difference in total sleep time between infants nursed in a cot or an incubator ($p= 0.271$), these findings are comparable to ours. However, sleep duration recommendations for infants are based on weak scientific evidence.

(369) The preterm infant may spend up to 90% of the day sleeping which decreases with advancing GA.(370) Whilst 14-17 hrs are identified as appropriate for term newborn any duration between 11 and 19 hours has been considered acceptable. (369)

In our TST analysis, we found that the majority of overnight sleep was AS, representing 63.07% (56.25–67.43%) of the TST. As sleep in the newborn begins with AS it is not surprising that overall, TST was dominated by AS, as following each. interruption (or spontaneous awakening) a new phase of AS sleep must begin. It is interesting that at 36 weeks in comparison to infants born in the lower GA groups had the longest duration of wakefulness versus those born at a higher GA age however, these differences did not reach a level of significance ($p = 0.128$).

The overall duration of AS was significantly lower amongst infants born in a lower birth GA group ($p = 0.007$), whilst the duration of QS was longer differences were not statistically significant (0.825). Consistent with other studies, our results support a trend towards changes in sleep organisation with advancing GA. EEG maturation occurs with increased chronological age, i.e. a decrease in the overall time spent in AS and an increase in QS (Table 4).(303, 371, 372) However, there is some conflicting evidence suggesting the opposite trajectory may be true. Han et al. utilised cEEG, aEEG, EOG, EMG over a 4-h period and described the changes that occurred with advancing GA. In comparing the percentage of each sleep state between a 34–35 GA group ($n = 20$) and a 36–37 GA group ($n = 23$), results showed an increase in the percentage of AS sleep duration from 39.8 to 46.6%, a decrease in the percentage of QS duration from 36.8 to 32.3%, a decrease in IS from 7.9 to 5.3%, whilst duration

of wakefulness differed minimally 15.5–15.8% between groups.(266) In a review of sleep state measurement, Werth et al described a plateau in AS duration between 30- and 36-weeks GA at 60–65% of sleep time,(258) which is similar to our results of a median of 63.07%. QS was described as increasing until 36 weeks to approximately 38% which is higher than our findings of a median of 27.88%. Thereafter, Werth et al. described both sleep states decrease as wakefulness increases. Similar to Foreman et al we found no disparities based on sex in our analysis of sleep state duration.(310)

In our methodology we defined an uninterrupted sleep cycle. (271) However, inconsistencies in sleep cycle definitions have been noted, (271, 373) whilst some studies did not define it but described sleep cycling in broad terms as fluctuations between sleep states. (305)

In our analysis of uninterrupted sleep cycles, the median (IQR) duration was 37 (32.00- 43.13) minutes which is shorter than previously reported for MLP infants at 36 weeks PMA.(271, 313, 373) The duration of QS was significantly longer amongst infants born at younger GA ($p<0.001$). While our sleep cycle analysis also showed differences in the duration of AS by GA group within an uninterrupted sleep cycle these differences did not reach statistical significance.

Learning and memory development are dependent on normal cycling.(374) There is extensive variability in the literature pertaining to the duration of a sleep cycle in preterm infants at 35-36 weeks PMA. Tsuchida et al reported a sleep cycle duration of 50 to 65 minutes for infants >35 weeks PMA.(271) Curzi-Dascalova et al. described a sleep cycle duration of a small group of participants (n=11 between 35- and 36-weeks GA) by the mean +/- standard error as 69.4 +/- 6.1 min. (313) Even for healthy

term born infants, sleep cycles typically last a mean of 50–60 min however, a wide range of 30–70 mins was also noted.(375) Dereymaeker et al also report extensive variability in uninterrupted sleep cycle duration amongst infants of 35 -36 weeks GA which ranged from 6.3–109 minutes with a mean (SD) of 50.9(19.2) minutes.(356)

Heterogeneity in methodology is evident in the literature making it difficult to compare like with like. Many sleep studies carried out have been based on behavioural observation, with or without inclusion of ECG, EMG and EOG,(180, 195, 310, 368, 376) or sleep state determined based on activity or actigraphy.(377) Whilst some studies did utilise cEEG with video, differences in inclusion/exclusion criteria, duration and timing of monitoring and the environment where monitoring was carried out were evident. (266, 312, 313, 367) Although environmental factors may impact the duration and intensity of sleep, intra-individual variation in sleep organisation may also be attributed to endogenous factors with genetic links.(310, 378) Shorter duration of monitoring incorporating one or two sleep cycles, may not be truly representative of sleep cycle duration for an infant group. No other study to date has utilised cEEG over a 12 hour to determine sleep state organisation of a healthy group of MLP infants at 36 weeks PMA. To date, the majority of studies using EEG to monitor preterm sleep have been during day time hours, recording duration has rarely been longer than 4 hours, inclusion/ exclusion criteria were variable, little information is provided in relation to mode of feeding and little detail provided about the lay out of the sleeping environment. (206, 266, 312, 376) All infants in this study were healthy at time of monitoring, and had no history of medical problems and were cared for under similar environmental conditions with no disturbances for clinical purposes.

Infant feeding in our unit is time based, generally 3 hourly for infants learning to feed or four hourly when larger volumes are tolerated well orally. Infants that had a mixed mode of feeding overnight (i.e. actively engaging with oral feeds combined with passively feeding via nasogastric tube) had a longer TST ($p<0.001$). This is the first study to report these findings. With respect to guidelines for establishing feeding in preterm infants we use an individual developmentally supportive approach. Infants are passively tube fed to varying degrees if feeding skills are not established with oral feeds offered on an individualised basis. Allowing the infant to sleep and grow through passive feeding may support neurodevelopment which in turn supports the development of the infants suck swallow coordination. When TST in both mode of feeding groups was broken down by sleep state, the more immature infants who were partially tube fed had a longer AS duration in comparison to infants who were more mature and fed exclusively orally ($p=0.001$). Achieving a balance between supporting the infant in establishing feeding and protecting sleep is key.

4.5 Strengths and Limitations

This is the largest study to date focusing on sleep of the healthy uncomplicated MLP infant through overnight cEEG monitoring carried out at a similar PMA, and time point of the day, under similar environmental conditions. These factors reduced the risk of sleep being compromised by morbidity or environmental exposure during EEG monitoring. The number of male and female participants was reasonably balanced as was the number of multiple and singleton pregnancy's, removing bias related to these factors in the study. We also identified mode of feeding for each infant, a factor that has not been widely reported in previous sleep studies. Previous EEG sleep studies

have based percentages of each sleep state on the interpretation of a single sleep cycle or short periods of EEG.(13, 258, 271, 321)

This study also had some limitations. Our study was conducted in an open plan neonatal unit where we aimed to minimise environmental stimuli by caring for infants in a quiet low-lit area of the unit during monitoring. However, we did not measure ambient sound or control for potential confounding factors such as environmental temperature, sleeping position or clinical workload. We did not use electrooculogram (EOG) like many studies but we did use video to assess overall behavioural observations including ocular and facial movements.

4.6 Future research

As variations in infant sleep play an important role in cognitive development and physical growth,(196) future sleep research incorporating sequential and prolonged EEG monitoring should be considered gold standard. This will provide an objective robust estimate of TST and a greater insight into sleep architecture unique to GA. It will also provide confirmation of achievement of sleep state organisation appropriate for GA which may be a potential biomarker of future outcome.(321, 379) Alteration in EEG-sleep organization or maturation may be an expression of brain dysfunction.(380) Further research is also required to assess any associations between sleep variables and long-term developmental outcome.(18)

Whilst much of the literature highlights the adverse effects of the neonatal environs on sleep and neurodevelopment, it must also be considered that the neonatal unit provides an opportunity for intervention that may positively influence the development

of the fragile immature preterm brain especially vulnerable to environmental influences. As neonatal nurses are possibly most in touch with the uncomplicated healthy MLP infant's needs, they are best placed carry out further research in this area.

Sleep staging in preterm infants is complex and time consuming and requires a degree of expertise in EEG. A growing body of researchers have used machine learning in health care to design algorithms such as seizure detection algorithms.(381) Sleep detection algorithms, detect specific features of sleep, (277) automate sleep staging, (382) and estimate brain maturity.(383) Automated approaches have shown great promise in providing robust quantitative data pertaining to sleep organisation and perhaps identify future biomarkers of developmental outcome. Identifying infants who warrant increased ongoing surveillance and provide early intervention if required would potentially improve outcome of infants, which is the ultimate aim of neonatal care.

4.7 Conclusion

This is the first study to describe overnight twelve-hour sleep architecture in a large homogenous group of MLP infants using video EEG. Our findings provide robust normative data for each week of GA unique to the MLP infant group (healthy at birth). Quantitative changes in sleep state may be used as a reference point in future studies and a potential biomarker of developmental outcome.

Chapter 5

Quantitative analysis of quiet sleep in moderate to late preterm infants with a normal developmental outcome at 18 months

Abstract

Background

Moderate to late preterm (MLP) infants are at risk of adverse outcomes. Brain function can be measured using electroencephalography (EEG) which changes between sleep states. Inter-burst intervals (IBI) are a specific feature of quiet sleep (QS) and a marker of brain maturation.

Aim

This study aims to describe features of IBI in MLP infants with a normal developmental outcome at 18 months and investigate potential influencing factors whilst patients in the neonatal unit.

Methods

MLP infants had overnight cEEG with video performed in the neonatal unit at 36 weeks post menstrual age (PMA). All parents were invited to return with their infants for a follow-up Griffiths III neurodevelopmental assessment at 18 months. Quantitative analysis of EEG (qEEG) of infants with a normal developmental outcome at 18 months was performed using an adapted IBI detection algorithm. Five quantitative features of IBI were extracted (maximum IBI length, median IBI length, IBI variability, number of IBIs/minute and percentage of epoch that was IBI) for each infant.

Results

Sixty MLP infants had overnight EEG and a normal developmental outcome at 18 months. The median (IQR) features were: IBI maximum length 6.89(5.97-7.86) secs, median length 3.00(2.60-3.27) secs, IBI length variability 2.49(2.00-2.90) secs, number of IBIs per minute 5.58(4.81-5.88) and percentage IBI 32.90(24.68-39.02) %. There was a significant reduction in values for all IBI features except IBI median length with PMA ($p=0.02-0.08$). All IBI features, were significantly longer/higher for infants nursed in incubators. Infants from multiple pregnancies had longer IBI length maximum ($p=0.030$), IBI length median ($p=0.038$) and a higher IBI length variability ($p=0.043$) than infants from singleton pregnancies.

Conclusion

We have described for the first time, detailed quantitative features of IBIs in QS of the MLP infant with a normal developmental outcome at 18 months PMA. This normative data which can be used in future studies of infants at risk of abnormal development. Automated analysis may be a useful pre-discharge screening tool for all preterm infants.

5.0 Introduction/Background

As described in Chapter 1 section 1.3, sleep has a critical role in optimising neural network development, functional connectivity, synaptogenesis, preservation of brain plasticity and has a strong correlation with brain growth.(282) Disturbance to the normal quality or quantity of sleep and the multiple neurological processes that are actively taking place, may lead to a reorganisation of neural networks. Structural and functional connectivity in the brain coincides with the ontogeny of sleep and brain maturation. Although the literature frequently describes sleep through behavioural observations, EEG monitoring is the gold standard for identifying sleep states (Chapter 1 sections 1.5.3, 1.5.4) (326, 384)

In the moderate to late preterm (MLP) infant group, the background EEG activity in AS is continuous mixed frequency activity of low to moderate amplitude (30-70 μV). A defining feature of QS in the EEG of the MLP is discontinuity or tracé discontinu. The degree of discontinuity is directly related to maturity and the degree of cortical folding.(385) On the EEG, a discontinuous EEG pattern displays high voltage bursts (50-300 μV) of mixed frequency activity separated by a period of quiescence or lower voltage activity on all EEG channels, termed an inter-burst interval (IBI) (Figures 1.22 A and 1.22 B). The high voltage bursts of activity between IBIs are generated by the interaction between the subcortical and cortical plate(373) or through environmental stimuli both of which support the normal construction of cortical networks, brain circuit development and the preservation of brain plasticity.(6, 386)

Vanhatalo and Kaila described 2 types of EEG activity in preterm infants to term age; spontaneous activity transients (SATs) which are intermittent, endogenously generated, mixed frequency bursts (with nested oscillations) of activity corresponding to the 'bursts' described above, and ongoing continuous lower amplitude oscillatory cortical activity occurring between SATs.(387) SATs occur in both AS and QS but QS is marked by a paucity of ongoing oscillatory cortical activity relative to AS, and this paucity defines the interburst interval (IBI) or inter-SAT interval seen in QS. SATs are proposed to be events triggered by spontaneous activity in pyramidal neurons connected by excitatory glutamatergic synapses and are fundamental to early neuronal network formation while sensory pathways are still developing. Ongoing oscillatory activity on the other hand, is believed to develop with maturation of inter-neuronal GABAergic signalling. While SATs often occur spontaneously, they are also modifiable; studies in both rat models and humans have indicated that a component of SATs called delta brushes, consisting of a delta slow wave crested by rhythmic fast activity, are elicited both following spontaneous myoclonic twitches in sleep and by somatosensory stimulation, and are involved with fine tuning the topographic mapping of the cortical somatomotor system.(202, 373, 386) Age-specific features or oscillations are identified on EEGs. Delta brushes are a normal feature of maturation and frequent in the EEG of the MLP infant group.(202)

AS (a precursor of rapid eye movement sleep, REM) is critical to normal development (Chapter 1 section 1.3.2.3). By providing the brain with endogenous stimulation, Mirmiran and Ariagno contend that AS promotes neuronal processes such as synapse formation and pruning. This theory is consistent with the fact that AS predominates in

infancy.(388) Mirmiran et al's animal studies have shown that AS deprivation during the neonatal period is associated with reduction in cortical and brain stem volume(389)

As described in Chapter 1 section 1.5.3.2, features of QS undergo significant changes in a short period of time. The focus of this study is on QS (a precursor of non-rapid eye movement sleep, NREM) and the IBI duration within QS because of the association between IBI and brain maturity. There is no universal definition of an IBI.(390) Heterogeneity exists in identifying cut-off threshold values for the minimum and maximal amplitude and duration of an IBI. (271, 373, 391) Thresholds for amplitude used include $< 50 \mu V$ (373), $45 \mu V$ (392), $<30 \mu V$ (291), and $<10 \mu V$ (393) but some researchers have advised against very restrictive criteria ($<10 \mu V$) which have shown to result in shorter measurements of IBIs in comparison to less restrictive criteria of $<25 \mu V$.(391) There is agreement that discontinuity in QS diminishes with age and the maturation of excitatory synapses, increased connectivity, the overall functional maturity of cortical activity and the degree of cortical folding. (385).(391, 394) Duration thresholds suggest normative values for IBI durations at different GA's include as ≤ 20 sec @30 -31 weeks GA, ≤ 10 -15 sec @32 -34 weeks GA and < 10 sec @ 35 -36 weeks GA. However, there is variability in these values in the literature. (260, 271, 373, 391, 395) Hence IBIs are a biomarker of central nervous system maturation(281, 385) and a useful parameter in determining whether brain activity is appropriate for GA.(14, 396)

Changes in background pattern in QS with advancing GA also include changes in amplitude of the IBI which progressively increases toward term.(397) By term age an alternating pattern develops, consisting of high voltage bursts (50 - $150\mu V$) of activity

which last for longer periods of up to 4-10 seconds, alternating with lower-voltage activity (but $> 25 \mu\text{V}$) on all EEG channels, termed *trace alternant*. (271, 273, 398)

Several recent preterm EEG studies have utilised automated quantitative analysis (qEEG) which provides an objective method of quantifying multiple EEG features (qEEG). (383, 399, 400) Whilst amplitude EEG (aEEG) is a valuable tool and the tool of choice adopted by many neonatal units, visual analysis of aEEG does not allow for the identification and quantification of IBI and duration.

5.1 Aim

The primary aim of this study is to:

- Describe features of IBI in the EEG of healthy moderate to late preterm (MLP) infants at 36 weeks PMA who have a normal developmental outcome at 18 months.
- The secondary aim is to investigate if factors such as birth GA, birth weight, sex, pregnancy type and location in the neonatal unit influence IBI features.

5.2 Methods

Chapter 2 describes, in detail, all methods used in this study (see sections 2.1 to section 2.12 inclusive). Two uninterrupted QS epochs at two different time points were pruned from each overnight EEG recording. The first epoch selected was as close to midnight as possible (before or after) and the second epoch was between 03.00hrs and 6.00hrs. Neonatal unit activity is at a minimum at these time points and hence the potential for sleep disruption is reduced.

5.2.1 The IBI detection algorithm- training and adapting

A computer algorithm previously developed by our group to detect burst and inter-burst intervals on the EEG of preterm infants < 30 weeks GA was used in this study. (401)To train and optimize the algorithm's performance in recognising IBI's in the MLP infant group, QS epochs (from the beginning to the end of a QS period) were randomly chosen from the EEGs of infants based on birth GA week group (32,33,34,35 or 36 weeks birth GA (n=12). We defined an IBI as a period ≥ 1 second with low voltage activity of < 25 μV . All IBIs were manually annotated by a researcher (MAR) with extensive EEG experience (Figure 5.1).

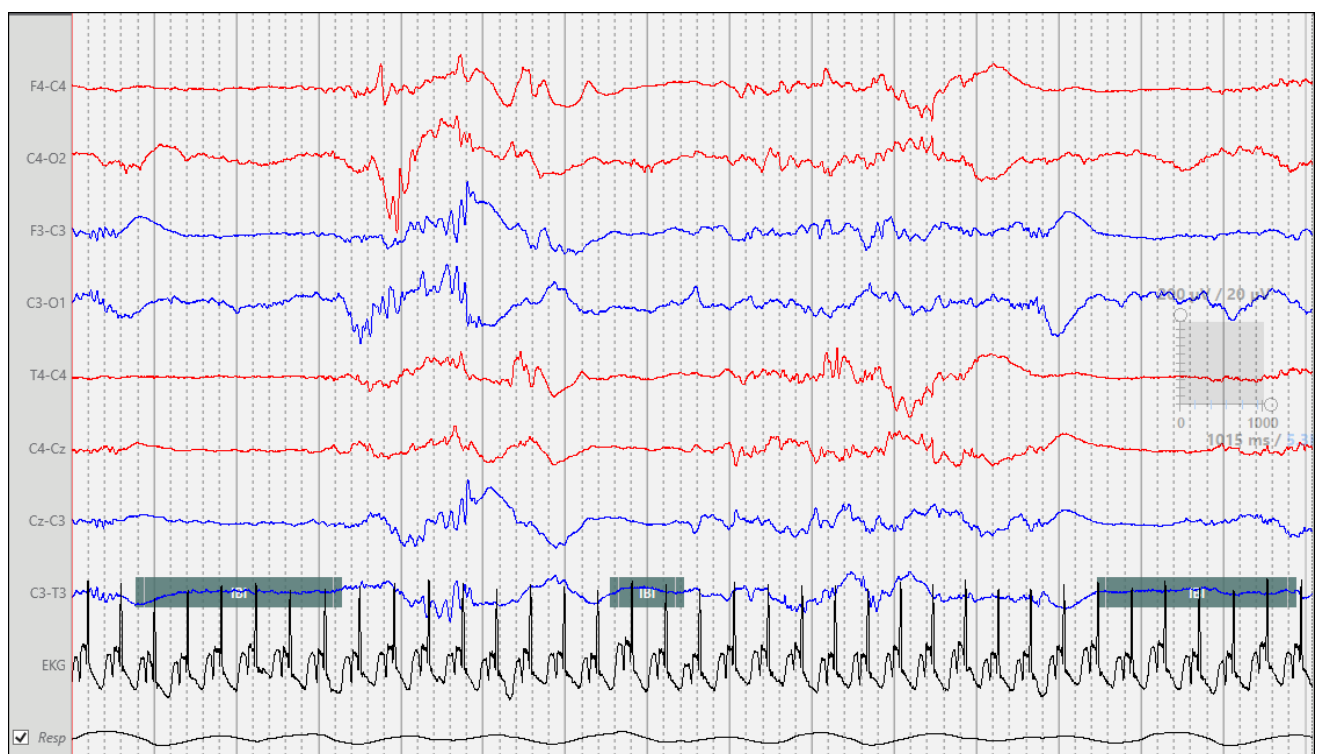


Figure 5.1 Post acquisition manual annotations of IBI on EEG of MLP infants for algorithm development. Grey bars indicate IBI annotated

Slow wave activity <1 Hz was noted to be more pronounced in the IBIs of the MLP group compared to the younger preterm group for which the detector was originally

designed.(401) Dr O'Toole, senior biomedical engineer at the INFANT Research Centre, applied a high pass filter to suppress this slow wave activity which improved the performance of the algorithm (Figure 5.2). Details of the filter used and optimisation process are given in Appendix (iv)

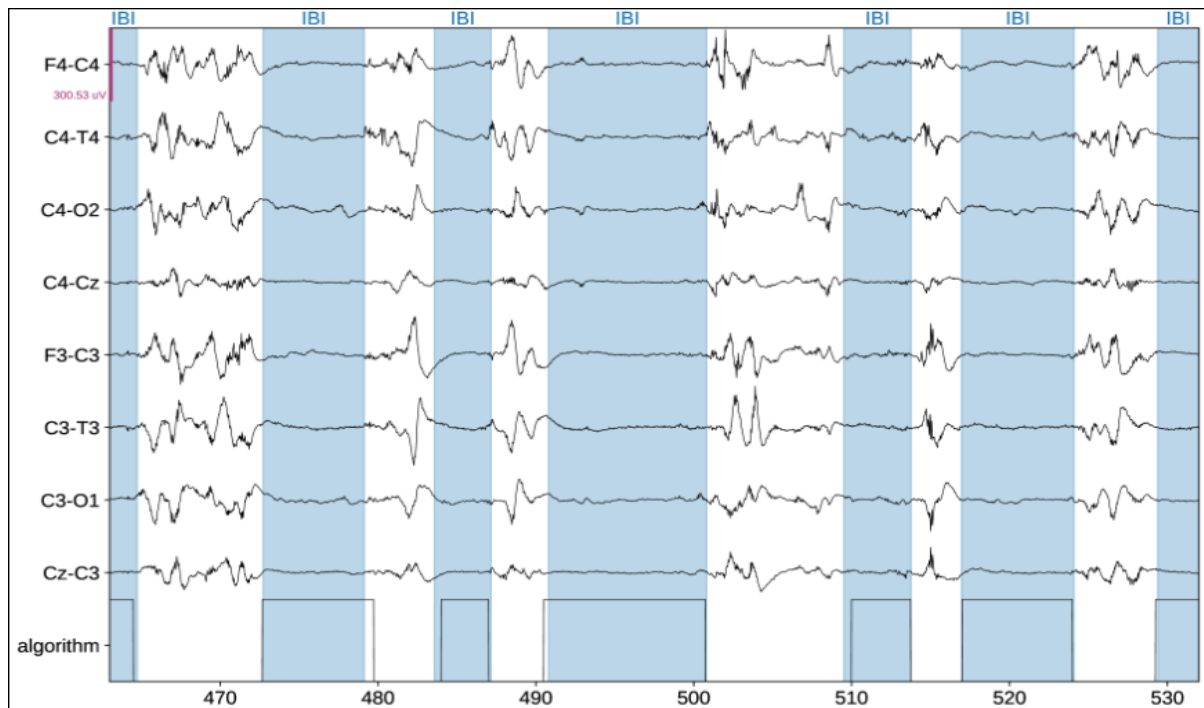


Figure 5.2 Annotation of IBI detection algorithm compared to human annotation. Screenshot of more than 60 seconds of quiet sleep (time point of epoch expressed in seconds, 470-530). Inter-burst interval (IBI) annotation by reviewer in blue. IBI algorithm detector outlined at end of the figure.

For supplementary material relating to algorithm development see Appendix (i)

5.2.2 IBI detection algorithm

All pruned QS epochs were analysed using the IBI detection algorithm and the five IBI features were extracted. For each infant, each IBI feature was averaged across two QS epochs and summary measures were calculated.

Summary IBI features included:

- IBI length maximum (secs), the maximum duration of an IBI in the QS epoch estimated as the 95th centile (95th centile used to avoid potential artefact outliers).
- IBI length median (secs), the median duration of the IBIs in the QS epoch.
- IBI length variability, the variability in the duration of the IBIs throughout the QS epoch(secs).
- IBI number (per minute), the number of IBIs per minute in the QS epoch.
- IBI percent, the percentage of the QS epoch that was quiescent activity ($\leq 25\mu\text{V}$) and identified as an IBI.

5.3.4 Developmental assessment

As described in Chapter 2 Methods, all infants returned at 18 months PMA for a Griffiths III Mental Developmental Scales (MDS) assessment.(402) Assessments were performed by trained assessors. Infants with a general developmental quotient score of <90 in one or more subscales of development were considered to be at risk of developmental delay and were excluded from this study.

5.3.5 Statistical methods

Statistical analysis was performed using IBM SPSS Statistics (version 25.0, IBM Corp, Armonk, NY, USA). All tests were two-sided and a p -value <0.05 was considered statistically significant. Continuous variables were described using the median and interquartile range (IQR) and categorical variables using frequency and percentage.

The Wilcoxon signed rank test was used to compare the differences between the algorithm and the reviewers IBI estimates. The Mann-Whitney U test was used for comparisons of IBI features between two groups (sex, mode of feeding, accommodation and pregnancy type). We grouped infants by birth weight (<1.5 kgs, 1.5-1.99kg, 2.0-2.49kg, 2.5-2.99kg and >3 kg) and by GA at birth group(32,33,34.35 or 36 weeks).The Kruskal-Wallis test was used to compare IBI features by birth weight group and GA at birth group. The Mann-Whitney U test was also used to compare birthweight and chronological age by accommodation. The Spearman's rank correlation coefficient (r_s) was used to measure the strength of the associations between chronological age and IBI features. Linear regression was used to investigate the relationship with PMA and IBI features. The chi-squared test was used to investigate the association between two categorical variables (accommodation and pregnancy type). All tests were two-sided and a p-value<0.05 was considered statistically significant.

Investigation of the relationships between accommodation type and IBI features was carried out using linear regression. An adjusted and unadjusted analysis regarding the potential confounding effect of pregnancy type was undertaken when looking at the relationships between accommodation type and IBI features.

5.3 Results

Seventy-two MLP infants with full overnight EEG monitoring returned for a Griffiths III developmental assessment at 18 months. Twelve infants had a developmental quotient (DQ) score <90 in one or more subscales and were excluded from the study.

Sixty infants with overnight EEG data and a normal developmental outcome were included in the final analysis.

5.3.1 Inter-rater variability between human and algorithm annotations

For the five IBI features, the algorithm's estimates were compared to the reviewer (MAR) estimates using a sample of 12 infants with a normal outcome (Table 5.1). There were no significant differences between the algorithm and reviewer estimate of the five summary IBI features ($p>0.05$ from Wilcoxon signed rank test for all features) which supports strong performance of the algorithm.

Table 5.1 Summary measures of Inter-burst interval features based on human annotation and algorithm performance, n=12

Feature	Algorithm median (IQR)	Human annotation median (IQR)	p-value ¹
IBI-length variability (s)	3.14 (2.32 to 3.69)	2.22 (2.11 to 2.98)	0.133
IBI-length maximum (s)	7.82 (6.03 to 9.61)	7.00 (6.09 to 8.54)	0.419
IBI-length median (s)	3.18 (2.68 to 3.45)	2.71 (2.56 to 2.96)	0.126
IBI number (per minute)	5.33 (5.07 to 5.98)	6.37 (5.59 to 6.64)	0.074
IBI%	35.30 (29.3 to 38.4)	33.20 (31.5 to 37.0)	0.817

¹p -value from the Wilcoxon signed rank test

IBI: inter-burst interval, IQR: interquartile range, s: seconds. A p-value<0.05 was statistically significant.

5.3.2 Infant characteristics

The characteristics of the infants are described in Table 5.2. Over half (58.3%, n=35) of the infants were female. The median (IQR) birth GA was 34 weeks (33-35 weeks) and birth weight was 2.05kg (1.80-2.30kg). The median (IQR) chronological age at time of monitoring was 10.00 days(4.00-14.00 days) and weight at time of EEG was 2.26kg(2.02-2.47kg).

Table 5.2 Characteristics of infants, n=60¹

Infant Characteristics, n=60 ¹		
	Median (IQR)	
Birth weight of group in total(n=60) (kg)	2.05(1.80-2.30)	
Weight at EEG (Kg)	2.26(2.02-2.47)	
Gestational age at birth (weeks)	34.00(33.00-35.00)	
Gestational age at EEG (weeks)	36.00(35.00-36.00).	
Chronological age of group at EEG (days)	10.00(4.00-14.00)	
Duration of stay (nights) in the Neonatal Unit	14.50(8.50-23.00)	
	n(%)	
Birth GA (weeks)		
	32	11(18.3)
	33	11(18.3)
	34	20(33.3)
	35	9(15.0)
	36	9(15.0)
Sex		
	Male	25(41.7)
	Female	35(58.3)
Pregnancy		
	Singleton	30(50.0)
	Multiple	30(50.0)
Accommodation		
	Cot	45(75.0)
	Incubator	15(25.0)
Mode of feeding		
	Exclusively oral	37(61.7)
	Mixed	22(36.7)
	NGT	1(1.7)
	Median (IQR)	p-value
Birth weight by pregnancy type		0.003
Singleton pregnancy	2.19(1.89-2.65)	
Multiple pregnancy	1.91(1.74-2.12)	
Birth weight by location at EEG		0.579
Birth weight infants in cots (n=45)	2.04(1.80-2.26)	
Birth weight of infants in incubators(n=15)	2.10(1.77-2.68)	
Head circumference at birth(cm) (n=59)	31.50(30.50-32.50)	
Chronological age by pregnancy type at EEG		0.164
Age of singletons on EEG(n=30)	9.00(3.75-14.00)	
Age of multiples on EEG(n=30)	12.50(8.00-19.25)	
Chronological age by location at EEG of:		<0.001
Infants nursed in cot on EEG (n=45)	13.00(8.00-19.00)	
Infants nursed in incubators on EEG(n=15)	3.00(5.00-10.00)	

Kg: kilogram, cm: centimetres, IQR: interquartile range

EEGs were generally recorded while infants were cared for in a cot (75.0%, n=45) and the majority were exclusively feeding orally (61.7%, n=37). Half the infants in this study were from multiple pregnancies (50%, n=30). Infants from a multiple pregnancy (n=30) had a significantly lower birth weight ($p=0.003$) and were older chronologically however, differences in age were not significant ($p=0.164$).

5.3.3 Quantitative analysis of QS

The median (IQR) duration of the pruned uninterrupted QS epochs (based on two QS sleep epochs per infant) was 21.1(17.1-24.3) minutes with a range of 8.8 -29.4 minutes. IBI features overall and by birth GA, infant weight, sex, mode of feeding, location (cot or incubator) and pregnancy type are described and compared in Table 5.3.

The median (IQR) for the five features were: maximum length: 6.89(5.97-7.86) seconds, median length: 3.00(2.60-3.27) seconds, variability: 2.49(2.00-2.90) seconds, number of IBIs per minute: 5.58(4.81-5.88) and percentage IBI: 32.90(24.68-39.02) %. IBI features did not differ significantly by birth GA group, birth weight group, sex, or infant's mode of feeding (Table 5).

Table 5.3 Quantitative analysis of IBI features on EEG in quiet sleep overall and by gestational age group, birth weight,, sex, location, mode of feeding and pregnancy type at 36 weeks PMA(n=60)

n=60	IBI length Maximum (secs) Median (IQR)	p-value ¹	IBI length Median(secs) Median (IQR)	p-value ¹	IBI length variability(secs) Median (IQR)	p-value ¹	IBI number/min Median (IQR)	p-value ¹	IBI percent (%) Median (IQR)	p-value ¹
Overall	6.89(5.97-7.86)		3.00(2.60-3.27)		2.49(2.00-2.90)		5.58(4.81-5.88)		32.90(24.68-39.02)	
Birth GA (weeks)		0.960		0.789		0.953		0.569		0.813
32 (n=11)	6.88(5.68-8.21)		2.98(2.63-3.34)		2.49(1.91-3.07)		5.65(4.90-5.90)		32.95(23.44-39.86)	
33 (n=11)	7.17(6.34-7.86)		3.09(2.76-3.35)		2.61(2.31-2.76)		5.66(4.80-6.25)		37.22(25.55-39.22)	
34 (n=20)	6.84(5.26-7.98)		2.90(2.40-3.27)		2.40(1.81-2.85)		5.46(4.53-5.64)		32.10(22.67-37.38)	
35 (n=9)	6.89(5.67-7.75)		2.64(2.46-3.25)		2.48(1.93-3.02)		5.64(4.79-5.99)		30.95(23.78-39.26)	
36 (n=9)	6.51(6.22-7.95)		3.07(2.82-3.30)		2.31(2.21-2.85)		5.61(4.76-5.71)		30.53(25.71-36.84)	
Birth Weight		0.317		0.336		0.545		0.226		0.232
<1.5 (n=3)	7.89		3.00		2.93		5.73		30.04	
1.50-1.99(n=25)	7.17(6.09-8.08)		3.03(2.64-3.35)		2.53(2.01-3.100)		5.65(4.93-6.16)		35.16(29.93-39.46)	
2.00-2.49(n=23)	6.84(5.68-7.74)		3.02(2.54-3.24)		2.58(1.98-2.87)		5.55(4.70-5.70)		32.61(24.31-37.62)	
2.50-2.99(n=7)	6.31(5.65-6.89)		2.73(2.22-3.00)		2.27(1.92-2.50)		4.88(4.08-5.50)		27.10(19.51-30.95)	
>3.00(n=2)	6.81		2.93		32.41		5.20		5.20	
Sex		0.544		0.905		0.472		0.290		0.440
Male (n=25)	6.84(5.36-7.79)		3.02(2.47-3.33)		2.50(1.85-2.83)		5.43(4.39-5.89)		31.58(21.09-38.50)	
Female (n=35)	6.89(6.03-7.89)		3.00(2.63-3.20)		2.49(2.05-3.02)		5.61(4.90-5.88)		33.36(25.31-39.10)	
Mode of feeding ²		0.433		0.088		0.173		0.256		0.183
Oral (n=37)	6.84(5.38-7.71)		2.87(2.43-3.20)		2.49(1.87-2.87)		5.60(4.41-5.78)		32.61(22.54-37.66)	
Mixed(n=22)	6.96(6.00-8.05)		3.05(2.86-3.37)		2.63(2.23-3.05)		5.50(4.90-6.16)		35.49(28.19-39.13)	
Location		0.024		0.014		0.014		0.048		0.026
Cot (n=45)	6.68(5.68-7.67)		2.87(2.52-3.20)		2.49(1.93-2.74)		5.48(4.54-5.71)		31.58(23.87-38.14)	
Incubator(n=15)	7.73(6.31-8.55)		3.07(3.00-3.69)		2.91(2.31-3.26)		5.88(5.38-6.16)		37.22(29.77-43.75)	
Pregnancy		0.030		0.038		0.043		0.188		0.089
Singleton (n=30)	6.39(5.67-7.10)		2.82(2.45-3.10)		2.29(1.92-2.64)		5.43(4.55-5.70)		30.28(24.17-35.83)	
Multiple (n=30)	7.54(6.15-8.07)		3.10(2.80-3.37)		2.70(2.24-3.07)		5.64(4.82-5.91)		35.98(27.12-39.28)	

IBI: inter-burst interval, IQR: interquartile range. Max: maximum, GA: gestational age. ¹ The p-value calculated from the Kruskal- Wallis test was used for gestational age and weight and a p-value calculated from Mann-Whitney U test was used for sex, location and mode of feeding and pregnancy type. A p-value<0.05 is statistically significant. A mixed mode of feeding included both oral and nasogastric tube feeds. ² One infant fed exclusively via nasogastric tube and was eliminated from the mode of feeding analysis.

All IBI features were significantly longer/higher for infants cared for in incubators in comparison to open cots. At the time of EEG monitoring infants nursed in incubators(n=15) were younger chronologically with median (IQR) age(days) of infants in cots(n=45) of 13(8-19)days in comparison to infants in incubators(n=15) of 5(3-10) days ($p<0.001$). However interestingly infants in incubators were significantly older by birth GA than infants nursed in cots($p=0.011$).

There was a significant effect of PMA age on IBI maximum length, variability, number and percent, with values decreasing with increased PMA ($p=0.002-0.008$) (Table 5.4, Figure 5.3-5.7). Changes in IBI median length with PMA were not significant ($p=0.083$).

Table 5.4 Results of a linear regression analysis of effect of PMA on IBI features

Variables	Beta coefficient	R ²	F	p-value
PMA vs IBI_MAX	-0.724	0.151	10.295	0.002
PMA vs IBI_MEDIAN	-0.173	0.051	3.114	0.083
PMA vs IBI_IQR	-0.342	0.158	10.896	0.002
PMA vs IBI_NUMBER	-0.439	0.115	7.554	0.008
PMA vs IBI_PERCENT	-4.892	0.136	9.158	0.004

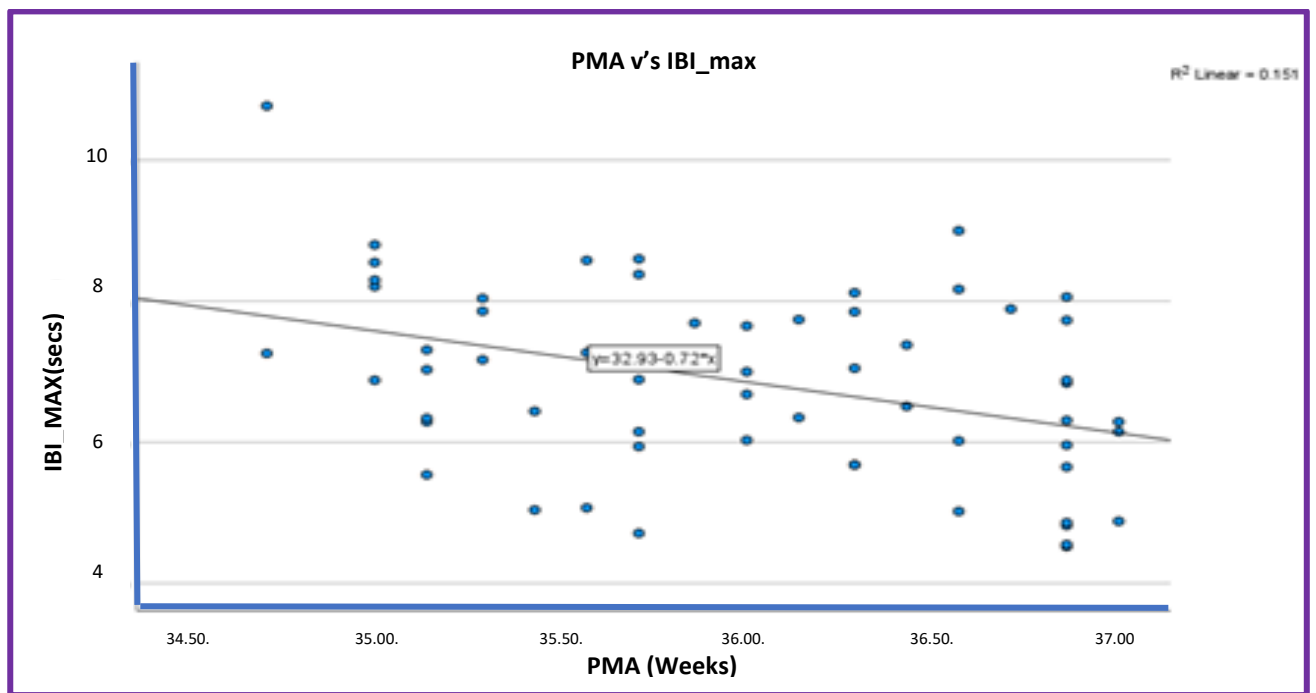


Fig 5.3 Scatter plot of IBI Max v PMA (weeks)

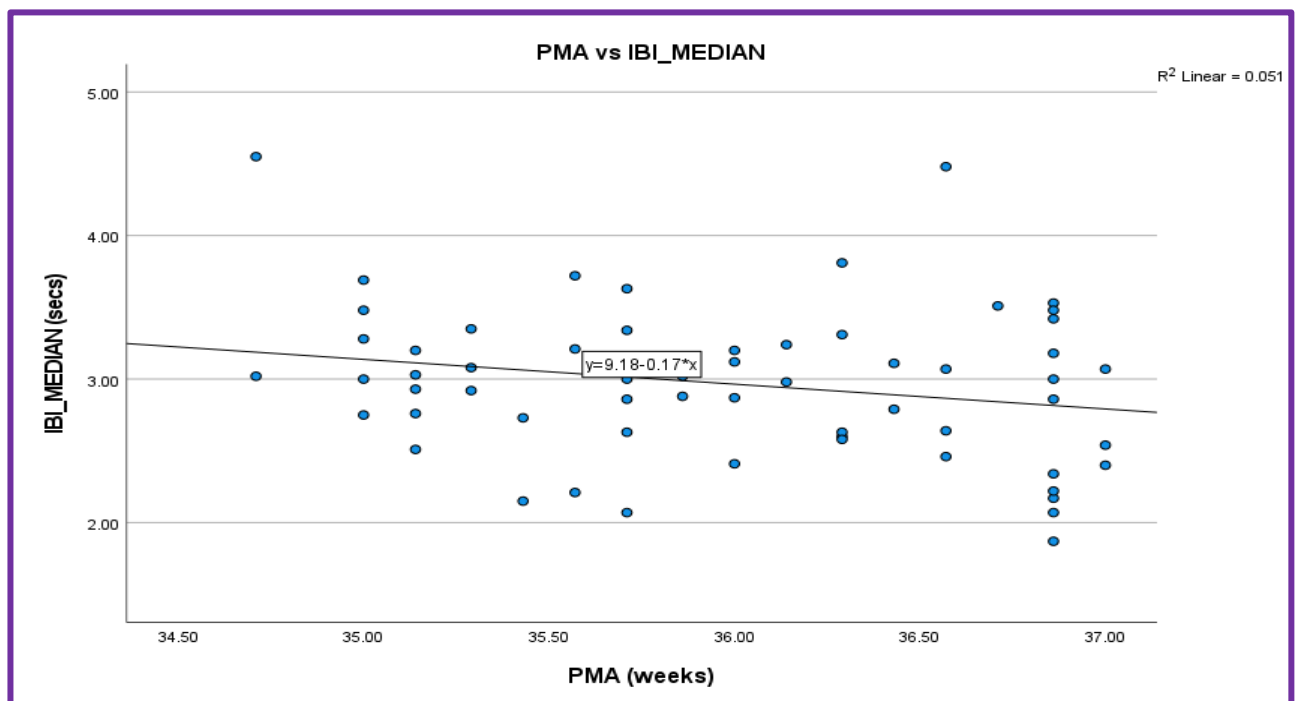


Fig 5.4 Scatter plot of IBI median v PMA (weeks)

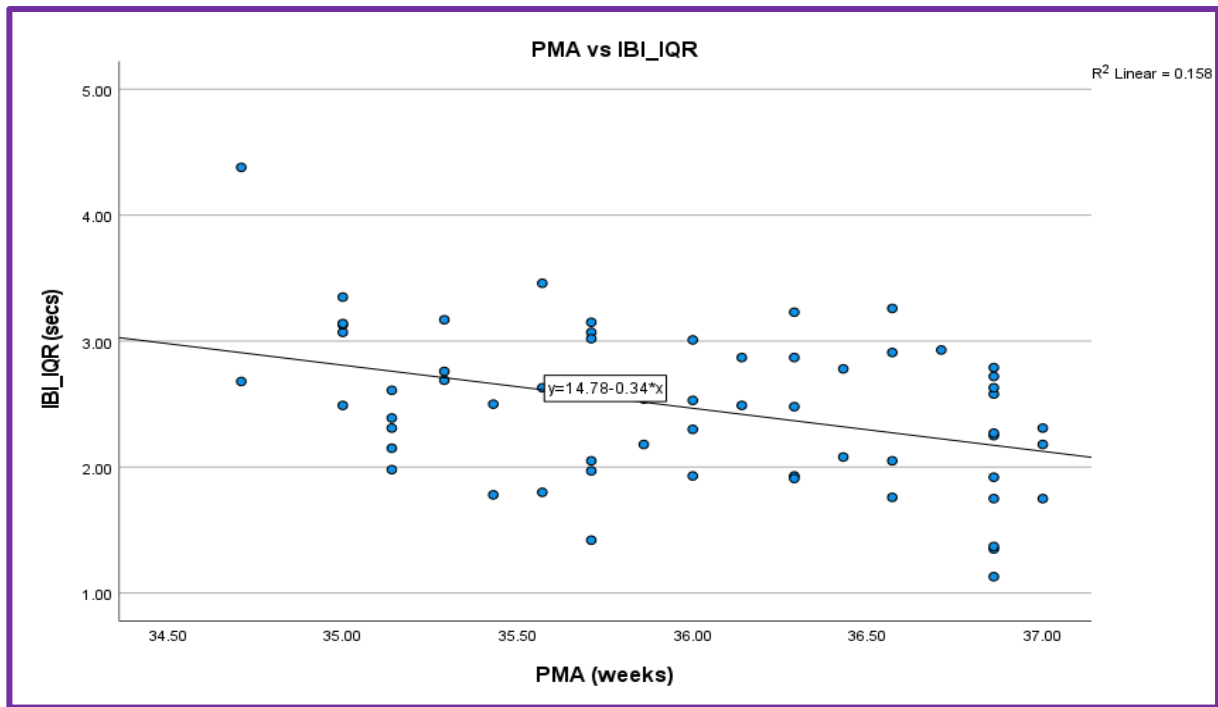


Fig 5.5 Scatter plot of IBI IQR v PMA (weeks)

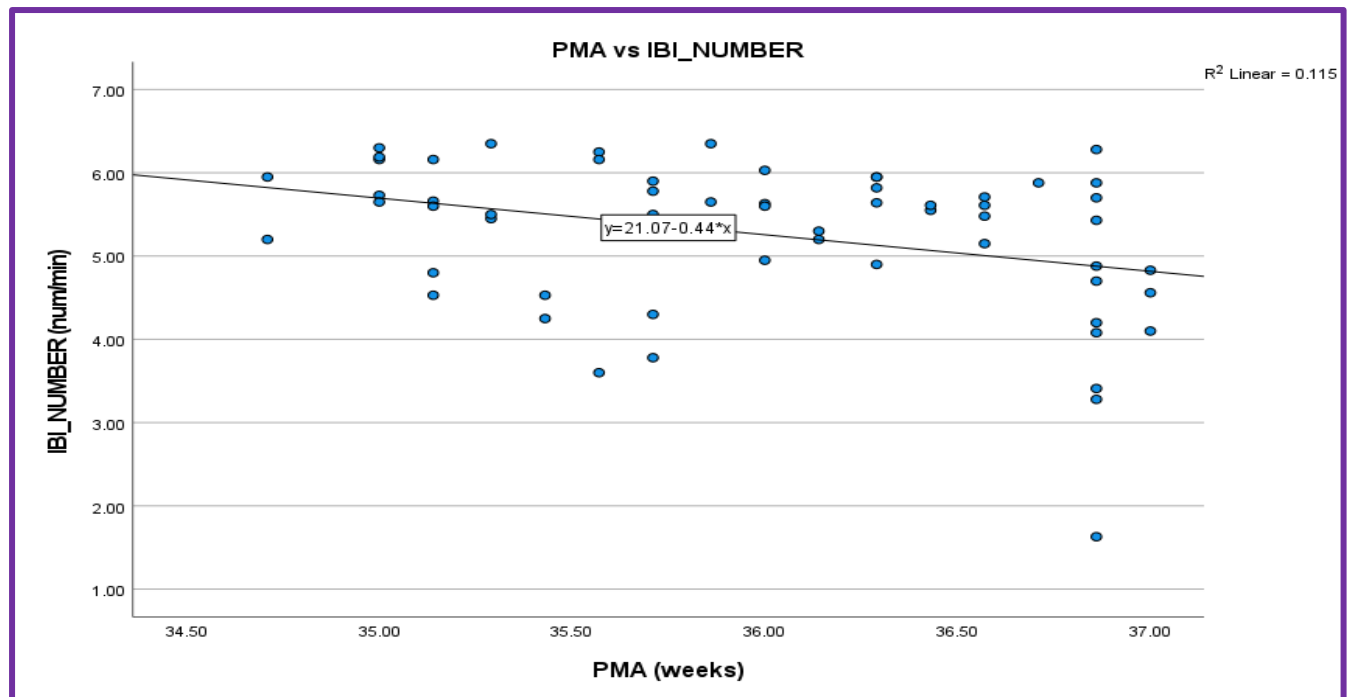


Fig 5.6 Scatter plot of IBI number v PMA (weeks)

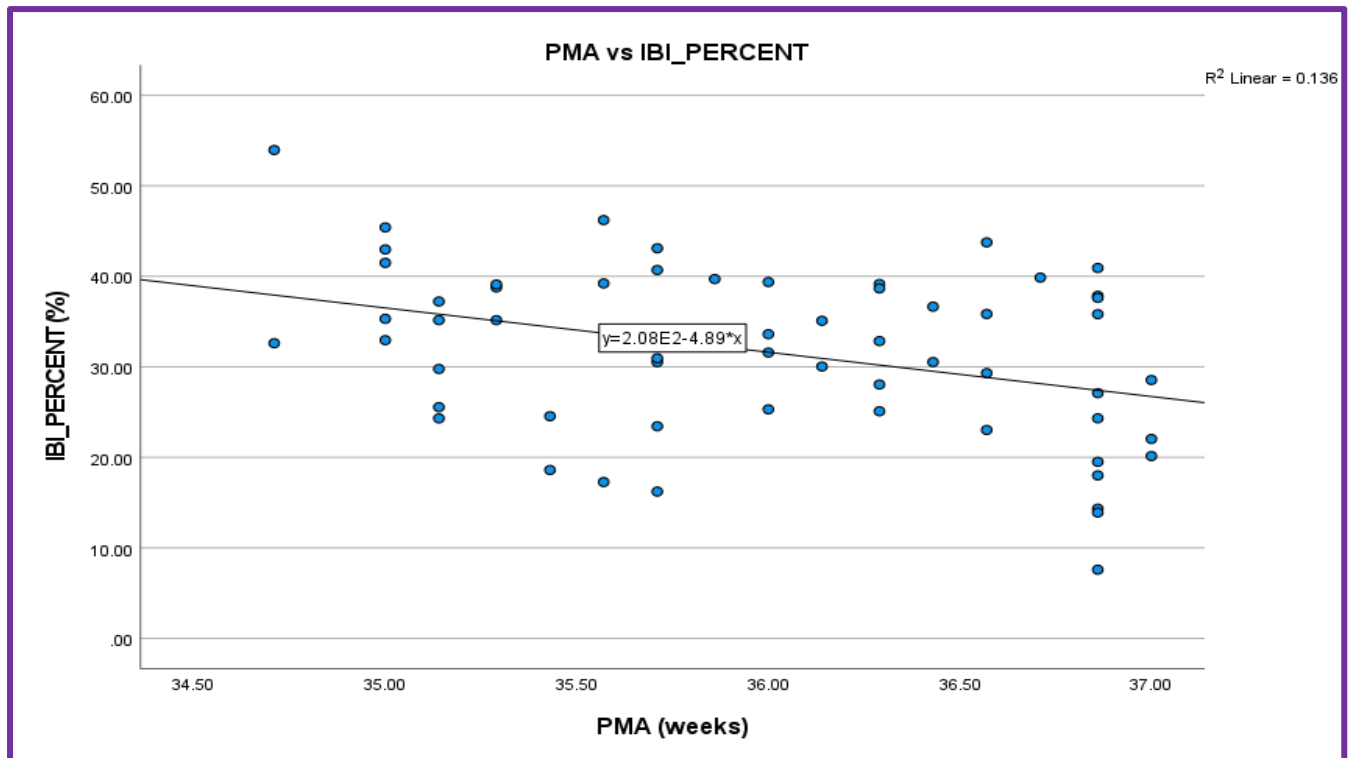


Fig 5.7 Scatter plot of IBI median v PMA (weeks)

Significant differences were also evident by pregnancy type for 3 IBI features. Infants from multiple pregnancies had significantly longer IBI max, IBI median and IBI variability(IQR) ($p=0.030$, 0.038 and 0.043 respectively) (Table 5.3). There was no difference in birth GA by pregnancy type ($p=0.107$). Infants from multiple pregnancies($n=30$) were chronologically older with a median (IQR) of 12.50 (8.00-19.25) days in comparison to infants from a singleton pregnancy($n=30$) with a median (IQR) of 9.00(3.75-14.00) days however, this difference was not significant ($p=0.164$) (Box plots Figure 5.8 and 5.9). In our group, 9/30 singleton infants and 6/30 multiple infants were nursed in incubators.

Given the differences in IBI features found between infants from singleton and multiple pregnancies, we used linear regression to determine if the relationship between IBI features and infant location (cot or incubator) remained evident when we controlled for pregnancy type. In an adjusted and unadjusted analysis controlling for pregnancy type (singleton or multiple) the relationship between all IBI features and location remain statistically significant. (Table 5.5).

Table 5.5 The relationship between IBI features and infant location when unadjusted and adjusted for pregnancy type

IBI Feature	Relationship	Relationship
	Unadjusted for pregnancy type	with location, Adjusted for pregnancy type
	<i>p</i> -value	<i>p</i> -value
IBI length Max	0.021	0.010
IBI length median	0.009	0.005
IBI length variability	0.01	0.005
IBI Number	0.019	0.012
IBI %	0.016	0.007

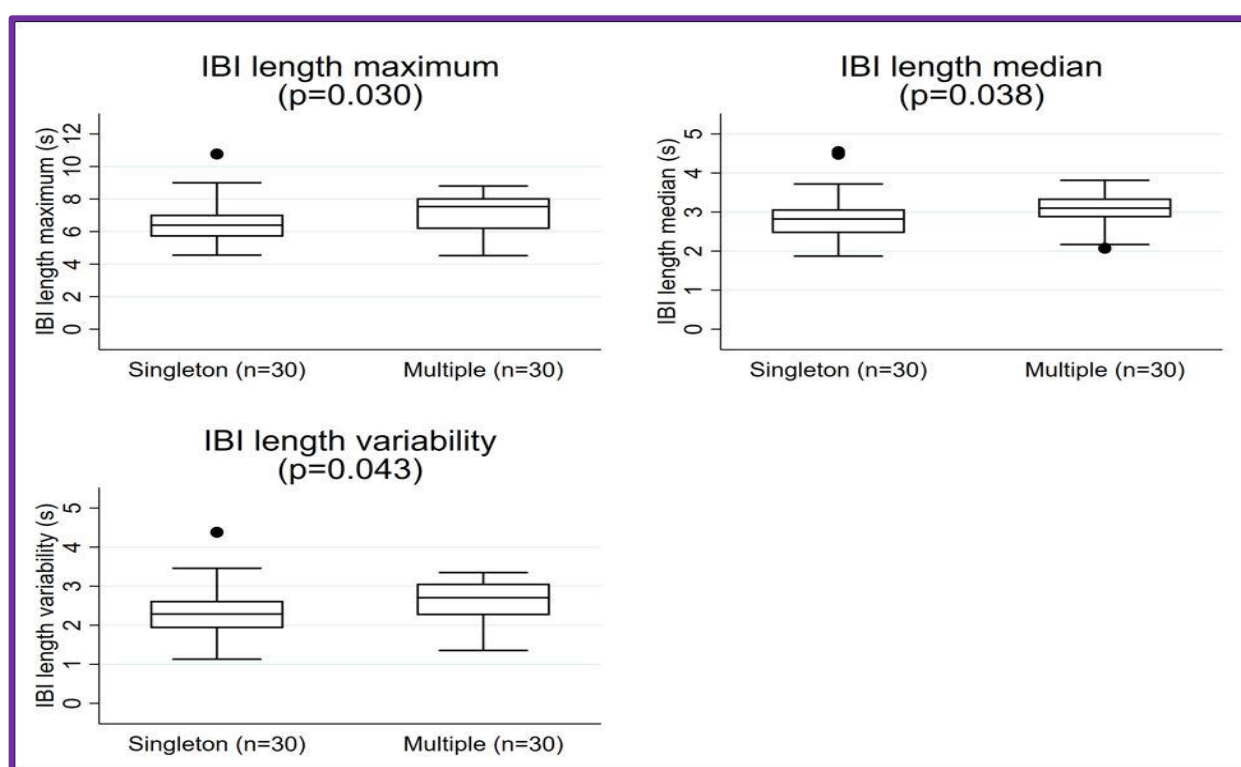


Figure 5.8 Box plot representation of differences in IBI features by pregnancy type

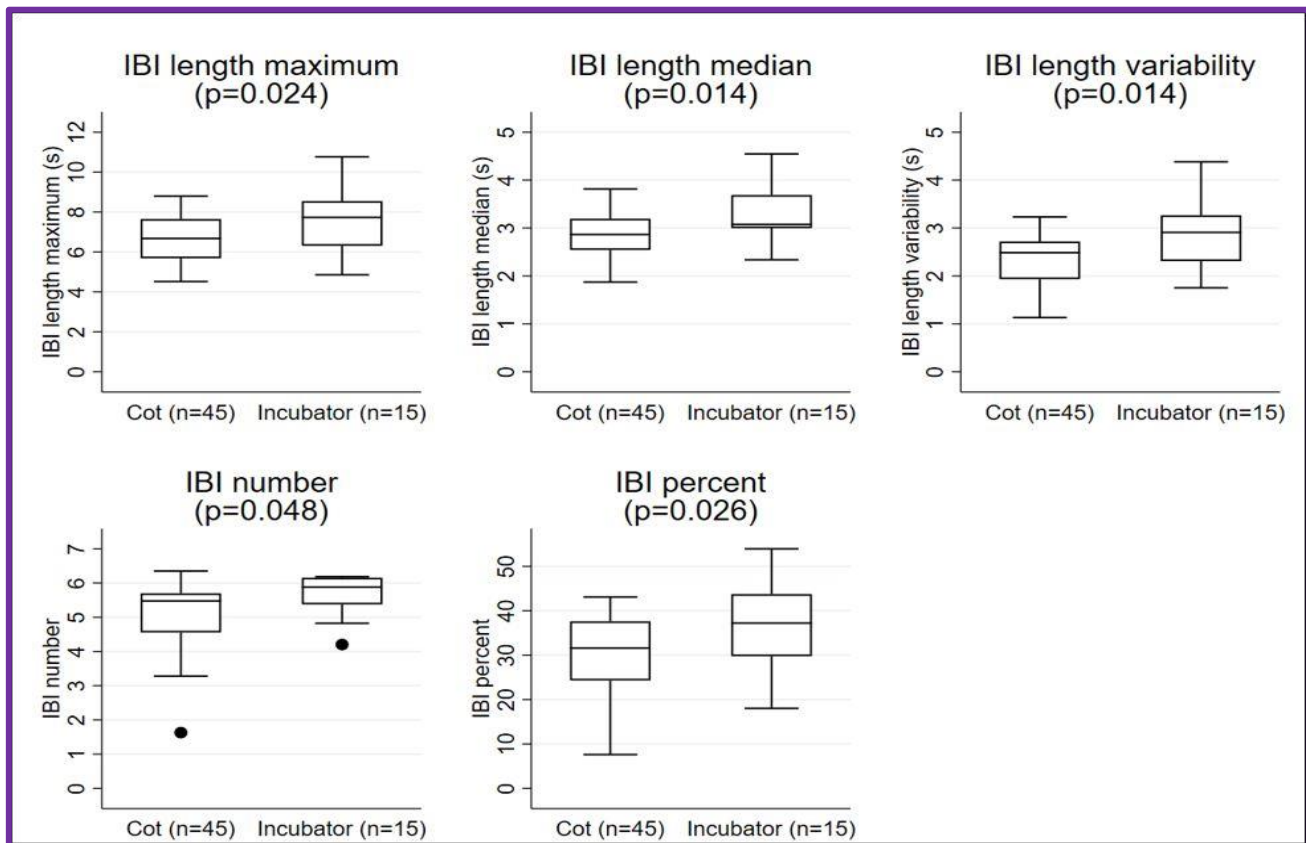


Figure 5.9 Box plot representation of differences in IBI features by location (cot/ incubator)

5.4 Discussion

In this study, we have described five IBI features of the EEG in healthy MLP infants with normal neurodevelopmental outcome at 18 months. As all infants were healthy at time of monitoring, 36 (35-36) weeks, QS was not affected by the infant's clinical status or prescribed medication. This is the first study to describe these features in a large group of MLP infants with a normal developmental outcome.

Sleep architecture undergoes quantitative and qualitative changes with maturation which is reflected in the EEG. As previously described in Chapter 4, the overnight duration of QS did not differ based on GA ($p=0.825$), sex ($p=0.089$), accommodation ($p=0.073$) or mode of feeding ($p=0.291$). However, in our uninterrupted sleep cycle analysis, the duration of QS significantly differed by GA group showing that infants

born at the earlier GA of 32 weeks had a median (IQR) of 19.50(17.00-22.50) mins in comparison to infants born at the later GA of 36 weeks 15.00(13.00-18.00) mins ($p=0.001$). As previously described also in Chapter 4, AS was significantly lower in infants born at earlier GA weeks groups suggesting a more mature sleep organisation amongst infants born at an earlier GA and chronologically older. These maturational effects based on birth GA do not seem to have extended to the features of IBIs we have described.

Maturational effects were noted however with increasing PMA as would be expected(14, 260), with 4 of the 5 IBI features showing decreasing values with increasing PMA. While advancing PMA did not significantly affect the median IBI duration, it did reduce maximum IBI length and perhaps consequent to that, reduce the variability (IQR). The interburst number/min also decreased which may be due to longer duration of bursts, which would be expected but are not measured here. A decrease in interburst percentage could be a combination of a shortening of the longer bursts and an increase in burst length.

The median (IQR) duration of a QS epoch in our analysis was 21.1(17.1-24.3) mins. This is similar to Dereymaeker et al who reported a mean of 19.0 (SD=3.6) minutes based on visual analysis and behaviour observation at 35–36 weeks PMA. (13) Our shortest and longest QS epochs (range: 8.8-29.4 minutes) were shorter than those reported by Andre et al(273) at 35-36 weeks GA (range:12.0-35.7 minutes).

The median (IQR) results of 6.88(6.00-7.05) sec for IBI maximum length and 2.99(2.59-3.25) sec for IBI median length at 36 weeks based on automated analysis are consistent with previously published results for this GA based on visual analysis of the EEG.(356)

An incubator allows preterm infants of all GA's to be supported and observed in a neutral thermal zone (irrespective of weight) while he/she adapts to the extrauterine environment. MLP infants who are healthy and clinically stable, such as the cohort under study are cared for in an incubator if they are low birth weight and/or require observation prior to transfer to a cot. However, in our study there was no significant difference in birth weight between the infants nursed in a cot or incubator ($p=0.579$) and the infants in incubators were significantly older chronologically adds weight to our suggestion that observation was the primary reason for incubator care.

As presented, IBI features in our healthy MLP infant group were significantly longer/higher amongst infants cared for in incubators compared to cots suggesting more immature brain wave activity. In the neonatal unit we aim to provide an environment close to the minimal sensory intrauterine environment. As per our unit policy, incubators are generally covered over, in part to reduce light exposure (but allow for observation) and muffle sound.(403).Infants cared for in incubators are generally handled less with less sleep interruptions and are in a neutral thermal zone. Our findings are possibly a reflection of an environment with less stimuli. IBI differences were also associated with pregnancy type (singleton versus multiple). While birth GA and chronological age were the same in multiples and singletons, birth weight and head circumference were smaller in the multiple pregnancy group. This needs further analysis and future research will examine other EEG features in this group such as amplitude and spectral power.

It must also be considered that infants in incubators (n=15) were significantly younger chronologically (p=0.001), many of whom had ≤ 3 days to adapt to the extrauterine environment. O'Toole et al explored how the preterm EEG evolved in the first postnatal 72 hours (infants <32 weeks birth GA). (404) During this period there was considerable post-natal adaptation with the EEG evolving rapidly in this period. This study describes the evolution of the EEG in 1 day as being equivalent to the slower maturation that occurs over 1 to 2.5 weeks GA. O'Toole et al also describe how the IBI duration decreases over the first 3 days in preterm infants. (404) (Fig 5.10) Preliminary analysis conducted by O'Toole et al suggest differences in IBI features remained significant between infants nursed in cots vs incubators after controlling for chronological age.

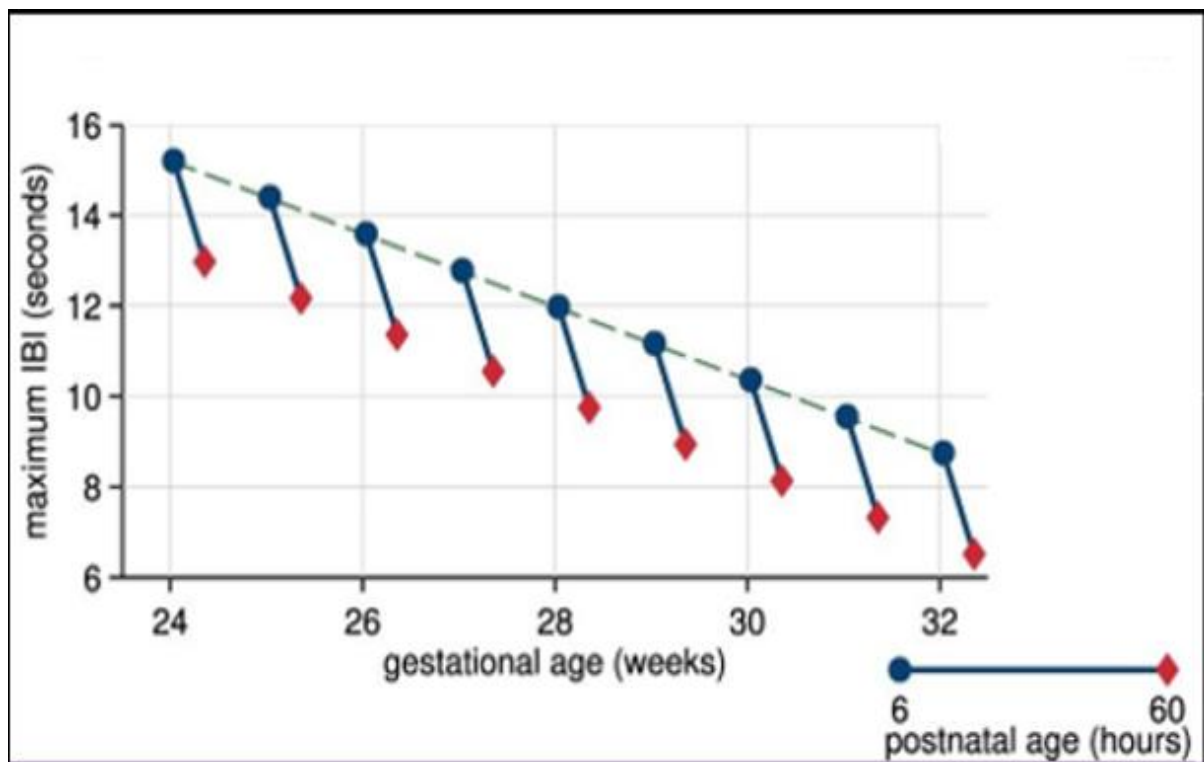


Figure 5.10 Decrease in IBI duration with by GA week and post-natal hours(404)

Based on sequential EEG's conducted on very low birth weight infants (<1500g) at a mean(SD) of 32(2.1) weeks and repeated at 39+4(1.2) Nunes et al described major developmental changes occurring in the first weeks of life.(405) However, she described the EEG of this low birth weight preterm infant group at term GA as delayed and had not reached the same level of maturity as a term born infant. By comparison Soubasi et al considered four features of aEEG (including continuity, bandwidth, the presence of sleep – wake cycling and the lower border) when investigating the maturity of clinical stable preterm infants of 25-34 birth GA(n=96).(406) Sequential weekly aEEG's concluded that preterm infants had a more mature or advanced EEG at the same PMA of infants born at a later GA,

There is a growing body of research using machine learning to design algorithms that provide a rapid and easily accessible trend of brain activity. Previously developed algorithms include sleep state detection algorithms (407) and multi-feature detection algorithms (401, 408) for use in preterm infant. The choice of features used in algorithm development is based on the research question being addressed and the advice and expertise of personnel in the area of signal processing. As we were investigating the maturity of QS amongst a group of MLP infants with a normal developmental outcome at 18 months, features of IBI were used. The algorithm used five features of the IBI, which are dependent on each other i.e. measuring the same feature using a different metric and providing complementary information. Other studies have incorporated burst detection algorithms (409), age specific wave forms and synchrony.(410) The novel value of automated technology is that algorithms may be trained to incorporate multiple EEG features on multiple channels unique to specific PMAs or infant groups with similar characteristics.

Overall our findings related to the differences between groups by location must be interpreted with caution as in absolute terms the IBI differences between groups by location were very small and of uncertain clinical significance except for IBI max where differences were of the order of 1 second. Also given the small numbers involved, it is difficult to draw definite conclusions about this finding which would need to be confirmed with a much larger study.

5.5 Strengths and limitations

Homogeneity is a major strength of this study. This homogenous group of healthy MLP infants were admitted to the neonatal unit and had overnight cEEG with video at 36 weeks PMA. All Infants had a benign neonatal stay and were healthy on discharge with no clinical or neurological concerns and a normal developmental outcome at 18 months. A limitation of this study was that although all infants were healthy, they required admission to the neonatal unit for specialist care. MLP infants cared for in an open plan NNU are separated from parents and have potential for greater exposure to a stressful environment and increased exposure to painful procedures which frequently interrupt sleep. All efforts were made to control environmental conditions and EEGs were recorded in a quiet area of the neonatal unit, and as per unit policy there were no overnight disturbances for routine procedures. Disturbances to an infant's sleep have been linked to unfavourable clinical outcomes, including growth and development. (267) A further limitation was that in the absence of clinical concern, MLP infants >35 weeks birth GA were cared for by mothers on the postnatal ward were excluded from this study.

We also described normative features of IBI based on a conservative threshold of $<25 \mu\text{V}$ for ≥ 1 second in defining an IBI (269) as opposed to more liberal threshold $< 50 \mu\text{V}$ which may increase the duration of IBI's. (373)

5.6 Areas for future research

In our primary analysis we quantified features of IBI in QS but excluded features of bursts of activity in QS. We did this as IBI features are easily identified based on visual analysis and as suggested previously one of the first parameters evaluated when making an overall assessment of the maturity of the brain wave activity displayed on the EEG. However, features of IBI's may not be the only useful indicators of maturation. Combining multiple features of EEG such as spectral power, changes in frequency bands of bursts of activity in QS with features of IBIs may provide a more accurate assessment of the overall functional maturity of cortical activity of the preterm brain.(411) Standardising the IBI definition based on amplitude and duration thresholds will provide more comparable normative values for different GA groups and facilitate collaborative research.(271)

Identifying the PMA of greatest prognostic value for a preterm group is key prior to introducing EEG as a pre-discharge screening tool to the neonatal unit. Future research should explore training of automated algorithms on infants also with abnormal outcome which may assist in identifying features predictive of abnormal outcome.

The clinical relevance of our secondary findings warrants further research. Does chronological age and accommodation type in the neonatal unit have developmental

effects? Are there differences in maturation of preterm infants born from multiple and singleton pregnancies? There is need for further studies with large sample sizes, of good design, and optimal measurement of all relevant variables with long term developmental surveillance in order to determine the clinical relevance for these findings. Finally, conducting a similar sleep study of MLP infants cared for on postnatal wards would make a valuable contribution to the literature. These infants are exposed to natural cycled light and mothers are also immediately on hand to comfort, hold and feed the infant on demand, all of which are more conducive to sleep and support normal sleep architecture development and maturation.

5.7 Conclusion

We have adapted an IBI detection algorithm for qEEG of QS in a healthy MLP infant group. This has provided robust normative data which may be used for reference in future studies. Pre-discharge EEGs are an important consideration in identifying infants that warrant close ongoing surveillance. There is a need to standardise EEG thresholds or cut-off parameters for IBI for each GA group under study.

The IBI algorithm in this study is freely available as Matlab code (https://github.com/otoolej/burst_detector commit: 573a641) and as Python code (https://github.com/otoolej/py_burst_detector commit: e31753c). A simple pre-processing filter, as described in this manuscript, can be added to this detector for the MLP cohort.

Chapter 6

Neurodevelopmental outcome of the moderate to late preterm infant at 4 months and 18 months

Abstract

Introduction

Moderate to late preterm (MLP) infants (32-36+6 weeks gestational age at birth) are the largest and fastest growing proportion of the preterm infant population. The availability of developmental outcome data in healthy MLP infants is often hindered by the belief that these infants are low risk and hence require little developmental surveillance.

Aim

The aim of this study is to describe the developmental outcome at 4 months 18 months postmenstrual age (PMA) of a cohort of healthy moderate to late preterm (MLP) born

infants admitted to the neonatal unit at birth and to compare their outcome to that of a healthy term born infant group at the same PMA

Method

MLP infants were recruited in the neonatal unit between July 2017 and September 2018 as part of a sleep monitoring study. A healthy term control (TC) group were recruited during the same period. Both groups had a neurodevelopment assessment (Griffiths III) at 4 months and 18 months post menstrual age (PMA).

Results

At 4 months 81 MLP infants and 97 TC infants returned for follow up whilst 75 MLP and 92 TC infants returned at 18 months for Griffiths III developmental assessment. MLP infants scored significantly lower in subscales A; Foundations of learning, ($p=0.004$), C; Eye/Hand co-ordination, D; Personal/Social and Emotional Development, E; Gross Motor Development and GD: General Developmental Quotient (all $p=0.001$) in comparison to the TC infant group with Cohen's d effect sizes ranging from -0.665 to -0.460. There was no difference in mean scores achieved in subscale B – language and communication between groups.

Conclusion/ interpretation

MLP infants are at risk of adverse developmental outcome. Ongoing surveillance of the developmental trajectory of this group of premature infants is require.

6.0 Introduction

The World Health Organisation (WHO) has acknowledged that whilst reducing mortality is the priority for newborns there is also a need to prioritise improving

As discussed in Chapter 1 Section 1.2.5, all infants born before term are at a greater risk for developmental delay and school-related problems in comparison to the term born infant. Whilst a more positive outcome is associated with the low-risk uncomplicated preterm infant, defining the term 'uncomplicated' may prove problematic as preterm birth itself is not normal and its aetiology may relate to deteriorating fetal or maternal health. The lower the GA of the infant the greater the impact on mortality and morbidity.(413, 414, 415) Very preterm infants may have more complex developmental needs, however all infants born preterm are at high risk for adverse development. Prematurity combined with low birth weight and intrauterine growth retardation (IUGR) further increases the risk of adverse outcome.(415)

Comprehending and producing language is a complex human cognitive skill which may be impaired by preterm birth. Difficulties experienced by preterm infants are not limited to cognitive functioning but a spectrum of social and emotional issues that have been identified (Figure 6.1). Infants born preterm are at risk of poorer orienting, alerting and executive attention skills (416) with a higher prevalence of neurological disorders related to psychological behaviour and emotional developmental.

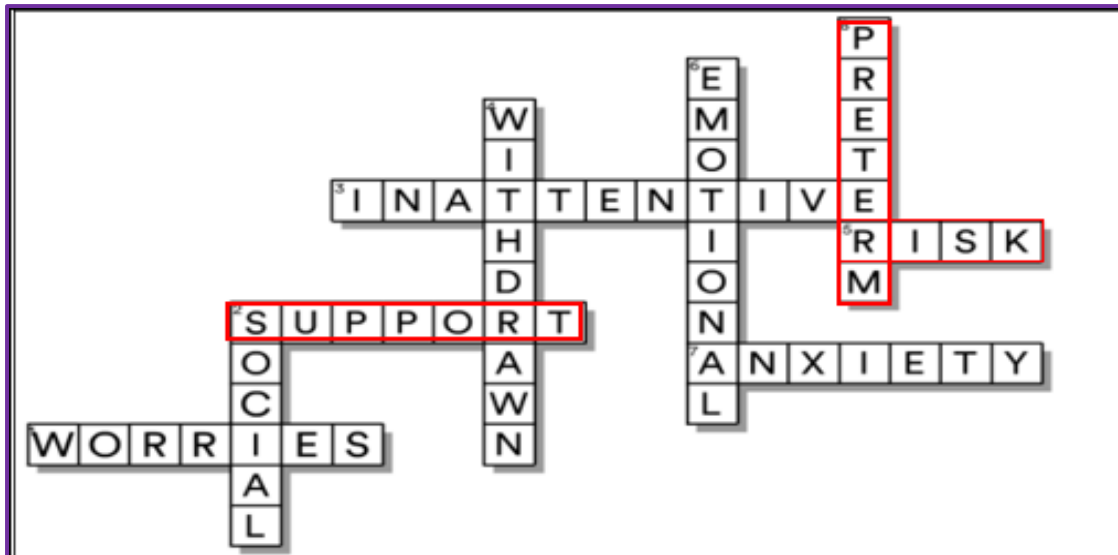


Figure 6.1 A summary of the key social and emotional difficulties that children born preterm might experience. An e-learning resource Preterm Birth Information for Educational Professionals accessed 23August 2021 www.pretermbirth.info (134)

A higher prevalence of CP (166) and non-CP motor impairment (417) are evident amongst preterm infant groups with the risk of impairment decreasing with advancing GA.(166) Although the risk of CP is low in the healthy MLP infant group, further research is warranted pertaining to subtle non-CP motor impairments such as developmental coordination disorder (DCD).(418) Williams concluded that children born preterm are at three to four times greater risk of non-CP motor impairment (two levels of impairment identified i.e., mild to moderate or moderate) in comparison to term born infants. For many DCD may only become evident at school going age. Children with DCA have also been shown to have psychosocial issues such as poor self-esteem and self-worth due to poor performance in physical education and sporting activities.(419)

Preterm infants from varying GA's have lower IQ (145) which correlates with problem-solving and educational performance.(146) An elevated incidence of special educational needs (SEN) has been described across the spectrum of GA's. (148, 149,

150, 151, 420) Mathematics is a frequently reported subject in which preterm infants experience difficulties and require support.(142) Any area of weakness could potentially have a negative impact on math ability including working memory, processing speed and ability for flexible thinking and exam performance.(143) In describing the outcome of any preterm infant group consideration must be given to factors such as cultural background and socioeconomic status (SES). SES metrics are derived from many different aspects of life include maternal education,(169, 421, 422, 423) income,(424, 425, 426) occupation,(427, 428) ethnicity,(429) maternal age,(429) and maternal marital status.(430) Social disadvantage is an added risk for adverse development.(431, 432)

Not all research supports adverse developmental outcomes in a MLP infant group in comparison to term born infant group. Odd et al described no variation in summary IQ measures between MLP and term born infants at 11 years.(147) Wang et al concluded no difference in attention/working memory between infants born 34-36 weeks gestation and term born infants.(107) Ramdin et al described no difference in the mean cognitive, language and motor scores on a Bayley's III assessment of late preterm infants at 16 months PMA (with a birth weight >1500 g) and term-born healthy infants who were discharged home with their mothers.(433)

Normative scores in developmental assessments of healthy moderate to late preterm (MLP) infants are less well established in comparison to the very preterm or the term-born infant.(21) However, the ever increasing proportion of MLP infants substantiates a need for a better understanding of the development outcome of this group. (434)

6.1 Aims

The aims of this study include

- To describe the developmental outcome of a cohort of healthy MLP infants at 4 months PMA and 18 months PMA
- To compare outcome of this MLP infant group to that of a cohort of healthy term born infants at the same PMA

6.2 Methods

Chapter 2 Methods, describes all aspects of this study including research setting (Section 2.1), participants (Section 2.3) and inclusion/ exclusion criteria (Section 2.4) This was a single centre study carried out in Cork University Maternity Hospital (CUMH) between July 2017 and September 2018. Ethical approval was granted by the Cork Research Ethics Committee (CREC) of the Cork Teaching Hospitals.

6.2.1 Participants

Proxy consent was sought from parents of MLP infants admitted to the neonatal unit as part of an observational sleep study. Inclusion criteria required infants to be clinically stable without neurological concern by the clinical team and no actual or suspected chromosomal abnormalities (See chapter 2, section 2.4). Infants from singleton and multiple pregnancies were included in the MLP infant group.

During the same period, term-born singleton infants (37-42 weeks GA with no metabolic or genetic anomalies) were recruited on the postnatal wards as part of a clinical trial of a massage intervention in the first months of life (Clinicaltrials.gov NCT03381027) (n=97). The non-intervention arm of this study served as the term control (TC) group for our study.

As described in chapter 2, section 2.9, parents of both groups were invited to return to the INFANT Early Life Lab for an infant neurodevelopmental assessment at 4 months and 18 months post menstrual age (PMA). Anthropometric measurements including weight, length and head circumference were also recorded at the follow up visits.

Information on maternal occupation was obtained from a questionnaire completed by the TC group whilst this information was obtained from the maternal electronic health record for the MLP group where documented. We categorised occupation as: professional, lower professional, manager/technical/administration, non-manual or skilled (based on Central Statistics Office 2016 criteria for categorising occupation groups).(435).

As both the MLP infant group and the TC group were recruited from the same hospital, within the same time frame and assessments carried out at the same age by the same small group of assessors, variability in sample groups and methodology was minimised. The mean (standard deviation) (SD) of the scores achieved by the TC group served as a reference test to determine the developmental outcome of the MLP infant group at the same PMA. Developmental achievements of both groups were compared to at 4 months and 18 months post menstrual age (PMA).

6.2.2 Griffiths III developmental assessment

In assessing developmental outcome of our MLP group we used the Griffiths III mental developmental scales. This allowed for an accurate comparison with the TC group (non intervention arm of the clinical trial) which used this developmental assessment tool in assessments at 18mths. The Griffiths III is a revision of the Griffiths II Mental Development

Scales which had a mean (SD) developmental quotient (DQ) score of 100(13). By comparison the Griffiths III has a mean (SD) DQ score of 109(19).(436) Griffiths III provides an overall general developmental score and an individual profile of strengths and weaknesses across five areas or subscales of development (Table 6.1).(437) Subscales of development include: learning (curiosity, communication creativity, learning and memory), hearing/speech and communication, eye and hand coordination, personal–social performance, and gross motor development (Table 6.1). Each subscale was equally weighted. Developmental quotient (DQ) scores were calculated for each subscale of development based on the mean score obtained for the group under study.(438)

Input from primary caregivers is required into the Griffiths test with some subscales requiring more input than others.(439) Caregiver input confirms the infant's ability to carry out specific activities in the home that may not be completed on day of assessment. In calculating scores achieved the Griffiths III uses a '*ratio transformation*', dividing developmental age in months by chronological age giving rise to slightly different means and standard deviations for each subscale of development.(440)

The same expert group of assessors performed the developmental assessments on both groups. Due to the nature of the study and the assessment used, we were unable to blind assessors to MLP group

Table 6.1 Subscales of development in the Griffiths III Mental Developmental Scales

Subscales of Development	Area of assessment
Subscale A: Foundations of learning	Assesses critical aspects of learning during the early childhood years including attention, processing speed, aspects of executive functioning such as flexibility, dimensions of reasoning, curiosity and creativity, organising and planning, concept formation and sequencing thought.
Subscale B: Language and Communication	Measures overall language development, including expressive, receptive, language to communicate socially. Memory has also been incorporated into the Scale.
Subscale C: Eye hand co-ordination	Considers fine motor skills, manual dexterity and visual perception skills
Subscale D: Personal Social and Emotional	Child's developing sense of self, growing independence, interactions with others, emotional development
Subscale E: Gross Motor development	Overall postural control, balance, coordination and other motor abilities
GD: General Development	A total overall development quotient score and to what estimated age the infant is achieving

Summary of information accessed 22Aug 2021 from accessed 22Aug 2021 [https://www.aricd.ac.uk/about-the-griffiths-scales/griffiths-iii/\(437\)](https://www.aricd.ac.uk/about-the-griffiths-scales/griffiths-iii/(437))

6.2.3 Statistical methods

Statistical analysis was performed using IBM SPSS Statistics (version 25.0, IBM Corp, Armonk, NY, USA). In the demographic data recorded, continuous variables were described using the median (IQR). Categorical variables were described using frequencies and percentages. Comparisons between continuous variables were made using the Mann-Whitney U test and between categorical variables using chi-squared test, A p -value <0.05 was significant.

Griffiths DQ scores at 4 months and 18 months were described by the mean and standard deviation (SD) and the independent samples t-test was used to scores in each subscale of development between MLP and TC groups. Cohens *d* formula was used to determine the standardized mean difference between the MLP and TC groups. A *d* of 0.2 was considered a small effect size, 0.5 a medium effect size and 0.8 a large effect size.(441)

As Griffiths is a norm-based test we also used the mean (standard deviation) (SD) of the scores achieved by the healthy TC group at 18 months as a reference test to determine the developmental outcome of the MLP infant group at the same PMA. Categories were defined as normal (within 1SD of TC group), mildly abnormal (>1SD below the healthy term group), moderately abnormal (>1-2 SD below the TC group) and severely abnormal (>2SD below the TC group).

Finally, we performed a post-hoc analysis of the MLP group to examine the effect of absolute GA in weeks, sex and pregnancy type on outcome at 18 months. Within the MLP infant group analysis, comparisons of scores between subgroups (e.g. sex or pregnancy type) were calculated using an independent sample t-test and outcome by gestational age week group using a one-way ANOVA

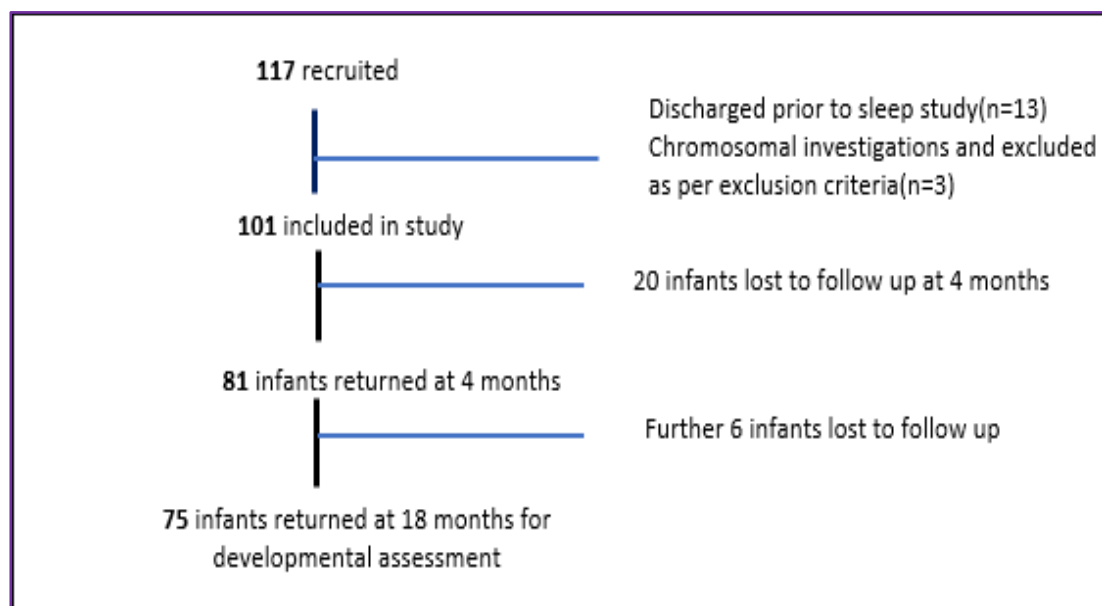


Figure 6.2 Recruitment of MLP infant group

6.3 Results

One hundred and seventeen MLP infants were recruited to this study in the neonatal unit and 101 infants participated in this study.(Fig 6.2) Ninety seven infants participated in the term control (TC) group. The median (IQR) birth GA (wks) of MLP infant group was 34.00 wks (IQR:33.75-35.21) and the TC group was 39.86 weeks (IQR:38.93-40.82). As expected, the TC group(n=204) had significantly higher birthweight and larger head circumference than the MLP group (n=101) (Table 6.2). The majority of infants in the MLP and TC groups were male (52.5.% and 55.9% respectively).

At time of recruitment, within the MLP group 52.5% of infants were from singleton pregnancies and 47.5% from multiple pregnancies. Nine infants had a birth weight<10th centile for GA i.e. small for gestational age (SGA)(442). All SGA infants had normal developmental outcome at 18 months. Infants from a singleton pregnancy had significantly higher birthweight than infants from a multiple pregnancy

(median (IQR): 2.27(1.95-2.65) vs 1.97(1.78-2.14), $p<0.001$); however, this difference in weight was not evident at 18 months (median (IQR): 11.29(10.80-12.49) vs 11.37(10.60-12.24), $p=0.666$).

At 4 months PMA 81 MLP infants and 97 infants in the term control returned for their first follow up visit and Griffiths III assessment. Anthropometric measurements including weight, head circumference and length were also recorded at the follow-up visit. There was no significant difference in these measurements between groups at this time ($p=0.057$, 0.133 and 0.423 respectively) (Table 6.2). In the MLP infant group, Infants from multiple pregnancies had a significantly lower birth weight than infants from a singleton pregnancy ($p=0.004$).

6.3.1 Maternal occupation

Data relating to maternal occupation of the MLP group was obtained from the maternal electronic health record. Data relating to maternal occupation for the TC group data was obtained from a parental questionnaire completed as part of the clinical trial. Occupation was not recorded in the electronic health record for 18.7% ($n=14$) of MLP infant mothers (Table 6.2). In the TC group 2.2% ($n=2$) of mothers did not disclose their occupation. Based on data recorded there was no difference based on category of occupation between study groups ($p=0.784$). Based on information recorded on employment, 71.4% of MLP and 64.0% of TC mothers were professionals with a university degree or higher, with 14.3% of mothers of MLP infant group and 15.7% of TC group were employed in managerial/ technical or administrative roles. All mothers of TC group were employed whilst 2.7% of MLP group were unemployed.

Table 6.2 Demographics of infants recruited to the study at birth, at 4 months and 18 months

	Moderate to late preterm group (MLP)	Term Control group (TC)	
	Median (IQR)(n=101)	Median (IQR)(n=97)	p-value
Maternal age in years	35.00(31.00-38.00)	35.00(32.50-37.00)	0.189
Gestational age at birth(weeks)	34.0(33.0-35.0)	39.86(38.93-40.93)	n/a
Birth Weight (Kg)	2.10(1.87-2.40)	3.53(3.23-3.83)	<0.001
Birth Head Circumference (cm)(n=100)	32.00(30.85-32.90)	35.20(34.50-36.15)	<0.001
	n(%)	n(%)	
Sex			
Male	53(52.5)	56(57.7)	
Female	48(47.5)	41(42.3)	
Pregnancy			
Singleton	53(52.5)	97(100)	
Multiple	48(47.5)	0(0)	
4 months assessment	Median (IQR)(n=81)	Median (IQR)(n=97)	
Chronological age	6(5-6)	n/a	
Corrected age(months)	4(4-5)	4(4-4)	
Weight (Kg)	6.78(6.28-7.25)	7.08(6.36-7.82)	0.057
Head Circumference (cm)(n=80)	43.00(42.00-44.00)	42.40(41.40-43.60)	0.133
Height (cm)(n=79)	64.00(62.38-66.20)	65.00(63.00-66.50)	0.423
	n(%)	n(%)	
Sex			
Male	39(48.1)	56(57.7)	
Female	42(51.9)	41(42.3)	
Pregnancy			
Singleton	41(50.6)	97(100)	
Multiple	40(49.4)	n/a	
18 months	MLP(n=75)	Term (n=92)	
Weight (Kg) ¹	11.36(10.75-12.40)	11.50(10.70-12.60)	0.518
Height (cm) ¹	81.50(79.10-84.00)	83.00(80.00-85.00)	0.079
Head Circumference (cm) ¹	48.00(47.90-48.50)	48.80(47.53-49.50)	<0.001
Chronological age *	19.65(19.32-20.44)	18.46(18.17-18.72)	<0.001
Adjusted age on assessment *	18.00(18.00-19.00)	18.46(18.17-18.72)	
	n(%)		
Sex			0.091
Male	35 (46.7)	55(59.8)	
Female	40(53.3)	37(40.2)	
Pregnancy			
Singleton	38(50.7)	92(100)	
Multiple	37(49.3)	-	
Maternal Occupation			0.383
Unemployed	2(2.7)	0(0)	
Not documented	14(18.7)	3(3.3)	
University Student	3(4.0)	1(1)	
Employed	56(74.7)	88(95.7)	
professional	38(65.5)	57(63.3)	
lower professional	2(3.4)	3(3.3)	
Manager/ technical/ admin	8(13.8)	14(15.6)	
Non-manual	6(10.3)	10(11.1)	
Skilled	1(1.7)	5(5.6)	

MLP: moderate to late preterm infants, IQR: interquartile range, Kg: kilogram, cm: centimetre

The Mann–Whitney U test was used for comparisons of continuous variables between groups. The Chi-squared test was used to determine the relationship between categorical variables. A *p*-value <0.05 is significant

* Delay in 18 months assessment for MLP (n=14) infants due to Covid restrictions. ¹Missing data as not recorded in electronic health record or failure to obtain at 18 months. In MLP group missing data includes: weight at 18

months (n=4), Head circumference (n=6), Height (n=1) and maternal occupation (n=14). In the term control group missing data includes, head circumference at birth (n=16) and at 18 months includes Weight(n=4), Head Circumference (n=16), Height (n=3) and Maternal Occupation (n=3)

6.3.2 4 months PMA assessment

At 4 months PMA 81 MLP infants and 97 of TC group returned for assessment. There was no significant difference in weight, length or head circumference between the MLP and TC groups at 4 months ($p=0.057$, 0.133 or 0.423 respectively) (Table 6.2). Almost half of the MLP infant group were from multiple pregnancies whilst singleton infants only were recruited to the TC group (exclusion criteria). There was minimal sex difference within the MLP group at 4mths PMA (48.1% were male and 51.9% were female) and the majority of the TC group were males (57.7%). Significant differences were evident in between groups in all subscales of development with the exception of Subscale A, Foundations of learning ($p=0.127$). (Table 6.3, Figure 6.3).

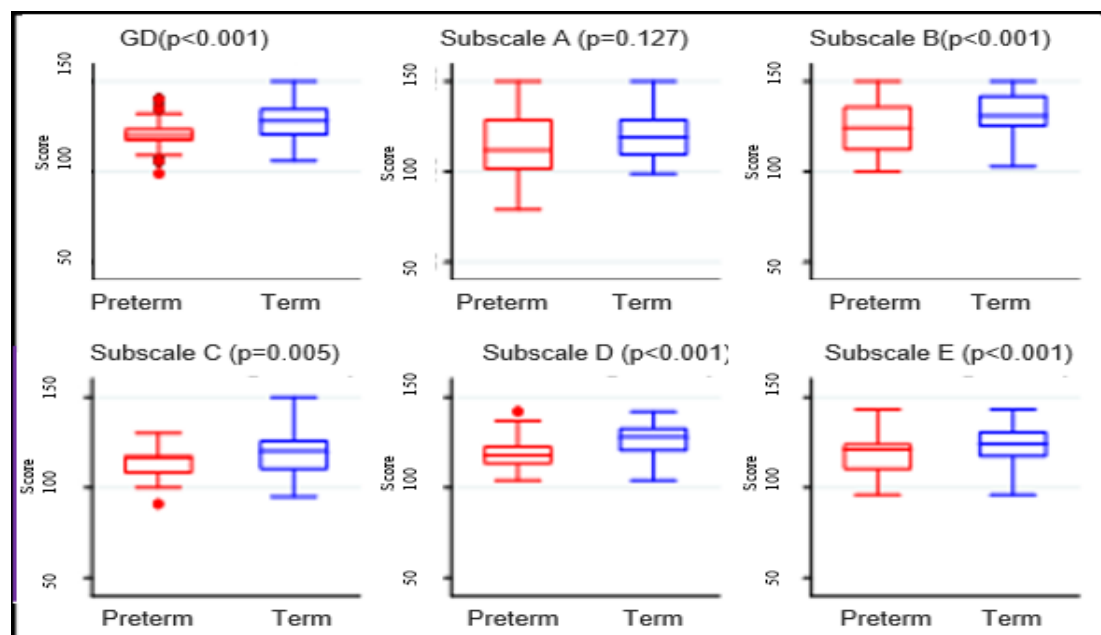


Figure 6.3 Boxplot representation of 4 month Griffiths assessment scores achieved by moderate to late preterm(n=81)(red) and term control group (n=97)(blue)

At 4 months PMA singleton infants (n=41) outperformed the multiples group in Subscale B (Language / Communication) (p=0.015) and Subscale C (Eye Hand Coordination) (p=0.022) with a higher overall General development scores (p=.044).

Table 6.3 Developmental outcome of moderate to late preterm (MLP) and term control (TC) groups at 4 months and 18 months post menstrual age (PMA)

Griffiths III Mental Developmental Scales	4 Months			18 Months		
	Preterm(n=81)	Term(n=97)		Preterm(n=75)	Term (n=92)	
Subscales of development	Median (IQR)	Median (IQR)	p-value	Median (IQR)	Median (IQR)	p-value
Subscale A: Foundations of Learning	112(101-129)	119(109-129)	0.127	110(97-118)	114(105-122)	0.002
Subscale B: Language / Communication	124(112-136)	131(125-142)	<0.001	106(96-114)	109(99-117)	0.286
Subscale C: Eye-hand coordination	117(108-117)	120(110-126)	0.005	109(103-118)	118(109-122)	<0.001
Subscale D: Personal Social/ Emotional	118(113-123)	128(120-133)	<0.001	115(108-122)	122(117-125)	<0.001
Subscale E: Gross Motor development	121(110-124)	124(117-131)	<0.001	124(112-130)	135(126-135)	<0.001
GD: General Development	120(117-126)	128(120-135)	<0.001	112(108-122)	122(112-127)	<0.001

GD: general development, IQR interquartile range, DQ; developmental quotient. a p-value calculated from the Mann-Whitney U test. A p-value <0.05 was significant

6.3.3 18 months follow-up assessment:

At 18 months PMA, 75/101 (74.3%) of the MLP infant group and 92/97 (95%) of the TC group returned for a second Griffiths III developmental assessment. At this time there was no difference in weight (p=0.518) or height (p=0.079) between groups but a significant difference persisted in head circumference(p<0.001) (Table 6.2). At this timepoint, 49.3%(37/75) of the MLP group were from a multiple pregnancy. At this assessment MLP infants continued to score significantly lower than TC group in all subscales of development and overall general development with the exception of Subscale B, Language and Communication (p=0.286) (Table 6.3, Fig 6.4).

All infants born with a birth weight $\leq 10^{\text{th}}$ centile ($n=9$) or small for GA (SGA) had a normal developmental outcome at 18 months. Five of these infants were from a multiple pregnancy whilst four infants were from a singleton pregnancy.

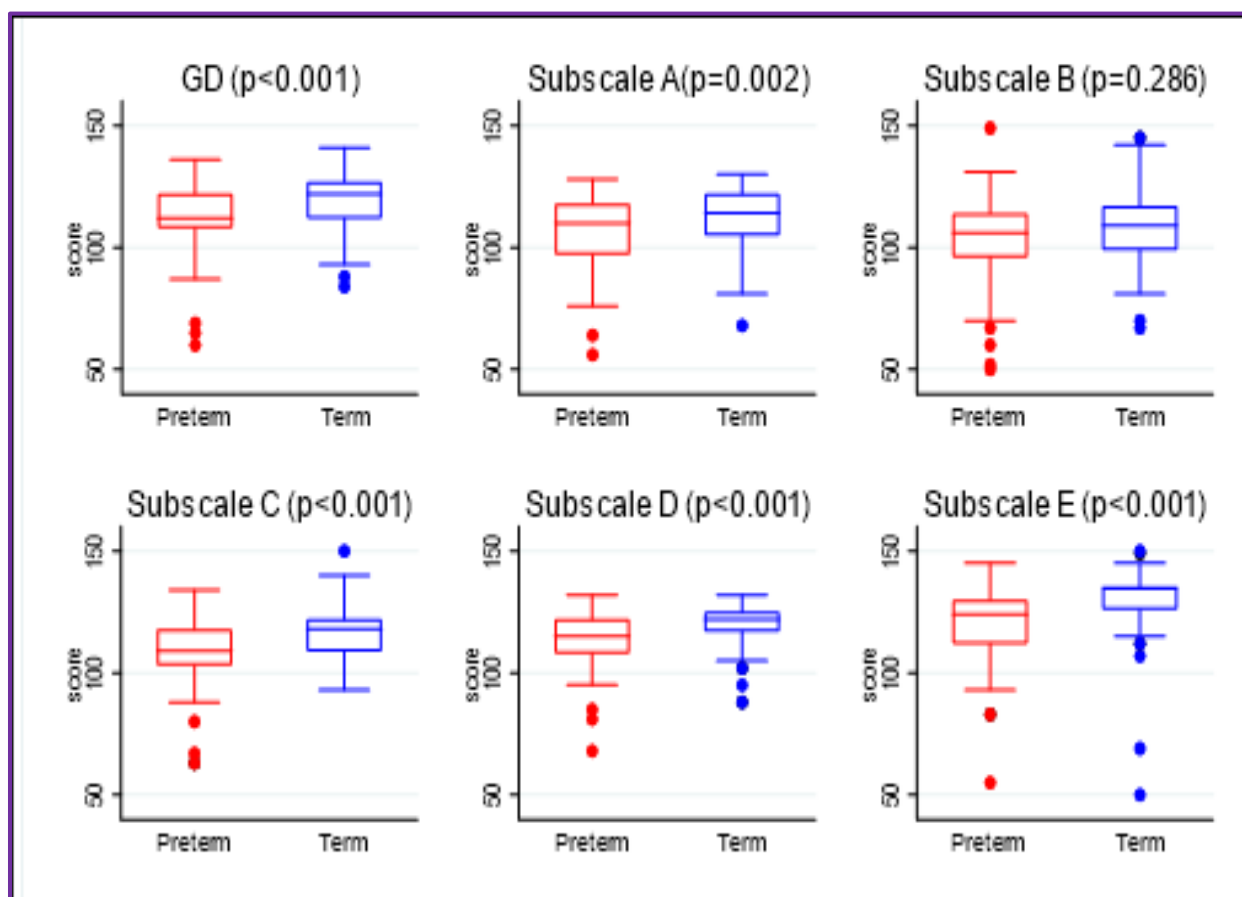


Figure 6.4 Boxplot representation of 18 months Griffiths assessment scores achieved by moderate to late preterm ($n=75$) (red) and term control group ($n=92$) (blue)

The Cohens d effect size calculations at 18 months showed a medium negative effect size difference was evident between groups in overall general development at 18 months ($p < 0.001$) with a Cohens $d = -0.602$ (Table 6.4). Differences were evident in scores achieved in Subscale C Eye-hand coordination ($p < 0.001$, with a Cohens d effect size of -0.648), Subscale D Personal /Social /Emotional ($p < 0.001$, with a Cohen's d effect size of -0.590) and Subscale E Gross Motor ($p < 0.001$, with a Cohen's d effect size of -0.665). These findings indicate that the

MLP group scored 0.648, 0.590 and 0.665, standard deviations below the TC group within these three subscales of development. A small-medium effect size was seen in Subscale A ($d = -0.460$) and a small effect size in Subscale B ($d = -0.252$).

Table 6.4 Comparison of Griffiths III general development DQ scores of the moderate to late and term control groups at 18 months and effect size difference described using Cohens d

	Term Control Group(n=92)	Moderate to late preterm (n=75)			
	mean (SD)	mean (SD)	Difference (95% CI)*	p -value**	Cohens d
Subscale A DQ ,Foundations of learning	112.46(11.92)	106.45(14.30)	6.00(2.00 to 10.01)	0.004	0.460
Subscale B DQ, Language / Communication	107.99(14.85)	103.97(17.19)	4.02(-0.88 to 8.91)	0.107	0.252
Subscale C DQ, Eye -hand co-ordination	116.16(10.75)	108.36(13.45)	7.80(4.11 to 11.50)	<0.001	0.648
Subscale D DQ, Personal/social/emotional	119.04(8.17)	113.56(10.51)	5.48(2.63 to 8.34)	<0.001	0.590
Subscale E DQ, Gross Motor	130.30(13.58)	120.85(14.94)	9.45(5.09 to 13.81)	<0.001	0.665
GD DQ , General Development	119.45(10.78)	112.12(13.67)	7.33(3.59 to 11.06)	<0.001	0.602

DQ: developmental quotient score, SD: standard deviation, CI: confidence Interval.

A p -value <0.05 is considered statistically significant. Cohens d formula was used to determine the standardized mean difference between the TC and MLP groups

*difference is TC-MLP

**from independent samples t-test

Table 6.5: Scores on Griffiths III MDS achieved at 18 months by moderate to late preterm infant group by birth gestational age, sex and pregnancy type

Gestational age group (n=75)	age (weeks)	Subscale A: Foundations of Learning	p-value	Subscale B: Language and Communication	p-value	Subscale C: Eye-hand coordination	p-value	Subscale D: Personal Social / Emotional	p-value	Subscale E: Gross Motor development	p-value	General Development	p-value
			0.978		0.450		0.741		0.573		0.713		0.585
32 weeks GA (n=12)		108.58(12.26)		109.17(7.38)		110.25(9.72)		116.50(6.95)		120.50(13.35)		115.25(6.09)	
33 weeks GA (n=13)		106.31(14.23)		101.54(20.70)		108.15(15.69)		114.38(16.52)		125.08(14.61)		112.46(16.86)	
34 weeks GA (n=28)		105.57(16.68)		100.14(18.31)		105.79(14.20)		111.00(10.02)		108.29(18.92)		108.82(15.14)	
35 weeks GA (n=10)		105.50(12.38)		108.70(20.99)		111.60(14.86)		115.10(7.94)		120.00(8.60)		114.00(12.98)	
36 weeks GA (n=12)		107.33(13.67)		106.42(13.49)		110.00(12.02)		114.42(8.36)		123.17(10.32)		114.75(12.69)	
Sex			0.075		<0.001		0.003		0.001		0.582		0.001
Male (n=35)		103.31(16.69)		95.80(19.38)		103.46(15.89)		109.26(12.41)		119.83(14.49)		106.66(16.53)	
Female (n=40)		109.20(11.34)		111.12(11.00)		112.65(9.08)		117.33(6.66)		121.75(15.44)		116.90(8.14)	
Pregnancy type			0.959		0.040		0.230		0.298		0.216		0.319
Singleton (n=38)		106.37(11.50)		107.97(14.51)		110.21(10.69)		114.82(7.48)		118.74(16.20)		113.68(10.41)	
Multiple (=37)		106.54(16.89)		99.86(18.89)		106.46(15.71)		112.27(12.90)		123.03(13.39)		110.51(16.36)	

The p-value was calculated using the one-way ANOVA for gestational age and the p-value calculated using an independent sample T-test for sex and pregnancy type. A p-value<0.05 was considered significant

In the MLP group we conducted a post-hoc analysis to investigate the effect of degree of prematurity (GA week group), sex and pregnancy type on outcome at 18 months (Table 6.5). Although numbers in each GA group (32, 33, 34, 35 and 36 weeks) were low, we found no significant differences in assessment scores, based degree of prematurity.

In the MLP infant group, sex was found to be associated with Griffiths scores with female infants outperforming male infants in Subscale B ($p < 0.001$), Subscale C ($p = 0.003$), Subscale D ($p < 0.001$), and overall general development (GD) ($p < 0.001$). There was however an imbalance between MLP and TC groups in terms of sex (i.e. 53.3% of MLP infants were females in comparison to 40.2% of TC group). To account for sex imbalance between MLP and TC groups we used regression analysis with both study and sex as independent variables. When we adjusted for sex imbalance scores achieved in Subscale B significantly differed between groups ($p = 0.028$). (Table 6.6)

Table 6.6 The relationship between Griffiths scores in each subscale of development between groups when adjusted and unadjusted for sex

	Unadjusted analysis		Analysis adjusted for sex	
	<i>Difference in means* (95% CI)</i>	<i>p-value**</i>	<i>Difference in means* (95% CI)</i>	<i>p-value***</i>
Subscale A	6.00(2.00 to 10.01)	0.004	6.55(2.55 to 10.55)	0.001
Subscale B	4.02(-0.88 to 8.91)	0.107	5.30(0.58 to 10.02)	0.028
Subscale C	7.80(4.11 to 11.50)	<0.001	8.56(4.93 to 12.20)	<0.001
Subscale D	5.48(2.63 to 8.34)	<0.001	6.20(3.44 to 8.96)	<0.001
Subscale E	9.45(5.09 to 13.81)	<0.001	9.76(5.36 to 14.16)	<0.001
Subscale GD	7.33(3.59 to 11.06)	<0.001	8.25(4.63 to 11.87)	<0.001

CI: confidence interval,

A p-value < 0.05 is considered statistically significant

*difference is TC-MLP

**from linear regression analysis with group as the independent variable

***from linear regression analysis with group and sex as the independent variables

Adjustment for pregnancy type: At 18 months PMA there was no difference in overall general developmental (GD) scores between infants born from singleton and multiple

pregnancies (p=0.319) (Table 6.5). Significant differences by pregnancy type were only evident in Subscale B (Language and Communication) (p=0.040) in which singleton infants out performed infants born from a multiple pregnancy. As the TC group was limited to singleton infants only, we conducted an analysis restricted to singleton infants only. Again there was a statistically significant difference in all subscales (except subscale B) between the MLP and TC groups (before and after adjustment for sex). We next adjusted for sex which again showed no difference in subscale B with or without adjustment. (Table 6.7)

Table 6.7 Restricted analysis to singleton infants before and after adjustment for sex

	Unadjusted analysis		Adjusted analysis	
	<i>Difference in means (95% CI) *</i>	<i>p-value**</i>	<i>Difference in means (95% CI)*</i>	<i>p – value***</i>
Subscale A	6.09 (1.59 to 10.59)	0.008	6.38(1.86 to 10.91)	0.006
Subscale B	0.016(-5.61 to 5.64)	0.996	0.79(-4.79 to 6.35)	0.779
Subscale C	5.95(1.86 to 10.05)	0.005	6.49(2.43 to 10.54)	0.002
Subscale D	4.23(1.19 to 7.27)	0.007	4.72(1.73 to 7.70)	0.002
Subscale E	9.45(5.09 to 13.81)	<0.001	11.80(6.26 to 17.33)	<0.001
Subscale GD	5.76(1.69 to 9.83)	0.006	6.39(2.40 to 10.39)	0.002

CI: confidence interval,

A p-value <0. 05 is considered statistically significant

*difference is TC-MLP

**from linear regression analysis with group as the independent variable

***from linear regression analysis with group and sex as the independent variables

Using the reported Griffith's cut off as < 90 for delayed development, 5/75(6.7%) of the MLP group were <90 compared to 2/92(2.2%) of the TC group at 18mths. When we standardised Griffiths III scores based on the general development DQ score of the healthy TC group (n=92) with a (mean=119, SD=10), 29.3% of MLP group had an abnormal outcome versus

18.5% of the TC group (Table 6.8). Cognitive skills are most affected in this group which may limit later academic performance and consequently, vocational opportunities.

Table 6.8 Developmental outcome of the Moderate to late preterm (MLP) standardised based on mean (SD) of the term control group at 18 months

	TC(n=92)	MLP(n=75)
	n(%)	n(%)
Normal Outcome (>109)¹	75(81.5)	53 (70.7)
Abnormal Outcome	17(18.5)	22 (29.3)
Mild delay, One SD below mean (100-109)	13(14.1)	14 (18.7)
Moderate delay, two SD below the mean (90-99)	2(2.2)	3 (4.0)
Severe delay, three SD below the mean (0-89)	2(2.2)	5 (6.7)

¹Scoring of Griffiths was standardised based on the general development developmental quotient score of a healthy term control group (n=92) with a (mean=119, SD=1)

6.4 Discussion

We have shown that MLP infants had significantly lower in Griffiths MDS at 4 months and 18 months PMA. No difference was seen in subscale A Foundations of learning at 4 months (p=0.127) and at Subscale B Language and Communication at 18 months (p=0.286) between the groups.(Table 6.3).

We did not find any difference in outcome at 18 months due to gestational age at birth (32-36 weeks). This may be because of low numbers in each gestational week and we were underpowered to examine this effect.. In a similar study that investigated the developmental outcome and social-emotional competence of MLP infants versus a TC infant group at 2 years, Cheong et al (135) also found no evidence of an association with birth gestational age. As all infants had an uncomplicated neonatal stay, factors such as maternal well-being, obstetric/fetal complications, level of maternal education,

socioeconomic and cultural background may come into play when considering the risk of adverse outcome and the level of surveillance warranted. (109, 171, 172, 443)

Outcome of infants born very prematurely and small for GA (SGA) (defined as a birth weight <10th centile (442) is described as significantly associated with hyperactivity scores (coefficient 0.81, $p < 0.04$) when assessed using the Strengths and Difficulties Questionnaire at 5 years($n=342$). (158). Bickle et al's study concluded that birthweight <10th percentile does not appear to be a risk factor for adverse outcome in MLP infants in the area of cognition and neurodevelopmental at 18 months. Longitudinal follow up will provide data on the longterm effects prematurity and SGA. An increase in neonatal complications and prolonged hospitalisation have been described in in SGA MLP infants (444).

Maternal level of education, has been described as having a significant and consistent relationship with the cognitive development of preterm children from birth through school years.(445, 446) Maternal education is also the most frequently evaluated indicator of SES.(447) Lower parental education and SES are associated with poorer developmental outcomes in preterm infants. (117, 137, 149, 168, 169, 445, 448) Detailed data on maternal education and maternal IQ were not available for our MLP cohort. However, similar profiles in occupation were seen in the MLP and TC groups($p=0.070$) and those employed ($p=0.784$), with 71.40% of MLP group and 64.0% of the TC group identified as professionals requiring a minimum of a university degree. It is reassuring that differences between the groups were not driven by differences in maternal education.

There is a higher risk of preterm birth with a multiple pregnancy (449) (supported by the fact that approximately half of the MLP infants in this study were from multiple pregnancies). In our analysis of the MLP infant group at 18 months DQ scores of singleton and multiple infants of MLP infants differed only in Subscale B Language and Communication ($p=0.040$) (Table 6.5).

Manuck et al, (controlled for GA at delivery and maternal education) described no difference in outcome of preterm singleton and multiple pregnancies (<33.9 weeks birth GA) using the Griffiths Mental Developmental Scales or Bayley Scales of Infant Development at 2-3 years.(132) However, Raz et al described twins < 34 weeks birth GA to have lower global language performance a lower visual processing scores compared to singleton infants during preschool years (3-6 years).(450)

MLP infants in this study also had significantly lower scores in the personal, social and emotional subscale of development ($p<0.001$) with a moderate effect size (Cohens d of -0.590) (Table 6.4). This finding supports Johnson et al's results of an increased risk of delayed social competence. (118) Cheong described social behavioural issues to be four times higher amongst MLP in comparison to term-born infants.(4) This may also suggest a predisposition to challenging behaviour, (64, 157) an increased risk of hyperactivity and a diagnosis of ADHD.(126) However, Huddy et al concluded that an association between prematurity and hyperactivity was not always inversely related to GA.(94) Children with learning, social, behaviour, motor and sensory impairments have a higher special educational needs (SEN) requirement.(151, 152)

Previous research studies have shown a strong correlation between prematurity and a diagnosis of cerebral palsy(CP) with the absolute risk of CP decreasing with

advancing birth GA.(162, 164) Pierrat et al. reported that 1.0% of infants born between 32 to 34 weeks GA weeks had a diagnosis of CP at 2 years of age. (165) Although more mature preterm infants generally have better outcomes, MLP infants have shown a six times higher prevalence of CP than children born at term (7 per 1000 vs.1.1 per 1000 live births). (166) (135) This study has shown that the greatest difference between groups was in the area of gross motor development with a moderate effect size of -0.665. Our findings support Williams suggestion that non- CP motor impairment may be the most common form of impairment in the preterm population.(418) Motor milestones in early infancy have been associated with children needing full time special education.(451) However, no participating MLP infants had a diagnosis of CP at 18 months. As mild motor impairment may only become apparent beyond 18 months, a diagnosis of CP or a non-CP motor disorder at a later age cannot be ruled out.

Identifying developmental deficits through early assessments of preterm and term-born infants supports the use of Early Intervention Services (EIS). The importance of early intervention has been acknowledged by international health organizations some of which recommend regular developmental surveillance screening for all children.(452) A Cochrane neonatal review group identified a positive influence of early intervention programmes on cognitive and motor outcomes which persisted into school age years.(177) Reichow et al acknowledged EIS as critical to optimizing language, socio-emotional development, educational success, and the prevention of additional future developmental delay.(453) EIS have demonstrated quantitative improvement in developmental outcomes for children with a moderately positive effect on children with established developmental deficits.(454) As the smallest improvement in abilities may have a major impact on life choices and earning potential,

EIS services have the potential to affect the quality of life of all infants born preterm into adulthood and reduce a potential long-term socio-economic impact.

This study has provided detailed information relating to the developmental outcome of healthy MLP infant and the strengths/weaknesses of the group in comparison to a healthy TC group. Maturation and development of all areas of the brain are both linear and non-linear and may accelerate or abate at different weeks of gestation.(455) Using the TC group means in a group similar in all aspects to the MLP infant group under study (with the exception of location of care), isolates the effect of GA on outcome. As our TC group scored high, a score within 1 SD of the mean of the TC group was considered a normal outcome (>109 general development DQ score). 29.3 % of MLP infants had an abnormal outcome. Putting this into perspective 3 out of every 10 MLP infants had an abnormal outcome, the majority of which showed mild delay.

6.5 Strengths and Limitations

This study provides robust data that describes the developmental outcome of a large group of healthy MLP infants. Homogeneity is a major strength of this study. All participating MLP infants had an uncomplicated journey through the neonatal unit and were considered healthy at discharge. Both MLP and TC groups were recruited over the same period (2017-2018) with the same standardised developmental assessment performed by the same small group of expert assessors(n=4) at the same PMA of 4 months and 18 months. From a research perspective, homogeneity is an important factor in terms of identifying norms for specific groups of preterm children.

Neurodevelopmental assessments were completed using a tool which has recently been validated a representative sample of infants born in Ireland and the UK. (456)

Limitations include the vast majority of participants (96%) of the MLP infant group were Caucasian, reflecting the local population. This limits the generalisability of our findings. The MLP infant group was inclusive of singleton and multiple births. Only MLP infants admitted to the neonatal unit were included in this study, to the exclusion of those cared for on postnatal wards. This may have had implications in terms of level of stress or sensory exposure for the MLP infant in comparison to the TC group

Research has shown that developmental delay is more prevalent amongst MLP infants from disadvantaged groups.(149, 423) As this data was not available in our study, we utilised education level (where documented) as a proxy for socioeconomic status which was similar between groups. A total of 18.7% of MLP infants did not have this data recorded another possible limitation includes the fact that developmental outcome assessors were not blinded to birth GA at time of assessment.

6.6 Opportunities to improve the outcome for MLP infants

At the outset, improved developmental outcomes for newborn infants must start with prioritising the health of women in general and specifically during pregnancy. Parents are central to children's health and development hence supporting parental wellbeing can only have a positive effect on a child's long-term physical and psychosocial development.(457) The weight of evidence supporting suboptimal neurodevelopment

of MLP infants also has implications for obstetric practice and discussions with parents in providing a case for early delivery during the MLP period versus increased monitoring and surveillance.

The architectural framework of brain development is predominantly genetically defined. However, brain development is experience dependent and relies on circuits reinforced and strengthened by repeated use. (458) Both genetics and the environment (nature with nurture) interact, playing a role in shaping the development and influencing maturation of neural circuits and the strength of connections within the brain. (51) This interaction commences in early fetal life and continues as the infant grows toward childhood and adolescence. This is particularly relevant to the preterm infant who may be exposed to high levels of stress at a sensitive period of development within an environment such as the neonatal unit. All environmental exposures (positive and negative) may potentially affect the preterm infants' well-being and impact the normal trajectory of brain network development. (459) The embedding of positive experiences in early life when developmental plasticity is at its highest will contribute greatly to establishing the foundation for either optimal or suboptimal brain development. (245)). Recent trends have shown slightly lower rates of certain neurologic and developmental conditions amongst preterm infants than previously recorded (162, 165, 460). Education on neuroprotective measures and advances in neonatal care are likely to be contributing to these improved rates(122).

To date research on developmental outcomes has placed a greater focus on very preterm infants, late preterm infants or the term hypoxic infants. By comparison there is a paucity of literature pertaining to the neurodevelopmental outcome of healthy MLP

infant as a stand-alone group. Further robust longitudinal research studies on neurodevelopment will have implications for resource allocation to assist in the provision of a supportive pathway of multidisciplinary surveillance for all MLP infants.

Early diagnosis of developmental delay presents a unique opportunity for surveillance, and intervention with targeted therapies. Further research will inform decisions relating to tools used and screening intervals for MLP infants at a local and national level

There does appear to be some positivity (although small) on the overall developmental outcome of preterm infants (including MLP group

6.7 Conclusion

There is a paucity in the research literature on the long-term developmental outcome of the uncomplicated MLP infant with benign neonatal stay and normal neurological exam on discharge. This study's findings confirm that MLP infants are at higher risk of adverse outcomes in comparison to term-born infants. This study also highlights the effect size of moderate to late preterm birth on developmental outcomes. Our findings support the need for a broader effort for ongoing surveillance of the 'at risk' healthy MLP infant group.

As developmental delay can be transitory or sustained we do not know the value of suboptimal scores at 4 months or indeed 18 months and how these results will play out long term. However, infants who do show an abnormal outcome at 4 months and/or 18 months may be identified as being 'at risk' and require ongoing close surveillance,

Chapter 7

Summary, conclusion and areas of future research

This chapter provides an overview of the various studies performed. This research programme consisted of an observational study using EEG technology to assess the sleep architecture of healthy MLP infants in the neonatal unit at 36 weeks post menstrual age (PMA). Participating infants had follow-up developmental assessments at 4 months and 18 months PMA using the Griffiths III mental development scales. Finally, we compared developmental outcomes of MLP to that of a term healthy cohort at the same PMA. Below is a summary of each chapter and highlights the strengths and limitations of the study. Areas that warrant further research will be explored and personal reflections are made.

7.1 Summary

This study described the sleep architecture of healthy MLP infants overnight at 36 weeks in the neonatal unit. Using cotside EEG with ECG /respiratory monitoring with video, sleep states were identified and annotated for MLP infants(n=98). Of the total overnight SWC's recorded (n=589), 23.3% (n=137) were interrupted.

A more detailed analysis of the nocturnal sleep architecture of MLP was also described. The overall total sleep time (TST) duration(hours) was 7.09(6.61-7.76), 1.58(1.18-2.13) feeding/ attending to care and 3.19(2.38-3.85) awake. Participants were grouped by birth GA week (32,33,34,35 or 36), sex, mode of feeding and location at the time of monitoring (cot or incubator) there was a statistically significant increase in AS noted with advancing birth GA ($p=0.007$). The median (IQR) duration of an overnight uninterrupted sleep cycle was 37.00 (32.00-45.13) mins. Whilst the duration of QS in sleep cycles was significantly higher amongst infants born at a lower GA ($p=0.001$),

An IBI detection algorithm was used to calculate the features of QS of MLP infants. Values for five summary measures of inter-burst intervals IBIs in QS at 36 weeks GA with normal developmental outcome at 18 months(n=60). Features described include: IBI length maximum (secs), IBI length median (secs), IBI length variability, IBI number (per minute) and IBI percent of the QS epoch. The values of these features amongst this very healthy homogenous preterm infant group provides valuable base line data which may be used in future studies.

Developmental outcome of the MLP infant group was compared to a healthy control group at 4 months and 18 months PMA. 75/101 MLP and 92/97 TC infants had a Griffith's Ill assessment at 18 months. With the exception of Subscale B - Eye and Hand Coordination (0.286) there was a significant difference in scores achieved in all subscales between the two groups. MLP infants scored significantly lower in all other four subscales of development and overall general development ($p \leq 0.002$) in comparison to TC infants with a Cohen's D effect size of - 0.460 to - 0.665. When standardised based on the general development DQ score of the healthy TC group ($n=92$) with a (mean=119, SD=10), 29.3% of MLP infants had an abnormal outcome with 18.7% were mildly delayed (one SD below the mean i.e. 100-109), 4 % moderately delayed (two SD below mean i.e. 90-99) and 6.7% were severely delayed (three SD below the mean i.e. 0-89).

7.2 Contribution to the literature

Moderate to late preterm (MLP) infants are the largest and fastest growing proportion of the preterm infant population yet are poorly represented from a research perspective. The development of sleep architecture is a highly dynamic process and progresses rapidly during the MLP infant period.(312, 461).

The main contribution of this thesis is in describing detailed sleep architecture for healthy MLP infants in the neonatal unit at 36 weeks post menstrual age. It also describes how factors such as birth GA, birth weight, sex, accommodation, mode of feeding impact sleep. EEG used in sleep studies contributes to our understanding of brain development and can assist in identifying infants with immature sleep organization and who warrant close ongoing surveillance.

Sleep has been identified as an important factor in cognitive and developmental outcome, physical growth and overall wellbeing.(196, 394) This thesis also describes the frequency of overnight sleep interruptions occurring in an open plan neonatal unit for a healthy MLP infant group at 36 weeks PMA, the majority of whom were preparing for discharge home. Whilst previous studies have described the handling of infants in the NICU, (212, 462) the number of night-time interruptions to a healthy MLP infant group for the purpose of feeding have not been previously described. Irrespective of the cause, frequent interruptions to preterm infant sleep may impact the pathway of normal neurodevelopment, compound underlying medical conditions (463) and potentially trigger many conditions such as anxiety disorders, attention deficit hyperactivity disorder,(ADHD) and depression.(212, 283)This work lays the groundwork for potentially optimising sleep in the neonatal unit in the future.

This study also describes the normative sleep architecture of a large cohort of the healthy MLP infant group at 36 weeks post menstrual age (PMA) over a 12 hour period combining visual analysis of EEG, behavioural observation and physiological parameters of cardiac and respiratory well-being. This is the first study to provide an overview of overnight normative sleep architecture MLP infant group increasing the dept of knowledge on what we know about MLP infant's sleep. Previous studies by Han et al(266) Curzi-Dasclova et al (313)and Georgoulas et al(197) have produced data on the duration of sleep cycles and sleep states. Whilst these studies did utilise EEG they were of shorter duration with recording of 1 to 4 hours and had fewer infants. Other studies relating to sleep state identification by Mirmiram et al (464) and Foreman et al(310) did not use EEG and scored sleep state based on behavioural observation (video) alone which may not give reliable data. Our prolonged recording

with sleep scored by the gold standard of EEG +video should provide accurate and reliable normative data for this group.

Inter-burst intervals (IBI's) are quiescent periods of activity in QS, which normally occur in the EEG of premature infants and decrease in duration with advancing GA.(391) Initial assessment of EEG normality is frequently based on the duration of discontinuity and if it is within normal limits for the GA of the infant.(328) Previous studies have described IBI features of very preterm infants with a normal developmental outcome. However, often the neonatal clinical course of participants varied as did the criteria and cut off cut-off threshold values for the minimum and maximal amplitude(179, 328, 393) and duration of an IBI.(179, 260, 271)

Automated technology and machine learning provide an objective means of quantifying EEG features, making EEG interpretation faster to interpret at the cot-side (356, 465) especially when there is limited access to the expertise of a specialist neurophysiologist.(466)This study has adapted an algorithm previously developed for very preterm infants for use in the MLP infant group. Through automated methods, features of quiet sleep (QS) of infants with a normal developmental outcome at 18 months are described. This normative data can be used as baseline data for future studies of IBI analysis in this age group to predict outcome.

The relationship between sleep activity and healthy cognitive development is difficult to demonstrate based on a neonatal stay which varied from 3 days to 6 weeks, and relatively wide gestational age groups. However, birth during a critical period of development and period of accelerated brain growth outside the normal uterine environment (8) in itself substantiates a need for developmental surveillance.

This study also investigated the developmental outcome of this group of healthy MLP infants at 4 months and 18 months PMA in comparison to term born infants using the Griffiths III developmental assessment tool. Despite recent trends in research describing the developmental outcome of the MLP infant group, in terms of long-term sequela there remains a paucity of literature in comparison to studies specific to developmental outcome of the very preterm or the term hypoxic infant. Amongst research studies that have been published differences were highlighted between MLP and term born infants in relation to short term neonatal course and admission to neonatal unit.(107, 111, 467) Despite a benign neonatal course and a normal clinical exam we have shown that outcome of the MLP infant group differed significantly at 18 months from a comparative healthy term control (TC) group.

This study identified the strengths and weaknesses of the MLP infant group using the Griffiths III mental developmental score. (437) Whilst assessment of an infant or toddler may be an important predictor of outcome, it is not always predictive of school-age cognitive functioning, long term learning disabilities or academic achievement.(127) Many behavioural problems do not become evident until preschool and school age years.(87, 92, 93, 117, 128) which highlights the need for ongoing surveillance with advancing age.

7.3 Areas requiring further research

This observational study does not provide sufficient evidence to recommend changes to clinical practice. However, it does highlight the number of areas that warrant further research. These include but are not limited to:

- Greater utilisation of EEG in the neonatal unit
- The impact of feeding on the duration of sleep
- Screening and surveillance strategies for MLP infants
- Long-term developmental studies unique to the MLP infant group
- The impact of training of education professionals to increase awareness of deficits that may be associated with prematurity
- Correlating 36 weeks EEG with outcome in MLP infants
- Assessing the relationship between 36 weeks EEG and 4 months EEG in MLP infants
- Determine if IBI features are a maker of abnormal brain development

7.3.1 Greater utilisation of EEG in the neonatal unit

EEG provides an objective sensitive measure of brain activity and accurate reliable data pertaining to sleep in comparison to the subjective reported measures. Cotside education surrounding sleep state recognition on real time EEG, will assist clinicians in identifying sleep states and inform clinical decisions relating to routine interventions such as feeding, clinical exam or procedures. Knowing the infants sleep state may help reduce sleep cycle interruptions and avoid disturbances that can wait until infant is in indeterminate or transitional sleep. Evaluating the impact of such measures as sleep education on overnight sleep disturbances and duration is worthy of further research

EEG is an underutilised tool that is a valuable adjunct to neurological clinical exam. As all healthy MLP infants may not be followed up in a specialist neonatal clinic, EEG with automated analysis may be a useful pre-discharge screening tool which will assist

in identifying the presence or absence of normal features appropriate to GA and identify how the preterm brain has adapted to the extrauterine environment.(411) Early prognostic information is valuable to parents and the health care team in identify infants at risk and warrant close ongoing surveillance. The value of providing EEG as a routine pre-discharge tool for all categories of prematurity (very preterm, moderate or late preterm) within the neonatal unit warrants further exploration.

7.3.2 The impact of feeding on the duration of sleep

Whilst some immature infants may not have established feeds and require occasional passive tube feeding, the majority of infants were fed orally and not passively tube fed. For many healthy MLP infants, the duration of stay in the neonatal unit is dependent on establishing oral feeding. Feeding in the neonatal unit is time and volume based (generally 3 or 4 hourly or demand) to ensure adequate hydration and nutrition. Infants in our study who woke spontaneously and were demand fed, fed more efficiently over a shorter period. In the absence of clinical concerns, studies that challenge MLP infants over 35-36 weeks PMA to wake spontaneously and declare themselves as ready to feed rather continue with a 3 or 4 hourly regime feeding will provide further information on the impact of feeding on total sleep time and duration of stay.

7.3.3 Screening and surveillance strategies for MLP infants

The Child Health Screening and Surveillance (CHSS) programme in Ireland provides a free systematic, evidence-based assessment of all preschool and primary school children.(468) This service provides seven contacts with this health screening and development services commencing at birth to 4-5 years. However, there is currently little in the way of nationally guided follow-up strategies for at risk preterm infant

groups. Further research is required relating to all preterm infant groups which may inform decisions relating to standardising such strategies, screening intervals and protocols for preterm infant groups. Referral criteria and pathways of care also warrant review, with high risk infant categories prioritised.

7.3.4 Investigating long term outcomes of the healthy MLP infant

The performance of the MLP infant group in comparison to the TC group at both time points substantiates a call for further research. An early assessment at 4 months and 18 months is valuable in highlighting infants who may warrant an intervention, developmental assessments at this age is but not always predictive of school-age cognitive functioning, long-term learning disabilities or academic achievement.(127) Assessment at five years of age and older will have a greater association with challenges which persist into adult life. (105, 139) Larger longitudinal research studies incorporating sequential assessments will inform the Department of Health and the Department of Education on budgetary requirements to provide resources required to support preterm infants. Without the resources to support and treat prematurity-related cognitive, motor and behavioural disabilities, assessment is of little benefit.(3)

7.3.5 Training and engagement with educational professionals

Investment in training for educational professionals of the potential challenges faced by children and adolescents with preterm birth histories. Future research may include identifying practices to support active engagement of educational professionals and parents which will contribute to the early identification of the child with learning difficulties. Identifying the early signs of learning difficulties with timely and effective

early intervention targeted therapies will have the greatest positive impact on children affected by impaired learning.

7.3.6 Correlating a 36 week EEG with neurodevelopment in the MLP infant group

Quantitative analysis of EEG has been used to predict outcome. Studies have shown that infants with abnormal EEG background activity are at high risk of neurological disorders. Detailed quantitative analysis of EEG, including features of both AS and QS of the MLP infant can provide valuable information about the development of infants. Sequential EEG's may track the trajectory of development and identify infants that may benefit from targeted therapies.

7.3.7 Assessing the relationship between 36week EEG and 4month EEG in MLP infants

At 4 months infants are in the early stages of development and their brains are highly plastic implying an infant's development is highly sensitive to experiences and environmental factors. Further research comparing features of a normal EEG at 36 weeks to that at 4 months will not alone determine normality but also may identify infants with an altered trajectory of development. Quantitative analysis of multiple features at 36 weeks may be predictive of EEG at 4 months which may also be predictive of neurodevelopment.

7.3.8. Determine if IBI features are a marker of abnormal brain development

Further research on IBI features is warranted (perhaps increasing the number of features) over prolonged periods and inclusive of infants with a normal and abnormal developmental outcome. This will assist in identifying specific features that are

associated with adverse outcome. Further study will also provide a better understanding of the underlying neural mechanisms. This information may be used to develop interventions which may protect the immature preterm infant brain from exposure to various stressors in a neonatal unit.

7.4 Strengths of this thesis

This study follows MLP infants from their time in the neonatal unit through their developmental follow-up at 4 months and 18 months. The detail of the study (Methods, Chapter 2) ensures it is readily reproducible which will help confirm the validity of the results and confidence in this study's conclusion. The study design allows for the observation of changes that occur over time providing a more comprehensive understanding of the developmental outcome of healthy MLP infants in comparison to the healthy TC infant group.

Homogeneity was a strength of this study. A high level of thoroughness was used in phenotyping this large group of MLP infants under study. This precision assisted in minimising measurement error whilst supporting the identification of subtle relationships between variables under study and outcomes of interest. All MLP infants were clinically stable, with no underlying medical or neurological concerns on discharge. The TC group were also healthy without a medical condition that warranted ongoing specialist care and hence considered to be at low risk of developmental sequelae.

All EEGs in the neonatal used were done by the researcher and monitoring was carried in a quiet area of the neonatal unit (which reduced stimuli experienced by participants) over a minimum 12 hour duration. Meticulous recordings ensured EEG's had low impedances and staff contacted the researcher if any concerns with regard to EEG leads or excessive artefact displayed. Post-acquisition all EEG's had sleep states scored by the same researcher based on background EEG activity, video observation and ECG / respiratory monitoring. Poor quality EEG's (i.e. with excessive artefact due to lead displacement) were excluded from the total sleep time analysis carried out (Chapter 4).

Conscious efforts were made to maintain communication with parents which included written feedback from developmental assessments, birthday cards on first birthday which there is little doubt contributed to 75% of infants active in this study at 18 months.

Developmental assessments of both groups were carried out by a small group of ARCHID, trained assessors at the same timepoints (i.e. 4 months and 18 months PMA). To reduce potential interrater variability, assessors updated their training before the commencement of this study. The time of assessments was coordinated with the mother and the infant's normal sleep-wake daytime pattern. This aimed at ensuring infants were not overly tired or hungry on assessment.

All scores recorded in developmental assessments were input meticulously to the study data base. These scores were audited by the organisational monitor to confirm accurate data entry. The results of this study provide important information of the

potential impaired domains of development of this 'low risk' MLP infant group in comparison to a full-term healthy control group

7.5 Limitations of this thesis

Firstly, this study was limited to the clinically stable MLP infant in the neonatal unit to the exclusion of comparative group of MLP infants with a complicated journey through the neonatal unit that may require specialist follow or up. It also excluded healthy MLP infants cared for by their mothers on the postnatal wards. A case for selection bias could be presented in the TC group which recruited healthy term infants from singleton pregnancies only. Both of these factors limit the generalisability of this study's findings.

A further limitation was that EEG monitoring was performed primarily during night time hours only, so daytime sleep was not analysed. Whilst monitoring was carried out in a quiet place in the neonatal unit we did not measure sound or light which may be considered confounding factors. As we wanted to minimise the number of electrodes used we did not use EMG or EOG electrodes but video assisted in identifying facial movements and REM activity. Clinical load did not always allow for infants (who were feeding well) to wake spontaneously for all feeds. We could not control the level of experience the parent/ nurse had in terms of feeding technique and supporting the infant in returning to sleep.

Whilst we conducted a comprehensive EEG at 4 months with the same precision, we did not use the data in this thesis. Sleep spindles as a feature of non-REM sleep(469, 470) were annotated by the researcher on all EEG's and those of the comparative group of TC infants in this study. However, these annotations will serve as an

invaluable resource for a further sleep analysis comparing MLP and TC groups by Ventura et al which is currently underway. (470) Future research may relate to the identification of features at 4 months that predict long term outcome

A further limitation was that assessors of Griffiths MDS were not blinded to the birth GA of participants hence there may be a bias in scoring the performance of infants of a lower birth GA.

Ideally, sequential monitoring throughout a neonatal stay may be considered the gold standard however, without resources this is merely aspirational. Timely use of EEG will identify infants with immature sleep organisation and require close ongoing surveillance.

7.6 Personal reflections

This reflection is based on my personal experience throughout this journey from the study design to the completion and publication of the results of this thesis.

I started this journey with timelines displayed on impressive colour coded highly organised wall charts allowing some extra time for the odd pot-hole I may encounter along the way. Who could have possibly predicted a global pandemic and the massive diversion it caused to many research projects including this study.

I believe there is no book that can explain the personal *buy in* that is necessary to complete a PhD. What I take away from this journey is so much more than its content. Whilst I undoubtedly take away a high level of knowledge, I also take away greater critical thinking skills. I have gained valuable research skills including experience in

project management, time management, academic writing and working independently. In addition to these academic skills and knowledge I have also experienced personal growth and development. I believe completing this thesis is a significant personal accomplishment that will have a lasting impact on my life.

I also take away from this experience nothing but admiration and respect for the researchers of this world. I also know I will always be involved in research for the remainder of my career.

7.7 Concluding remarks

EEG provides valuable accurate insights into brain activity however its interpretation requires expertise. A lack of resources that support expert timely interpretation is a deterrent to the use of EEG in many neonatal units. I suggest this has led to a hesitancy to engage with EEG technology, limiting its use to critically ill infants.

With advances in technology machine learning has provided automated 'add-ins' built into the EEG hardware such as seizure detection algorithms. These algorithms immediately alert the clinical team to seizure activity, providing prompt treatment and ultimately reducing the seizure burden. This current and future application of artificial intelligence (AI) may incorporate analysis of multiple normal and abnormal EEG features reducing the expertise required by human interpreters. This makes routine pre-discharge EEG a powerful, non-invasive tool and potential biomarker of developmental outcome and possibly more acceptable and usable in the neonatal community. On-going research incorporating machine learning is the way forward. With the buy in from organisations and resources available a pre-discharge EEG on

'an opt- in' basis could be offered to parents prior to discharge. With the buy in from organisations and resources available a pre-discharge EEG on 'an opt- in' basis could be offered to parents prior to discharge. Currently pre-discharge EEG's conducted over one sleep cycle may be a starting point for introduction of this service which may be interpreted by experts and highlight potential abnormalities. However, this would require resources.

Prematurity is a public health issue. Abnormal outcome is not limited to very preterm infants or infants with a complicated history from birth but all preterm infants are at risk of adverse development. Obstetricians need to adopt a high threshold for preterm delivery whilst parents and educators need to be aware of potential risks of adverse outcomes with any degree of prematurity. Surveillance and resources for infants born before term warrant greater government investment.

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Supplementary material re algorithm development

The algorithm to detect periods of inter-burst intervals (IBIs) in quiet sleep was developed and tested on the EEG of preterm infants with a gestational and postmenstrual age less than 30 weeks. During testing with the older moderate-to-late preterm (MLP) cohort, we found that applying a pre-processing high-pass filter improved detection performance of the algorithm.

The pre-processing high-pass filter was designed using a Butterworth filter with an infinite-impulse response. Two parameters, cut-off frequency and filter order, were selected from a brute-force two-dimensional grid search. This grid extended from 1, 2, ..., 8 for filter order and from 0.2, 0.3, ..., 1.2 Hz for cut-off frequency. Performance was assessed using Matthew's correlation coefficient (MCC) in a leave-one-baby-out cross validation across the 12 epochs. MCC was used to account for class imbalance between ratio of the IBIs and burst activity. After applying the high-pass filter, the IBI detector was applied to the pre-processed EEG. Testing performance of this modified algorithm was evaluated using the left-out-baby.

Selection of filter parameters

The algorithm's performance improved when applying the high-pass filter before detecting the IBIs: median (95% confidence interval) MCC without the filter was 0.593 (0.468 to 0.752) versus 0.776 (0.638 to 0.824) when the filter was applied, $p < 0.001$ ($n=12$). The most common parameters selected during the grid search was 0.9Hz for filter cut off, selected 9/12 times, and filter order of 1, selected 12/12 times. Figure S1 shows the performance grid for 1 iteration.

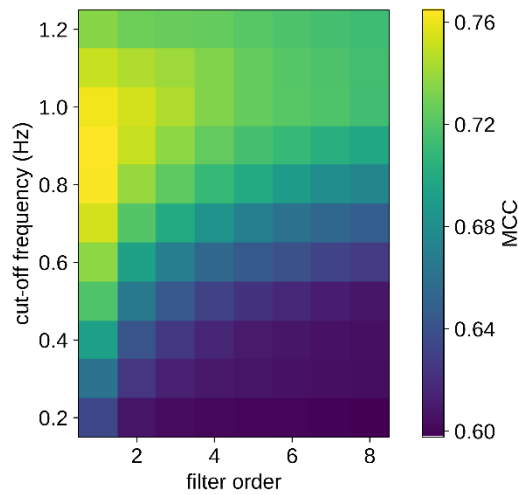


Figure S 1 Performance of the IBI detection algorithm dependent on 2 parameters of a pre-processing high-pass filter. Best filter parameters in this example are a 1st order filter at a cut-off frequency of 0.9 Hz. This figure shows 1 iteration of a 12-fold cross validation process to find the best parameters. MCC: Mathew's correlation coefficient.

Algorithm Implementation

All EEGs were down-sampled from 512 Hz to 64 Hz. An anti-aliasing filter, with a cut-off of 30Hz, was applied before down-sampling. The modified algorithm was applied to all EEG records using the high-pass filter parameters selected from the grid search. The 5 IBI metrics, detailed in the previous paragraph, were used to capture the temporal organisation of IBIs across the QS epoch. Each metric was generated on a 5 minute segment of EEG, with a 50% overlap, for each channel. The median value of all segments, and then over all channels, was used to calculate the metric per QS epoch.



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Clinical Research Ethics Committee

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Our ref: ECM 4 (w) 09/05/17

11th May 2017

Professor Geraldine Boylan
Professor of Neonatal Physiology
INFANT Research Centre
2nd Floor
Paediatric Unit
Cork University Hospital
Wilton
Cork

Re: Sleep architecture development of mid to late premature infants (32-36+6 weeks gestation) and correlation with neurodevelopmental outcome.

Dear Professor Boylan

This application will be reviewed subject to receipt of the following:

- **Revised Parent Information Leaflet:** Add name and contact details of Chief Investigator.

The following documents have been approved:

- Cover Letter dated 10th April 2017
- Application Form signed 11th April 2017
- Study Protocol
- CV for Chief Investigator
- Insurance Details
- Consent Form Version 1 dated 4th April 2017.

I note that the co-investigators involved in this project will be:

- Mary Anne Ryan, Research Nurse, Professor Eugene Dempsey, Dr Sean Matheson, Research Support Officer, Dr Andrea Pavel, Clinical Research Fellow, Anne Marie Cronin, Physiotherapist, Leanna Fogarty, Psychologist, Deirdre Twomey, Psychologist, Dr Peter Filan, Consultant Neonatologist, Dr Liam O'Connell, Consultant Neonatologist, Dr Brendan Murphy, Consultant Neonatologist, Ita Herlihy, Research Nurse, Claire O'halloran, Research Nurse and Darina Sheshan, Research Nurse.

Yours sincerely

Professor Michael G Molloy
Chairman
Clinical Research Ethics Committee
of the Cork Teaching Hospital

The Clinical Research Ethics Committee of the Cork Teaching Hospitals, UCC, is a recognised Ethics Committee under Regulation 7 of the European Communities (Clinical Trials on Medicinal Products for Human Use) Regulations 2004, and is authorised by the Department of Health and Children to carry out the ethical review of clinical trials of investigational medicinal products. The Committee is fully compliant with the Regulations as they relate to Ethics Committees and the conditions and principles of Good Clinical Practice.

Ollscoil na hÉireann, Corcaigh - National University of Ireland, Cork.



The 'Sleepi' study

Parent Information Leaflet

Introduction

My name is Mary Anne Ryan, and I am conducting a study called '*Sleepi*' (monitoring Sleep through EEG in Premature infants). I am a midwife and paediatric nurse who has worked in this neonatal unit for many years. I am now involved in research within the unit. This study I am conducting is looking at sleep in the premature infant and developmental outcome.

This study is focused on the sleep patterns of healthy premature infants born between 32- and 36+6-week's gestation. Sleep plays a huge part in the growth and development of your premature baby. Your baby was born early and some of his/her body systems are immature. The brain is one such area. We want to know more about the brain activity of the premature infant during sleep and how the brain waves of your premature baby develop as they get older.

How will I do this?

To obtain this information I will firstly need your permission for your baby to participate. Your consent is completely voluntary and you are under no obligation to part take. Your Clinical Research Ethics Committee decision not to be involved in this study will not affect the care you will receive. You are also free to withdraw from the study at any point.

We will need to access your medical notes and your baby's medical notes to obtain details your past medical and obstetric history and your overall health. We will also require information about your baby's delivery and any medical issues that may have occurred when in the neonatal unit.

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I will monitor your baby's brain activity by carrying out an electroencephalogram (EEG). The EEG is a reading of your baby's brainwaves which we measure by applying a number of soft pad type sensors to your baby's head. This is a painless procedure and will not distress your baby, in fact the greater majority of babies will sleep throughout the process.

During this EEG test we are specifically trying to record your baby's sleep patterns. Premature infants have their own unique brain wave patterns. Through this study we will identify the EEG pattern of your baby and

others born at similar gestation (32 to 36+6 weeks gestation) and compare patterns of all babies who partake. You will see the brain waves converted into wavy lines on a computer screen alongside your baby.

When will it be carried out?

If you consent to your baby being involved in the study, the first EEG (electroencephalogram) recording will be made while your baby is in the neonatal unit. We would like to carry out this EEG when your baby is 36 weeks gestation or prior to discharge. This will be carried out overnight, ideally over 12 hrs in the unit. This allows the research team to look at your baby's brain waves over a prolonged period.

Is this the end of my involvement in this study?

No, you will be contacted and your baby will be followed up at 4 months (corrected age). At this time, we ask that you to bring your baby to Cork University Hospital to the INFANT research centre, baby sleep lab where a further EEG will be carried out. We are aiming to conduct this EEG when your baby is sleeping and will work it around what suits you and your baby's nap time. Your infant (s) will be weighed with length and head circumference also measured. A developmental assessment will be carried out which will take approx. 30 minutes.

We would also like to monitor your infants developmental and carry out a second assessment when your infant is 18 months corrected age. We will contact you about this follow up nearer the time.

Will you tell me if there is a problem?

If there are any concerns at any stage of the study about your baby's EEG, your infant's growth and development you will be informed and referred to the appropriate medical team. You will receive a copy of the results of your infant's developmental assessment by mail within 2-3 weeks of assessment.

What happens to the information and recordings collected?

All information collected about you and your baby will be stored in a secure web-based database. The information will be coded and any information that could identify you or your baby will not be stored on the database. EEG recordings will be stored in the INFANT Research centre in Cork University Hospital. Initially EEG's will be analysed by research staff based in the INFANT centre. We will publish results from the study in International medical journals. In the future, researchers and commercial companies may apply to the study to access and use the anonymised data and samples collected for related research projects. There will be no financial gain from this request.

Confidentiality

The '*Sleepi*' study will be managed by a team of health care professionals. Your identity and the identity of your baby will remain confidential at all times. Neither your baby's or your name will be published or disclosed to anyone outside the research team. With your permission we will keep your family doctor up to date with any relevant health issues should they arise.

Statement of approval

The '*Sleepi*' study has been granted ethical approval from the Cork University Hospitals Research Ethics Committee. We have the necessary insurances as required by law.

For further information

Should you have any further questions or require any further information pertaining to the study please do not hesitate to contact Professor Geraldine Boylan Cork University Maternity Hospital ph: 0214920509 or email g.boylan@ucc.ie: or maryanne.ryan@ucc.ie

Parental consent form***The 'Sleepi' study***

Please indicate Yes or No to each of the following	Yes	No
I have read the information leaflet about this research and have been given a copy to keep. The information has been fully explained to me.		
I am aware that participation is voluntary and I may withdraw consent at any time. I am aware that my decision not to participate or to withdraw at any stage will not affect the treatment my baby will receive, or restrict my access to health care services normally available to my baby.		
<ul style="list-style-type: none"> • I agree to an overnight EEG being performed on my baby prior to discharge from the neonatal unit • I agree to a repeat nap EEG at 4 months corrected age • I agree to a developmental assessment being carried out at 4 months and 18 months corrected age 		
I understand that research team and ethical committees may look at my baby's or my maternal medical (labour and delivery) notes for research and/or audit purposes.		
I agree that my GP or other specialist doctor will be contacted if any health issues arise for my baby and that a copy of my baby's neurodevelopmental assessment will be sent for information purposes.		
I give permission for my baby's EEG and information collected about my baby to be stored for possible future research <u>unrelated</u> to this study without my further consent being required and subject to approval by a research ethics committee.		
I have been assured that all information collected pertaining to me and by baby will be kept confidential. I understand that all information will be coded and the results of this study will be published.		
I agree that I can be contacted to arrange a follow-up of my baby's progress.		

Signed: _____
Name of research participants parent

Date: _____

Signed: _____
Name of research participants parent

Date: _____

Signed: _____
Name of person taking consent

Date: _____

An Introduction to Neonatal EEG

Mary Anne Ryan, RGN, RSCN, RM; Sean Mathieson, PhD; Eugene Dempsey, MD; Geraldine Boylan, PhD

ABSTRACT

Newborn care has witnessed significant improvements in survival, but ongoing concerns persist about neurodevelopmental outcome. Protecting the newborn brain is the focus of neurocritical care in the intensive care unit. Brain-focused care places emphasis on clinical practices supporting neurodevelopment in conjunction with early detection, diagnosis, and treatment of brain injury. Technology now facilitates continuous cot-side monitoring of brain function. Neuromonitoring techniques in neonatal intensive care units include the use of electroencephalography (EEG) or amplitude-integrated EEG (aEEG) and near-infrared spectroscopy. This article aims to provide an introduction to EEG, which is appropriate for neonatal healthcare professionals.

Key Words: amplitude-integrated electroencephalography, electroencephalography, neonatal intensive care unit, newborn

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The electroencephalogram (EEG) characterizes and quantifies the electrical activity of the brain and serves as a unique marker of neurological well-being in adults, children, and newborn infants. EEG is the noninvasive real-time monitoring of brain function recorded continuously using a standard bedside/cot-side monitor.¹ With the advent of neuroprotective therapies for newborn infants such as therapeutic hypothermia, its use has gained importance in the neonatal intensive care unit (NICU).² While many NICUs use a more limited version of EEG called the amplitude-integrated EEG (aEEG), conventional EEG (cEEG) with simultaneous video recording remains the gold standard for neuromonitoring in the neonatal unit, providing information about the overall functional integrity of the brain with simultaneous recording of behavior.^{3,4}

The aim of this article is to provide a general introduction to EEG monitoring for neonatal healthcare professionals. We first discuss the clinical utility of EEG and the differences between conventional EEG and amplitude-integrated EEG. Standardized systems of EEG application have been described as well as an introduction to general terms used to describe the features of EEG.

UNDERLYING NEUROPHYSIOLOGICAL PROCESSES OF EEG

An understanding of the neurophysiological processes that underpin EEG is a prerequisite to understanding its use. Neurons are structural and functional units of the central nervous system.⁵ The function of neurons is to receive and transmit information to other neurons, muscles, and glands. Millions of neurons form synapses with other neurons, leading to trillions of connections between cells creating a sophisticated information

processing network within the central nervous system.⁶ Neurons communicate through an electrochemical process, and the normal function of neural networks is determined by a balance between excitatory and inhibitory synaptic input.⁷ Electrical inputs are received via the dendrites into the cell body and if strong enough will trigger an all or nothing signal, the action potential, which will travel down the axon to the terminal buds of the neuron.

EEG is a measure of the summed synchronous electrical activity generated by populations of neurons (pyramidal cells) in the more superficial layers of the cortex.^{8,9}

NEUROMONITORING—THE CLINICAL UTILITY OF EEG IN THE NEONATAL UNIT

Neonatal EEG is most frequently used for diagnosing and managing seizures. Neonatal seizures are caused by sudden, abnormal, and excessive electrical discharges in the brain and are often the sign of a more serious underlying condition. A large proportion of neonatal seizures do not have any associated clinical signs and are only seen on EEG.¹⁰ As a result, continuous brain monitoring is essential to accurately diagnose seizures, to determine the area of the brain where the seizure originates, to assess how the seizure propagates, and to determine how frequently they occur and their duration. Seizures are associated with a significant risk of mortality and poor long-term neurological outcome.¹¹ EEG can also assess the severity of newborn encephalopathy^{12,13} and is also very useful for preterm infants at risk of brain injury.¹⁴

Features of the neonatal EEG are uniquely associated with gestational age (GA).^{9,15,16} The presence of rhythms and activity outside the normal range for GA can be associated with long-term adverse developmental outcomes.^{17,18} EEG differentiates between levels of consciousness and provides a unique overview of sleep state organization and an opportunity to gain further insight into the complex nature of sleep. The EEG can accurately identify disorganized and dysmature sleep-state organization of newborn infants.¹⁹ Sequential EEG recordings of healthy preterm and term infants show the alterations that occur with maturation and advancing GA.^{20,21}

Amplitude-integrated EEG (aEEG) is derived from conventional EEG and is a simplified version that uses a limited number of channels.² The EEG signal is filtered, processed, and displayed in a time-compressed scale producing a representation of activity based on the amplitude of the EEG waveforms (see Figure 1). aEEG provides an easily interpretable visual trend of EEG activity and sleep-wake cycling.²²

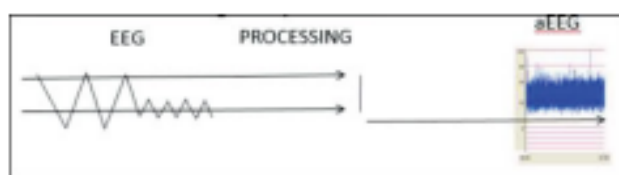


Figure 1. Raw EEG signal that is filtered, rectified, processed, and displayed on a time-compressed scale. This figure is available in color online (www.jpnnjournal.com).

Research findings support the prognostic value of aEEG in assessing general neurological well-being and in determining the long-term outcome of both the term and preterm infants.^{23,24} However, its use for neonatal seizure detection is more limited.^{4,25}

Although EEG has been recommended as the gold standard for identifying neonatal seizures by the World Health Organization and the American Neurophysiology Society, it is not consistently used in neonatal units. In a survey of neonatal units ($n = 25$) in Canada, 92% of neonatologists reported using continuous EEG; however, significantly more neonatologists favor the use of aEEG alone or in combination with cEEG.²⁶ A survey of neonatologists in China ($n = 251$) found the only 64% used EEG and 78% used aEEG. A UK survey of NICUs ($n = 57$) reported that only 4% did not have access to some form of EEG monitoring (aEEG or conventional EEG), with 46% having access to an out of hours formal EEG monitoring.²⁷ An Irish national neonatal unit survey ($n = 18$) reported that 5 units used aEEG, which included all level 3 neonatal units and 75% of level 2 units ($n = 4$).²⁸ Across all surveys, barriers to implementing EEG in neonatal units included cost, access to technology, and lack of formal training.

STANDARDIZED ELECTRODE PLACEMENT OF EEG ELECTRODES

The EEG is measured using electrodes or sensors attached to the scalp at various predefined spatial points and displayed as channels of activity that provide information about underlying brain activity. This activity is amplified, and digitized and displayed continuously on the computer screen of an EEG machine. Electrodes are identified by symbols (generally 2-3 symbols consisting of 1 or 2 letters and one number). The first symbol is the first letter, which reflects the underlying brain region (eg, F for frontal and C for central), and the second is a number also indicating its exact position and hemisphere (odd numbers on the left hemisphere and even numbers on the right hemisphere).²⁹

To ensure standardization of EEG electrode placement, recordings adhere to an international electrode placement system referred to as the 10/20 system.

This system serves as a reference map for electrode placement and the distance between each electrode is proportionate to skull size and shape and covering all underlying brain regions.³⁰ The 10 and 20 system represents either 10 % or 20% of the distance from landmarks of the head³¹ and typically involves the placement of a set of 21 recording electrodes (see Figure 1).^{9,32} Each EEG channel commonly displays the difference in electrical potential between 2 electrodes (bipolar). The bipolar channels are displayed, either in chains from anterior to posterior or transverse from one side of the head to the other and are called montages (see Figure 2).

Electrode placement for neonatal EEG recording

A multichannel assessment of neonatal EEG is required to provide spatial information about EEG activity in different regions of the brain. Considering the size and surface area of the neonatal head and the need for minimal handling, a modified version of a standardized 10/20 system is recommended for neonates (see Figure 2).

Despite a reduction in electrodes, this placement design has been identified as sufficient for the assessment of background EEG activity and the detection of seizures in neonates.³³ Reduced electrode neonatal montages generally contain both anteroposterior and transverse elements, and there may be institutional variations in how montages are configured.^{33,34} A typical neonatal montage includes electrode placement at positions Fp3, Fp4, C3, C4, Cz, Pz, T3, T4, O1, O2, plus a reference (R) and ground electrode (G) placed behind the left ear (see Figure 2A). As per American guidelines a viable alternative to Fp3 and Fp4 is Fp1 and Fp2.³⁵

Amplitude-integrated EEG (aEEG)

Many centers that do not have access to conventional video EEG benefit from the use of aEEG. Electrodes used for aEEG monitoring may use a single channel (2 electrodes, 1 on each side of the head, eg, C3-C4 central electrodes) or 2 channels (4-electrode placement with 2 at either side of the head, eg, Fp3-C3 or Fp4-C4) (see Figure 2B). A viable alternative of Fp1 and Fp2 in may also be used instead of Fp3 and Fp4 as per American guidelines.³⁵

APPLICATION OF EEG METHODS

Obtaining a technically satisfactory EEG is critical to accurate interpretation.³⁵ Electrical activity generated must propagate through brain tissue, the skull and scalp to be recorded on EEG; hence, adequate preparation and technique is a prerequisite to accurate electrode placement.

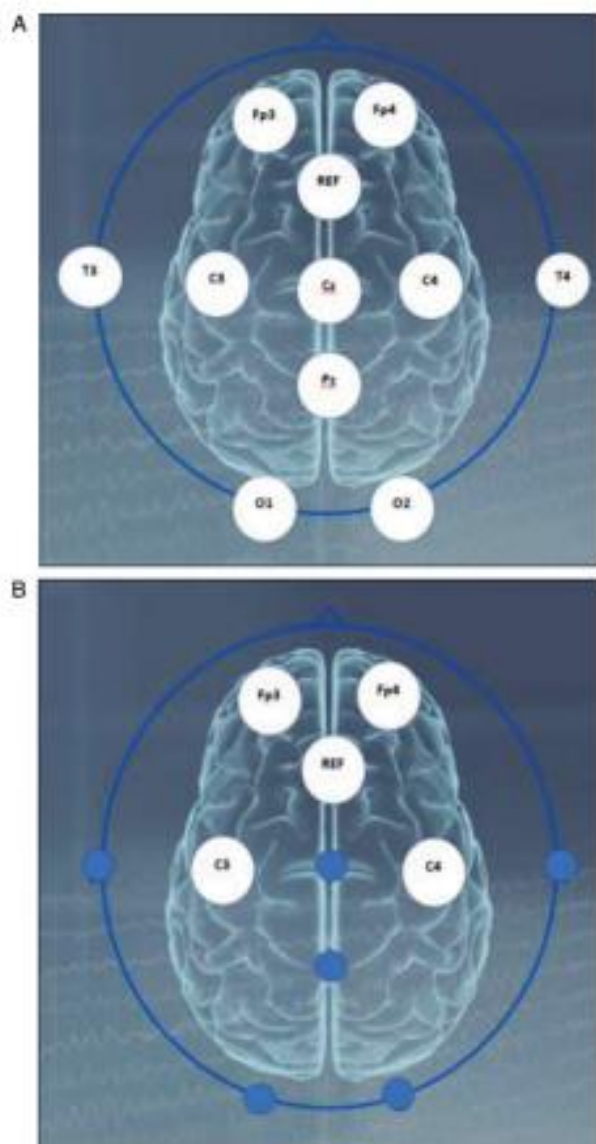


Figure 2. (A) Neonatal montage of electrode placement as per the modified version of the 10/20 system for neonates.⁹ (B) aEEG electrode positions. In both A and B, the left side mirrors the right side, with odd numbers on the left side of the head and even numbers on the right side. This figure is available in color online (www.jpnnjournal.com).

Flat disposable gel electrodes may be used on preterm and term neonates although institutions may also use cup-type electrodes.³⁶ Scalp sites for surface electrode placement are prepared by parting hair and rubbing the skin (3–4 strokes) with a sterile cotton tip stick and a slightly abrasive gel (such as Nu Prep gel), removing dead cells and debris from the scalp. Whilst electrode paste is a prerequisite for cup electrodes applying an appropriate quantity of conductive fixative

paste to the electrode before application will assist in reducing interference on EEG recording.³⁷ Adding a small amount of easily removable breathable medical tape (eg, Mefix) along with a well-fitting adjustable hat will reduce the risk of electrode displacement with movement and handling. The aim is to achieve and maintain a scalp electrode interface impedance of less than 10 k Ω on all electrodes throughout EEG recording. Regular checks of the EEG by trained personnel will ensure electrode placement is maintained and impedances remain low.

EEG monitoring generally also incorporates heart rate and respiration monitoring. Electrocardiogram (ECG) electrodes are applied to each shoulder to monitor the heart rate and to provide a reference channel to aid the identification of any ECG artifact. A micromovement sensor may be attached to the infant's abdomen to monitor respiratory activity.

SOURCES OF INTERFERENCE WITH THE EEG RECORDING

The EEG may be easily overwhelmed by unwanted biological or environmental signals or artifact.^{38,39} Sources of artifact include:

Physiological sources such as eye movement, tongue movement, sucking, head movement, muscle activity and sweat, ECG, and pulse. Infant response to loud noises, flashes of light, and handling for nursing or parental care can also be seen as general movement artifact on the EEG (see Figure 3).

Environmental sources include mains interference due to poor electrode contact with the scalp, surrounding interference from electrical devices such as oscillating ventilation incubators, and intravenous infusion pumps.⁹

Whilst environmental artifact may be minimized or removed, physiological sources such as movement, sweat, or ECG cardiac artifact are more difficult to control. Where possible sources of artifact are identified and removed or minimized to support accurate EEG interpretation.

VISUAL ANALYSIS OF EEG

Neonatal EEG is most accurately interpreted with knowledge of the infant's GA, medical history, and overall clinical condition.^{9,32} EEG interpretation also requires knowledge of recent medication use, some of which may alter background EEG activity and mask abnormalities.⁹ Accurate interpretation of the EEG requires many years of specialist training. This section provides a general overview of the most common features used to describe the EEG.

Features of EEG

As EEG features vary with degree of maturation, a visual analysis will determine whether the EEG pattern is appropriate for the GA. This is accurately interpreted through expert knowledge concerning specific rhythms, waveforms, and developmental features unique to GA.

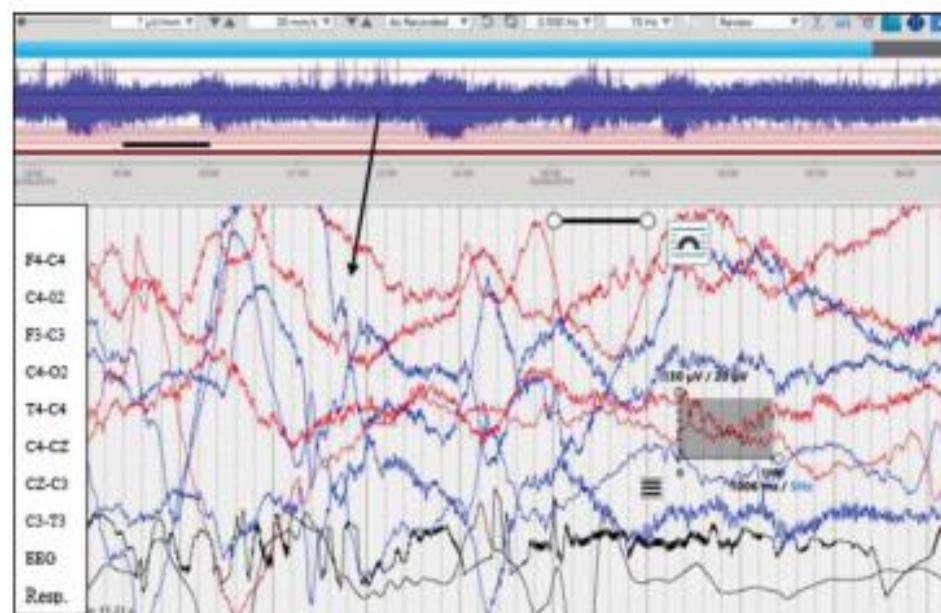


Figure 3. Movement artifact during feeding. The black line on aEEG indicates 1 hour of recording while the black line on EEG indicates 1 second of recording. This figure is available in color online (www.jpnnjournal.com).

To describe all of these features is beyond the scope of this article. Background or baseline EEG activity is described in terms of features of activity including frequency (Hz), amplitude (μV), continuity, and the presence or absence of sleep-wake cycling.^{40,41}

Frequency

The frequency of the EEG refers to the number of waves of activity seen per second. Frequency is measured in Hertz (Hz) and classified into 4 main bands: delta activity (the slowest frequency of 0.5-3.5 Hz—dominant frequency in neonates), theta (4-7.5 Hz per second), alpha (7.5-12 per second), and beta activity (12.5-30 Hz per second).⁴²

Amplitude

The amplitude of the activity seen on EEG is measured in microvolts (μV) and can be described as low (0-25 μV), moderate (25-75 μV), or high (>75 μV) (see Figure 4). Amplitude varies with GA, sleep state, and underlying conditions but may also be affected by artifact and/or caput or localized edema at the site of electrode placement or by the interelectrode distance.⁴³

Continuity

Continuity of activity refers to the degree to which background activity is continuous and showing an amplitude

of more than 25 μV ⁴⁴ (see Figure 5), or discontinuous with periods of activity of less than 25 μV ⁴⁵ (see Figure 4). Period of discontinuity is a normal phenomenon in the preterm infant; however, duration and patterns of discontinuity outside normal parameters may be associated with underlying pathology in the preterm term. Discontinuity is not normal on the healthy term infant EEG and its presence may signify an underlying pathology.

Sleep-wake cycling

Sleep state may be determined based on behavioral observation or visual analysis of the EEG. Sleep-state organization evolves with maturity, and fluctuations or cycling between sleep states (sleep-wake cycling) become evident from 30 to 32 weeks' GA.⁴⁶ Sleep cycling is also seen on the aEEG with quiet sleep showing a wider band, due to alternating high- and low-amplitude activity, than the narrower bands of active sleep and waking² (see Figure 6). However, the latter 2 states are not easily distinguishable on the basis of the aEEG alone.

Conventional EEG, however, provides a more detailed display of features of each sleep state. In modern EEG machines, the aEEG trend is also available and can be displayed above the conventional EEG display (see Figures 4 and 5). Sleep-wake cycling is a prerequisite to normal neurosensory and cortical development, supports the normal neurodevelopmental process, and is

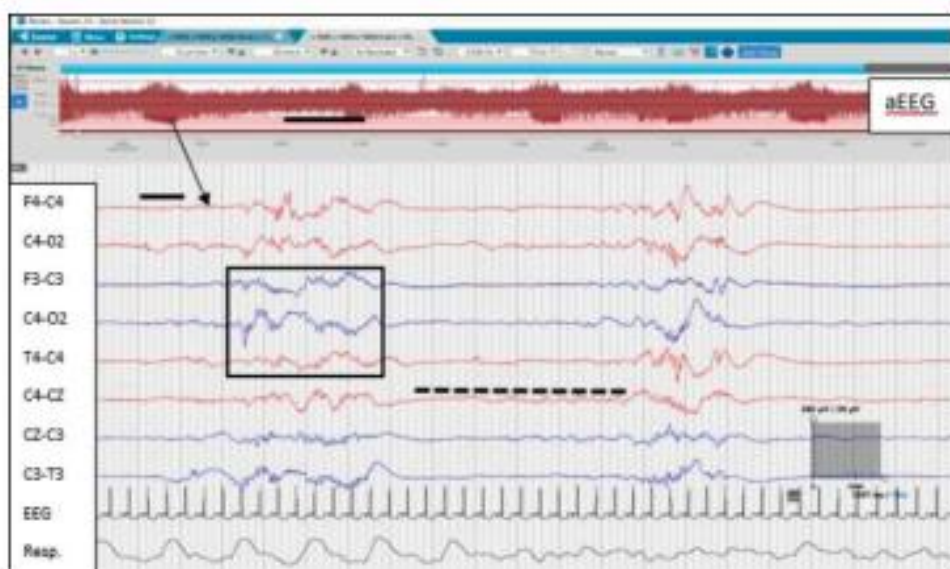


Figure 4. Periods of low amplitude or discontinuity (background activity <25 μV indicated by the black dashed line) seen on EEG channels in quiet sleep (of a preterm infant at 36 weeks' gestational age) with high-amplitude bursts of activity (ie, >75 μV) (indicated in the black box). The black line on aEEG indicates 1 hour of recording while the black line on EEG indicates 1 second of recording. This figure is available in color online (www.jpnnjournal.com).

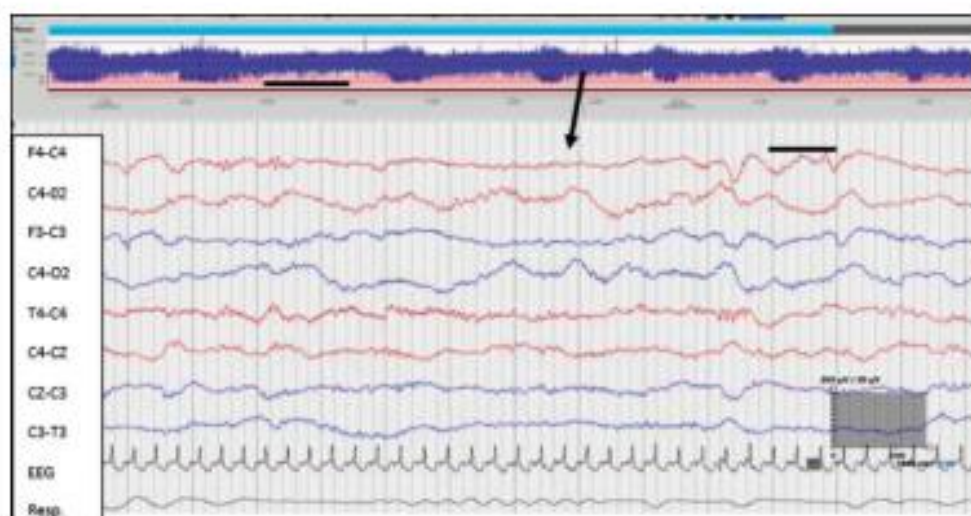


Figure 5. Period of continuous activity of mixed amplitude in active sleep. The black line on aEEG indicates 1 hour of recording while the black line on EEG indicates 1 second of recording. This figure is available in color online (www.jpnnjournal.com).

considered a surrogate marker for neurological well-being.^{47–49} Delayed cycling or disorganized sleep in term or preterm infants is associated with neurological dysfunction such as poor emotional regulation and cognitive development.^{46,48,50}

DISCUSSION

EEG is a safe and noninvasive tool providing real-time monitoring of brain activity.

EEG monitoring causes little if any discomfort to the infant as evidenced by the fact that many infants sleep through EEG application and removal. Despite being recognized as a very valuable tool for neuromonitoring in the NICU, training and education for healthcare providers is limited and the majority are self-taught with little formal training in EEG.^{28,51} Hence, organizational investment in EEG technology requires investment in specialist education and training.

Prolonged continuous monitoring is a prerequisite for adequate assessment of brain function.⁵³ EEG monitoring may challenge the resources of the NICU, as if providing a service supporting prolonged EEG monitoring, access to EEG technologists, and an expert neurophysiology team 24/7 is required to ensure accurate real-time interpretation of brain activity and prompt treatment if required.^{28,51} American guidelines recommend 24-hour EEG monitoring when screening for seizures and if detected to record for 24 hours after the last seizure seen.⁵⁵ Identifying seizure activity on EEG will potentially expedite treatment response and reduce the overall seizure burden.

The prognostic value of EEG lies in its ability to identify infants at risk of abnormal outcome and ensure intervention occurs early in life when the brain is most likely to adapt and learn. Effective early support may reduce the incidence or severity of adverse outcomes that may threaten a child's development

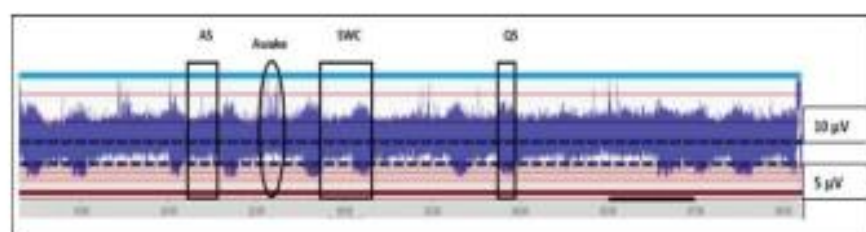


Figure 6. aEEG displaying sleep-wake cycling. A complete sleep-wake cycle. AS and QS are highlighted in rectangular boxes. Awake state in the circle. An upper amplitude of 10 μ V and a lower amplitude of 5 μ V are indicated by the dashed line. Black unbroken lines indicate 1 hour of EEG recording (9 hours displayed on recording). This figure is available in color online (www.jpnnjournal.com).

or improve the infant's life chances and overall neurodevelopmental outcome.⁵² For infants with hypoxic ischemic encephalopathy, EEG at 36 and 48 hours had the highest predictive value for an abnormal outcome.⁵³ EEG carried out at approximately 35 weeks post-menstrual age has been identified as a strong predictor of outcome at 2 years for infants less than 32 weeks' birth GA.⁵⁴

However, infants undergoing therapeutic hypothermia over a prolonged period and the very preterm infant with fewer skin layers have higher skin protection needs.^{54,55} Checking and recording electrode-skin contact site and frequent regular repositioning of the infant head will ensure skin integrity is not compromised at electrode placement sites.⁵⁶

Finally, irrespective of its clinical purpose, all technology surrounding an infant in the NICU may potentially limit a parent's physical proximity to their infant and parent-infant interaction. This may be further exacerbated by parental fear of the technology-rich environment. Cot-side parental education may reduce fear and encourage parental involvement in routine care.

CONCLUSION

We have provided a broad introduction to EEG monitoring and its clinical utility that we feel is relevant to all healthcare professionals. We have discussed the underlying neurophysiological processes and standardized practices associated with EEG. While skilled and trained personnel are required for the application, maintenance, and interpretation of EEG, it is important for all neonatal staff to be familiar with EEG and acknowledge its value in neonatal practice.

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Nocturnal sleep architecture of preterm infants in the NICU

Sleep is the main behavioural state of the preterm infant and a prerequisite for normal neuronal maturation and cortical development. Neonatal electroencephalography (EEG) is a useful bedside tool providing a unique view of sleep state organisation. This observational study describes the sleep architecture of mid to late preterm infants in a neonatal intensive care unit (NICU) setting. As the preservation of sleep supports neuronal maturation, routine overnight disturbances are recorded and quantified.

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Keywords

preterm neonatal EEG; sleep architecture; NICU; sleep interruptions

Key points

Ryan M.A., Mathieson S., Livingstone V., O'Sullivan M.P., Dempsey E., Boylan G.B. Nocturnal sleep architecture of preterm infants in the NICU. *Infant* 2020; 16(5): 209-14.

1. Disturbances of nocturnal sleep in preterm infants were frequent in the NICU, predominantly for feeding.
2. Infants who woke spontaneously, fed more efficiently within a shorter time-frame compared to infants who were disturbed from their sleep for feeding.
3. Optimising sleep in the NICU will support the normal trajectory of development.

Sleep is a prerequisite for neuronal maturation, normal growth, development, cognitive function and memory consolidation at any age but particularly during the accelerated period of brain development that occurs in the last trimester. Sleep architecture or sleep organisation in the healthy newborn infant is characterised by regular sleep-wake cycling (SWC), alternating between periods of active sleep (AS) (precursor of rapid eye movement sleep, REM), quiet sleep (QS) (precursor of non-rapid eye movement sleep, NREM), indeterminate (IS) or transitional sleep (TS) (showing features of both AS and QS) and wakefulness (W). Continuous video encephalography (vEEG) during sleep allows for the accurate identification of SWC.

Moderate to late preterm infants (MLP) are defined as infants with a gestational age between 32 and 36⁶ weeks at birth.¹ This single homogenous group are the largest and fastest-growing proportion of the preterm infant population and represent 75-80% of all preterm births. This study

aims to describe the nocturnal sleep architecture of MLP in the NICU and to quantify the number and purpose of SWC interruptions during this period.

Sleep architecture development

While both AS and QS are prerequisites for neuronal development, the contribution and role of each are likely to be different and unique to gestational age. AS is the dominant state in the MLP with the percentage of QS increasing with advancing gestational age.^{2,3} Sleep state organisation evolves with maturity during which fluctuations or cycling between sleep states support the normal neuro-developmental process and is considered a surrogate marker for neurological wellbeing.^{4,5} SWC patterns are clearly recognisable on EEG from 31 weeks' gestation⁶ although elements of differentiated states precede this time frame. Delayed cycling or disorganised sleep in the low-risk preterm infant is associated with neurological dysfunction such as poor emotional regulation and cognitive

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TABLE 1 Author details.

development.^{38,9} Sleep state may be determined based on behavioural analysis and/or visual analysis of EEG activity.

Behaviour sleep analysis

Behavioural sleep state analysis focuses on attributes of the infant, presenting a unique pattern of behaviour for each sleep state (TABLE 2).¹⁸ These states are readily observable and can be reviewed later if video is used with EEG monitoring.

Behavioural observations during sleep in a preterm infant are less obvious in comparison to the term born infant. Difficulty identifying the exact timing and onset of AS and QS may arise due to frequent body movements and rapid alterations between states in the preterm infant.^{11,12} A decrease in body movements and respiratory patterns becoming more regular reflect the preterm infant's neurological maturation.

Visual analysis of sleep on EEG

EEG provides an opportunity to gain further insight into the complex nature of sleep, particularly the microstructure. While interpretation of the complex features of the immature preterm EEG requires a specialist neurophysiologist, sleep state identification may be achieved with minimal cot-side training/education and is a useful marker of brain function and maturity.¹⁹

Prior to evaluation of the EEG, information pertaining to gestational age at birth, postmenstrual age (PMA), medication and physiological condition is crucial. Non-cerebral changes in the EEG may be due to artefact due to technical (eg rhythmic vibration caused by a high frequency ventilator) or physiological (eg sucking or movement related or muscle related or blinking)¹⁴ causes and may be sustained, transient or repetitive. Correct identification of artefacts will ensure accurate interpretation of recordings.^{13,18}

Modern EEG devices display both the raw EEG and a compressed EEG trend called the amplitude-integrated electroencephalogram (aEEG), as seen in FIGURES 1-4. The aEEG is derived from the raw EEG by filtering and processing the signal to produce a compressed representation of the maximum and minimum amplitude range of the EEG. The narrower band areas of aEEG represent the low amplitude high-frequency activity that occurs in AS and W and the wider band representing QS. Long-term trends or SWC over many

Active sleep	Quiet sleep
Eyes closed +/- rapid eye movements	Eyes closed. No rapid eye movements
Irregular Breathing (rapid and shallow)	Regular abdominal respiration
Variable heart rate	Regular heart rate
Reduced muscle tone	Muscle atonia
Facial grimacing, smiling, groaning, cry	Occasional startles, sighs or sucking movements
Occasional twitches, variable movement	More difficult to rouse

TABLE 2 Behavioural features of sleep.

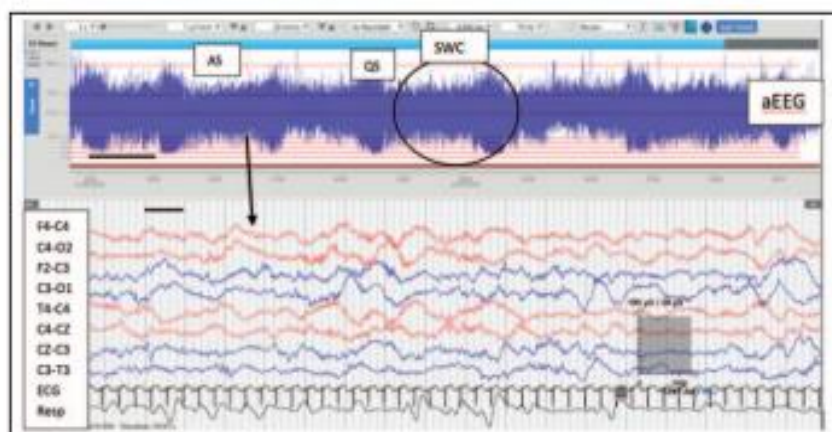


FIGURE 1 Active sleep. Infant born at 33⁺ weeks' gestation, recorded at 35⁺ weeks' PMA (day 16 of life). One complete SWC circled on aEEG. Raw EEG showing high frequency continuous activity of low to medium amplitude. Irregular respirations observed.

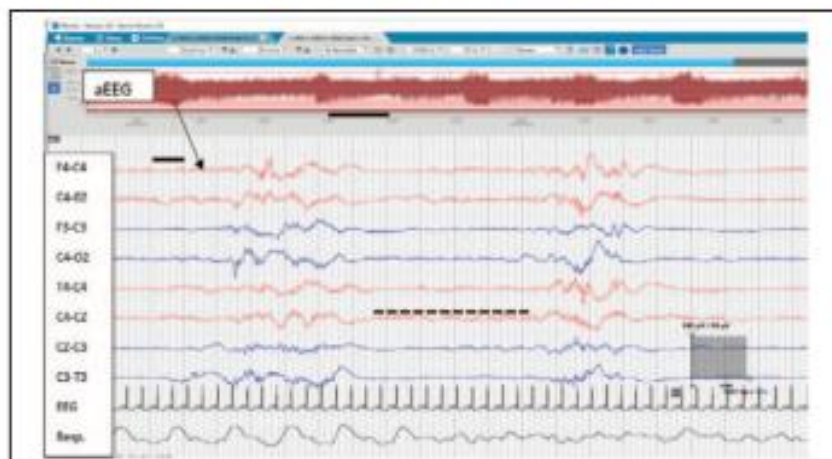


FIGURE 2 EEG recording during QS. Infant 36⁺ weeks gestation at birth and recorded at 36⁺ weeks' PMA (day 3 of life). The broken black line indicates the IBI duration. An IBI or period of discontinuity for 5 seconds is evident in and within normal range for a this gestational age infant.

hours are best seen on aEEG.

In the healthy MLP infant, EEG background activity varies and is dependent on sleep state and degree of prematurity.^{21,18} Knowledge of age-appropriate features of prematurity will assist in assessing sleep states on the EEG. In general terms, the EEG can be assessed within the following parameters:

Frequency of brain wave activity per second (Hz). The four main frequencies of

interest in neonates are: delta activity, which has a frequency of 4Hz or less; theta 4-7Hz; alpha 8-13Hz, and beta activity of 13-30Hz. Higher frequencies exist but are minimally present in neonatal EEG.

Amplitude or size of the waveforms. On the EEG amplitude is measured in microvolts (μ V). Amplitude may be described as low (0-25 μ V), moderate (as 25-75 μ V) or high (>75 μ V). In FIGURE 1 the amplitude of the recording during AS is

low to medium. In FIGURES 2 and 3 the amplitude in QS is low with intermittent bursts of high amplitude activity.

Continuity of background activity. This describes whether EEG activity is continuously present or is intermittent (discontinuous). The amount of continuity increases with advancing gestational age and is dependent on sleep state. From 32 weeks' gestation, continuous activity is evident in AS (FIGURE 1). In QS, discontinuity is seen on EEG of the preterm infant, which reduces with advancing gestational age (FIGURES 2 and 3).

Origin (location) of activity. For example, frontal, temporal, parietal, occipital or central.

Degree of synchrony and symmetry. The extent of synchrony (brain wave activity of similar type occurring simultaneously over both hemispheres) and symmetry (similar EEG amplitude and frequency content over both hemispheres) of EEG activity.

The EEG in AS in the MLP infant shows continuous low to moderate amplitude, higher frequency activity with frequent physiological artefacts due to movement and REM activity (FIGURE 1). In QS, periods of low voltage activity alternating with periods of higher amplitude activity (bursts) are seen (FIGURES 2 and 3). The period between bursts of activity is termed the interburst interval (IBI). In healthy preterm infants, the IBI decreases with advancing age as the EEG becomes more continuous.¹² The expected IBI for MLP is 10–15 seconds at 34 weeks' PMA and <10 seconds at 36 weeks' PMA. The black broken line on FIGURES 3 and 4 highlights the duration of the IBI.

Spontaneous activity transients are independent waveforms of activity, acknowledged as having distinct functional properties, evident on EEG and associated with particular gestational age.^{23,28} The most recognisable spontaneous activity of the preterm EEG are delta brushes, which are seen as slow delta waves (1–2 Hz) with an amplitude of 100–200 µV (peaking at 34–35 weeks' PMA) with superimposed fast or high-frequency alpha or beta activity.^{13,18} The peak frequency of this superimposed activity is 16–20 Hz between 32–35 weeks' PMA, falling to 10–15 Hz from 36 weeks' PMA.²⁹ Delta brush activity occurs in both AS and QS (more dominant in QS of the MLP) with the fast frequencies most frequently appearing in the ascending arm of the delta wave.²⁹ The presence of delta

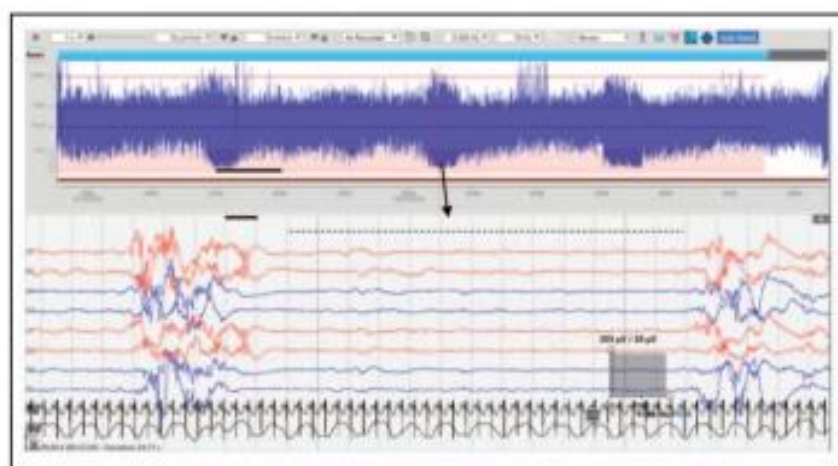


FIGURE 3 EEG recording during QS. Infant 33rd weeks' gestation at birth and recorded at 35th weeks' PMA (day 13 of life). A more immature EEG in comparison to that illustrated in FIGURE 2. A longer IBI of approximately 13 seconds is seen.

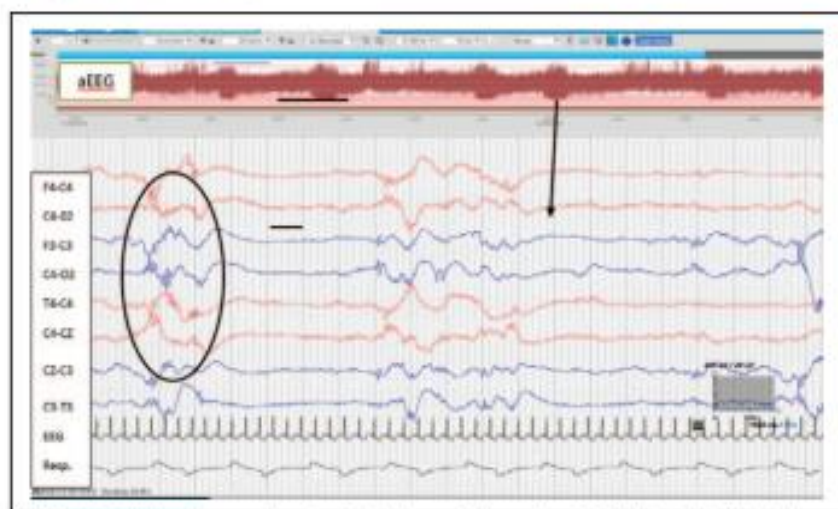


FIGURE 4 Delta brushes – a characteristic feature of the preterm EEG indicated in the black circle. Delta brushes are seen as delta wave activity (<4 Hz) with superimposed fast activity.

brushes is most evident between 32–35 weeks' PMA and disappearing by 38–42 weeks' PMA (FIGURE 4).²¹

The disappearance of delta brushes occurs in conjunction with the development of clear sleep state differentiation, suggesting an association with neuronal maturation and development.^{20,22} Other normal spontaneous transient waveforms of prematurity include: premature temporal theta; encoches frontales, and anterior slow dysrhythmia, as described by Pavlidis et al and Andre et al.^{13,18} The aim of this study was to identify and quantify SWC on EEG of a cohort of MLP infants and quantify sleep disturbances overnight in the neonatal unit.

Methods

This is a single centre observational study focusing on sleep patterns of healthy MLP

infants who required admission to the neonatal unit at Cork University Maternity Hospital (CUMH), which has a birth rate of approximately 7,500 babies per year. All infants with a gestational age of 32–36th weeks at birth, who were clinically stable with no actual or suspected congenital or chromosomal abnormality and no neurological concerns were eligible for inclusion in the study. Ethical approval was granted by the Cork Research Ethics Committee.

All EEGs were performed in a quiet area of the neonatal unit between 16.00hrs and 18.00hrs for a minimum of 12hrs. A reduced neonatal montage was used based on a standardised international 10/20 system of electrode placement proportional to skull size.^{23,28} Electrode positions included F3, F4, C3, C4, T3, T4, O1, O2, Cz, reference (R) and ground (G) with

ECG and an abdominal respiratory sensor was also applied. Impedances were $<10k\Omega$ before the commencement of EEG recording. All efforts were made to keep EEG artefact at a minimum for accurate interpretation of recording.

A SWC was defined as the commencement or onset of AS followed by QS (+/- IS) with cycling between states occurring until awakening. Sleep states were annotated by a research nurse (Mary Anne Ryan - MR) with extensive EEG experience and based on vEEG which combined typical infant behaviours and visual analysis of EEG activity. Annotations from a random sample of recordings were also independently reviewed by two neurophysiologists to verify sleep annotations by MR. SWC was quantified by visual inspection of the EEG and the duration of all sleep-wake states and feeding periods were annotated on each recording. As per unit policy, no routine procedures or examinations were carried out overnight and the neonatal unit standard of care was not altered throughout the monitoring. All recordings were reviewed by a neurophysiologist for any abnormal incidental findings. All recordings were then pseudo-anonymised before transfer to a secure server for long-term storage.

Statistical analysis was performed using IBM SPSS Statistics (version 25.0, IBM Corp, Armonk, NY, USA). Continuous variables were described using the median and interquartile range (IQR) and categorical variables using frequency and percentage. For infants who fed orally with ≤ 1 nasogastric tube (NGT) feed overnight, a random-effects model was used to investigate if the duration of oral feeding (feed given closest to midnight) differed by type of awakening (interrupted versus spontaneous). Type of awakening was a fixed effect in the model, subject was a random effect and an unstructured covariance was used. The Mann-Whitney U test was used to investigate if the duration of oral feeding differed by sleep state from which the infant was woken (quiet versus active). All tests were two-sided and a p-value of <0.05 was considered statistically significant.

Results

Informed parental consent was obtained for 117 MLP infants. Due to early discharge home or transfer to provincial hospital (14 infants), later exclusion criteria (two infants) or poor quality EEG

	Median (IQR)
Birth gestation (weeks)	34.1 (33.5-35.1)
Gestation at time of EEG (weeks)	36.0 (35.6-36.6)
Birth weight (g)	2,100 (1,868-2,378)
Weight at the time of EEG (g)	2,270 (2,123-2,503)
Postnatal age at the time of vEEG (days)	11 (5-17)
Sleep-wake cycles per infant per night	6 (5-7)
Range	3-11
	n (%)
Gender Male	52 (53.1)
Residence on EEG	
- Cot	79 (80.6)
- Incubator	19 (19.4)
Participants displaying SWC on EEG	98 (100)
Total number of sleep cycles recorded overnight	589 (100)
Total number of SWC interruptions	
- Clinical purposes	2 (<1)
- Feeding purposes	135 (22.9)

TABLE 3 Demographic data and overnight SWC recorded over a 12hr period (n=98).

(three infants), 98 overnight vEEGs were available for analysis. All participants were clinically stable at the time of the recording. Demographical data and overnight SWC of infants are shown in **TABLE 3**. The number of male and females were similar (52 males, 46 females) the majority of whom were nursed in a cot (80.6%).

SWC was identified on the EEG recording of all participants. The median (IQR) number of SWCs overnight was 6 (5-7) (**TABLE 3**). Of the total overnight SWCs recorded (n=589), 23.3% (n=137) were interrupted. On two occasions sleep was interrupted for clinical reasons. The remaining interruptions were exclusively for feeding and occurred during both AS (77.8%) and QS (22.2%).

Feeds were given either orally (78.8%) or via a NGT (21.2%, **TABLE 4**). On 68.8% (194/282) of occasions when infants were being fed orally, the infant woke spontaneously prior to the feed. For the remaining 31.2% (88/282) of occasions, infants were actively woken. When infants were fed via a NGT, they had their sleep

disturbed prior to the NGT feed on 61.8% (47/76) of occasions. For the remaining 38.2% (29/76) of occasions, infants were undisturbed and slept throughout the NGT feed (**TABLE 4**).

Based on the feed given closest to midnight, the duration of the oral feed was significantly longer when the baby was interrupted from their sleep compared to when the infant woke spontaneously (n=83). The estimated average feed duration was 24.13 minutes (95% confidence interval: 20.55 to 27.71) when the baby was interrupted from their sleep and 17.85 minutes (95% confidence interval: 15.38 to 20.31) when they woke spontaneously (difference in means: 6.28 minutes; 95% confidence interval: 2.22 to 10.35 minutes; p=0.003).

Further analysis investigated if the duration of feeding depended on the sleep state from which the infant was interrupted (AS versus QS). A total of 44 babies were included (n=34 in the AS group, n=10 in the QS group). Although the feeding duration was higher in the group woken from QS (median: 28.0; IQR: 15.8 to 40.8) than the group woken from AS (median: 22.5; IQR: 14.0 to 26.3), the difference between groups was not statistically significant (p=0.102).

Discussion

All MLP infants in this study displayed periods of AS, QS and W with SWC seen on all recordings.^{23,24} Interruption of overnight sleep was primarily for feeding purposes (both oral and NGT feeds) and equates to interruption of 23.3% of all overnight SWC. It is not unreasonable to assume an increase in this percentage of interruptions may occur during day-time hours when routine procedures and examinations are carried out. In a study carried out by Levy et al.,²⁵ vEEG monitoring of clinically stable near-term and term infants in the NICU (n=25) showed that provider contact occurred for 27% (65 minutes) of the four hours of recording time and only 50% of infants engaged in a full SWC prior to interruption for care. Similar to our study, the majority of infant contact/disturbances occurred during AS, a state which has a greater association with the growth and development of complex neural networks.^{23,24}

MLP infants frequently display disorganised feeding behaviours with difficulties evident in ability to co-ordinate 'suck, swallow and breathe' when feeding.²⁶

	n (%)
Total number of feeds given overnight	358 (100)
NGT feeds (n=76)	
Uninterrupted sleep	29 (38.2)
Interrupted sleep	47 (61.8)
Oral Feeds (n=282)	
Woke spontaneously	194 (68.8)
Interrupted sleep	88 (31.2)
Timing of interruptions (n=135)	
Active sleep	105 (78.8)
Quiet sleep	30 (21.2)

TABLE 4 Overnight feeding interruptions.

This factor is reflected in this study as 21.2% of feeds were given via NGT (n=76). Feeding ability may be further diluted by inadequate sleep in the NICU impacting clinical condition, growth and development, neurobehaviour and the establishment of oral feeding.^{20,21} Sleep disturbances for routine care in the NICU affects brain maturation,²² hence in the absence of clinical need, disturbing sleep for routine care when the infant is not actively feeding warrants further research.

In the absence of specific direction, the mode, frequency and schedule of feeds and sleep interruptions are very much guided by the bedside nurse and relate to feeding volumes taken/tolerated. Longer feed duration, as reported here when sleep is interrupted, has a knock-on effect on human resources, duration of stay, cost to the healthcare provider and warrants further consideration. Allowing a growing preterm to wake spontaneously for feeding will ensure little interruption to the natural course of a SWC, which may positively impact neurodevelopmental outcome. However, further research is warranted in relation to the impact of this practice on nursing resources, parental visiting and overall health and wellbeing of the infant.

A healthy *in utero* environment provides optimal conditions for normal central nervous system development.²³ Preterm birth brings with it a sudden change in the quality and intensity of sensory information received, which may lead to disrupted and fragmented sleep, impacting sleep organisation and developmental outcome.^{27,28} Prolonged and continuous exposure to the multisensory environment and stress-related events of the neonatal unit and/or sleep interruptions may impact not only the physiological status but the long-term neurodevelopmental outcome of the preterm infant, including long-term sensory processing disorders.^{12,29,34-38} Preterm birth is associated with significant

morbidity^{29,39} including long-term consequences, such as difficulties with cognition, language development, motor skills, behaviour and overall physiological wellbeing.^{8,29,37,40} A priority to creating a culture of 'sleep protection' requires knowledge about SWC and recognition of sleep states, which will assist in identifying the appropriate timing of handling for infant cares.^{9,11,25,41} Despite being closest to the point of care of the preterm infant in the NICU, a low level of knowledge of behavioural features and sleep state identification has been found among neonatal nurses and this may lead to misinterpretation of sleep activity (ie active sleep) for waking.⁴²

Strengths of this study include the number of participants, similar clinical status, duration and timing of overnight vEEG and the use of video-supported visual observation with respiratory monitoring supporting sleep state identification, ie rapid irregular respirations in AS (REM) with slower regular breathing in QS (NREM).² A limitation of this study is its focus on overnight sleep only. Further research should incorporate a full 24-hour monitoring period, providing insight into both day and night SWC and interruptions observed. The prognostic value of EEG in uncomplicated medically stable MLP infants remains unclear.^{33,43} Hence the significance of outcome to the number of SWC seen on EEG. The duration of each sleep state has yet to be determined and will constitute part of a future study.

In conclusion

EEG provides detailed real-time information about brain activity of preterm infants and the changes that occur during sleep wake cycling. Sleep disturbances may affect the development and organisation of sleep-wake patterns of the preterm infant and impact sleep state

organisation and potentially long- and short-term developmental outcomes.

Preterm infants are at risk of adverse neurological development especially during the critical phase of brain development that occurs during the MLP period. Preserving and supporting optimal sleep organisation among infants will support a normal neural pathway of development and help improve long- and short-term outcomes. This study has provided an introduction to preterm infant states as seen in vEEG, providing a window through which we can view brain activity during each sleep state.

This study has quantified overnight SWC and interruptions. With minimal exception, overnight disruptions to sleep were found to be a consequence of routine care, accounting for disturbance to 23.3% of the total SWC recorded. Reducing this number and preserving sleep provides an opportunity to enhance brain development but presents a real operational challenge in the NICU.

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CLINICAL RESEARCH ARTICLE

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Sleep state organisation of moderate to late preterm infants in the neonatal unit

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BACKGROUND: Sleep supports neurodevelopment and sleep architecture reflects brain maturation. This prospective observational study describes the nocturnal sleep architecture of healthy moderate to late preterm (MLP) infants in the neonatal unit at 36 weeks post menstrual age (PMA).

METHODS: MLP infants, in the neonatal unit of a tertiary hospital in Ireland from 2017 to 2018, had overnight continuous electroencephalography (cEEG) with video for a minimum 12 h at 36 weeks PMA. The total sleep time (TST) including periods of active sleep (AS), quiet sleep (QS), indeterminate sleep (IS), wakefulness and feeding were identified, annotated and quantified.

RESULTS: A total of 98 infants had cEEG with video monitoring suitable for analysis. The median (IQR) of TST in the 12 h period was 7.09 h (IQR 6.61–7.76 h), 4.58 h (3.69–5.09 h) in AS, 2.02 h (1.76–2.36 h) in QS and 0.65 h (0.48–0.89 h) in IS. The total duration of AS was significantly lower in infants born at lower GA ($p = 0.007$) whilst the duration of individual QS periods was significantly higher ($p = 0.001$).

CONCLUSION: Overnight cEEG with video at 36 weeks PMA showed that sleep state architecture is dependent on birth GA. Infants with a lower birth GA have less AS and more QS that may have implications for later neurodevelopment.

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IMPACT:

- EEG provides objective information about the sleep organisation of the moderate to late preterm (MLP) infant.
- Quantitative changes in sleep states occur with each week of advancing gestational age (GA).
- Active sleep (AS) is the dominant sleep state that was significantly lower in infants born at lower GA.
- MLP infants who were exclusively fed orally had a shorter total sleep time and less AS compared to infants who were fed via nasogastric tube.

BACKGROUND

Of the 15 million preterm births that occur worldwide each year, >80% occur between 32 and 36 weeks GA.¹ Current trends have shown that the rise in the rate of preterm birth is primarily attributed to the moderate to late preterm (MLP) infant group, defined as those born between 32 and 36 + 6 weeks gestation.² MLP infants have a higher risk of adverse neurodevelopment in comparison to term-born infants.^{3,4}

As the main behavioural state of the newborn infant, sleep is a prerequisite for normal neural network development, synaptogenesis and synaptic plasticity, particularly during the accelerated period of brain development that occurs in the last trimester.^{5,6} Sleep contributes to neural restoration, supports body repair, energy conservation and wellbeing.⁷ As sleep is an important basic need and determinant of brain development, supporting sleep and positive early life experiences for infants born during the MLP period, will support optimal neurodevelopment.⁸ Sleep in the newborn infant is comprised of active sleep (AS) (a forerunner

to rapid eye movement or REM sleep), quiet sleep (QS) (a forerunner to non-rapid eye movement or NREM sleep) and indeterminate sleep (IS).

Sleep architecture can be assessed using continuous electroencephalography (cEEG) with video.^{9,10} Each sleep state is associated with a distinct pattern of cEEG activity that alters between sleep states (Figs. 1 and 2). EEG activity describes background continuity (continuous or discontinuous), frequency of activity, and amplitude or voltage (μV) of activity, with associated alterations in behaviour and cardiorespiratory parameters (Table 1).^{11–13} Frequency of activity measured in Hertz (Hz) includes delta (0.5–4 Hz), theta (4–8 Hz), alpha (8–12 Hz) and beta (12–35 Hz) activity. Amplitude is described as either high (>50 μV), medium (between 50 and 25 μV) or low amplitude, which for the purposes of this manuscript we have identified as <25 μV .¹⁴

Sleep architecture undergoes quantitative and qualitative changes with maturation that is reflected in the EEG.^{15,16}

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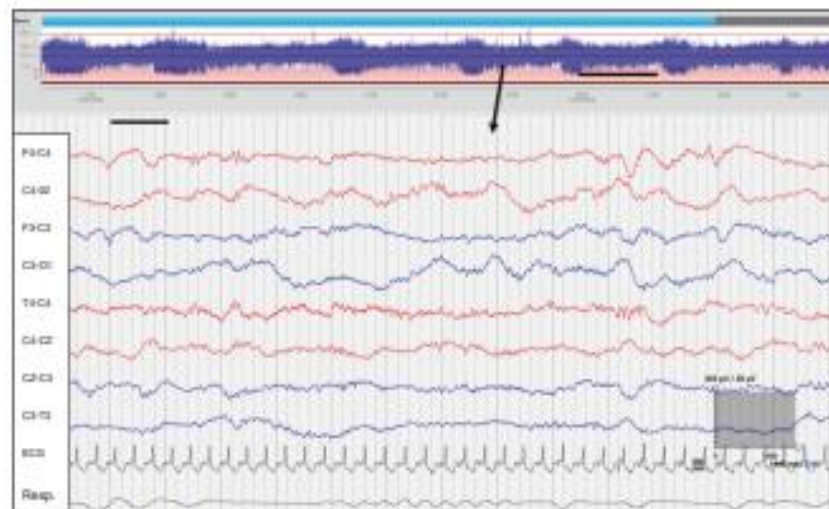


Fig. 1 Period of continuous activity of mixed amplitude in active sleep. The black line on aEEG indicates 1 h of recording whilst the black line on cEEG indicates 1 s of recording. The arrow indicates the point on the aEEG that is displayed on cEEG. The scale of the cEEG is displayed in a grey box with amplitude measurement expressed in μV vertically and duration expressed in milliseconds horizontally.

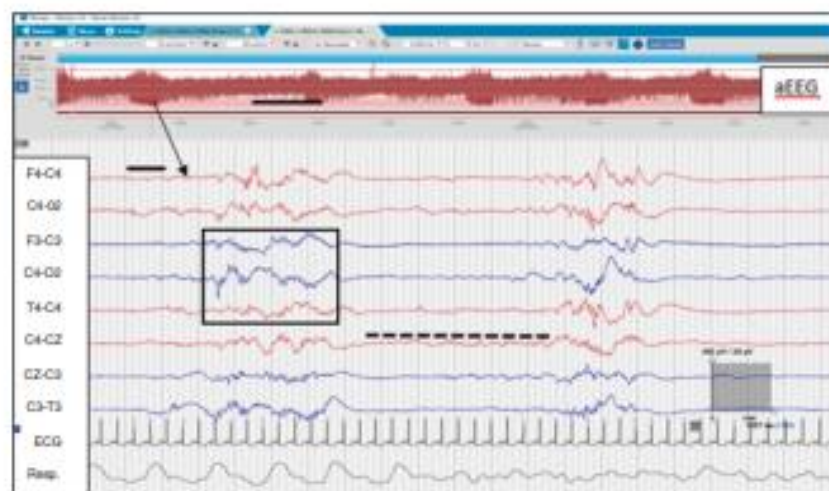


Fig. 2 Periods of low amplitude or discontinuity (background activity $<25 \mu\text{V}$ indicated by black dashed line) seen on cEEG channels in quiet sleep, with high amplitude bursts of activity, i.e., $>75 \mu\text{V}$ in a black box. The black line on aEEG indicates 1 h of recording whilst the black line on cEEG indicates 1 s of recording. The arrow indicates the point on the aEEG that is displayed on cEEG. The scale of the cEEG is displayed in a grey box with amplitude measurement expressed in μV vertically and duration expressed in milliseconds horizontally.

Alterations in sleep architecture correlate with measurable structural changes in the brain.¹⁷ Cycling between sleep states requires the integration of multiple brain networks.¹⁸ Sleep cycling is a surrogate marker of neurological wellbeing. Delayed cycling is associated with neurological dysfunction in preterm and term infants.^{19,20} cEEG provides accurate identification of changes in sleep states or cycling that is clearly evident from 30 to 32 weeks gestation. This in itself is reassuring in terms of a normal trajectory of sleep organisation and may be a useful biomarker of outcome.

There is a paucity of research on sleep architecture, particularly in the at-risk MLP infant group. We designed a prospective observational study to describe and quantify sleep of the healthy MLP infant in the neonatal unit at 36 weeks post menstrual age (PMA) (defined as birth GA plus chronological age) using cEEG with video. We hypothesised that the sleep architecture of all healthy MLP infants at 36 weeks PMA would be the same, irrespective of birth GA, sex, mode of feeding or accommodation at time of monitoring.

METHODS

This was a single-centre prospective observational study carried out in our open-plan neonatal unit in Cork University Maternity Hospital, Ireland, between July 2017 and September 2018. Ethical approval was obtained from the Clinical Research Ethics Committee of the Cork Teaching Hospitals prior to the commencement of the study.

Participants

Clinically stable MLP infants admitted to the neonatal unit, with no chromosomal/congenital abnormalities and no neurological concerns were identified as potential participants. Whilst the development of sleep may be compromised by the morbidity of the preterm infant, all infants in this study were healthy with no physiological or neurological concerns throughout their neonatal unit stay. An invitation to participate was extended to parents whose infants satisfied the inclusion criteria. Written informed parental consent was obtained for all participating infants.

Monitoring sleep

All infants had cEEG with video for a minimum of 12 h in the neonatal unit (pre-discharge) and as close as possible to 36 weeks but not exceeding

Table 1. Behavioural observations, cardiorespiratory parameters and EEG features identifying sleep states and wakefulness.

	Active sleep (AS)	Quiet sleep (QS)	Indeterminate sleep (IS)	Wakefulness
Behaviour	Eyes closed, may open slightly, $\pm$ REM	Eyes closed, no eye movement	Periods of opening/closing eyes	Eyes open
	Range of intermittent sporadic motor activity	Infant lying still/tonia	Slow eye movements	May be quiet, still or active
	– Low motor activity $\pm$ myoclonic twitching	May have some mouth/sucking motion	Slow startles	Fussing or crying
	– Upper and lower limb movement	May have slight startle, jerk	Intermittent sucking	High-intensity gross motor movements
	Occasional sucking movements	More difficult to rouse	Regular respirations	Irregular respirations with movement
	Quiet and low muscle tone between movements	Respirations slower, more regular than AS		Variable irregular heart rate
	Irregular shallow respiration, variable heart rate	Decreased heart rate variability than in AS		
EEG activity	Continuous activity above a threshold of 25 μ V	Periods of discontinuity evident in QS (≤ 25 μ V)		Similar to AS in quiet wakefulness
	Less than 3 s voltage attenuation < 25 μ V	High amplitude bursts 50–300 μ V, minimum 3 s	Continuous activity high voltage (100–200 μ V)	Mixed continuous activity
	Mainly high frequency > 8 Hz, low/mixed amplitude (30–70 μ V)	Inter-burst intervals decrease with advancing PMA		Increased artefact with movement
	Artefact with movement	Artefact with movement		Duration increasing with advancing age
aEEG activity	Lower amplitude represented by narrower portion of band	High amplitude represented by wider portion of band	Variable bandwidth	Quiet wakefulness displayed similar to AS
	Normal: a lower margin > 5 μ V and upper margin > 10 μ V	Lower margin < 5 μ V in QS	Not discriminated on amplitude EEG	High amplitude activity with movement

Table compiled based on findings in the literature.^{11,20,22,24}

36 $\pm$ 6 weeks PMA. We used gold standard cEEG with video to score sleep state or wakefulness based on minute-by-minute visual analysis of electrophysiological features. As heart and respiratory rate are influenced by sleep state, we also incorporated ECG (one electrode placed on each shoulder) and a respiratory sensor was secured to the abdomen with hypoallergenic tape. Together these provided a visual display of alteration in heart and respiratory rate and rhythm. We did not use electrooculography (EOG) or electromyography (EMG) as we wanted to minimise the recording channels for overnight monitoring but the video was utilised to observe behaviour including ocular and facial movements. The standard of care was not altered throughout the monitoring period.

cEEG monitoring was conducted in a quiet area of the neonatal unit. To facilitate feeding schedules and parental visits, all EEG monitoring commenced between 17:00 and 20:00 h and ended the following morning between 07:30 and 10:00 h. Lifelines iEEG (Lifelines Neurodiagnostic Systems, UK) and the NicoletOne monitor (Carefusion, Wisconsin) were used for cEEG monitoring that also displayed the amplitude-integrated EEG (aEEG) as a trend of two channels above the cEEG. Whilst aEEG can be useful for the identification of sleep cycling, the information provided is a general overview of EEG activity from which it is not possible to distinguish AS from periods of quiet wakefulness and IS.^{21,22}

All recordings adhered to a standard modified neonatal version of the International 10/20 system that ensured consistency in the positioning of EEG electrodes.¹² Scalp electrode sites were prepared by cleaning with Nuprep gel and eleven sterile disposable hydrogel electrodes were applied with additional water-soluble conductive paste. Electrode placement positions were at F3, F4, C3, C4, Cz, T3, T4, O1, O2, plus a reference electrode on the frontal region of the head (R) and ground electrode (G) placed behind the left ear. An adjustable cotton hat was placed on the head over the electrodes to assist in securing electrodes overnight. cEEG monitoring commenced when all electrode impedances were < 10 kOhms.

Post-acquisition cEEG annotation

When monitoring was complete, recordings were pseudonymised and transferred to a secure server and stored for further analysis. Post-acquisition an experienced neonatal neurophysiologist (S.R.M.) reviewed EEGs for any incidental abnormal cEEG activity. Many infants exceeded 12 h of monitoring; however, only 12 h was used in the analysis (commencing with the onset of AS and as close as possible to 18:00 h).

As there is a lack of consistency pertaining to the starting point of a sleep cycle in the literature,^{23,24} we defined a sleep cycle as commencing with an AS phase, with transitioning and completion of a QS phase without interruption.²⁴ A complete sleep cycle may end in wakefulness or the infant may enter another phase of AS. IS occurs during the period of transition between sleep states incorporating features of AS and QS but not clearly identifiable as either.²³ Sleep states on each cEEG recording were identified and annotated as AS (Fig. 1), QS (Fig. 2) or IS based on minute-by-minute visual analysis of cEEG activity, behavioral observation by time-locked video and cardiorespiratory parameters (see Table 1 compiled based on existing literature^{9,23–25}).

Sleep interruptions that resulted in wakefulness were identified as continuous irregular or mixed EEG pattern with movement and muscle artefact, whilst cardiorespiratory observations recorded an irregular heart rate and respiration. Behavioural observations on a time-locked video provided a visual confirmation of sleep interruption and wakefulness. In quiet wakefulness, a continuous low to medium voltage mixed frequency pattern was seen with regular heart rate and respirations (Table 1).

Each 12 h cEEG with video was annotated by the same reviewer (M.A.J.R.) and a 2-h epoch of each EEG was also annotated by a second reviewer (S.R.M.). A two-way random effects intraclass correlation was calculated between the two raters on a random sample of half the EEGs. Results showed the correlation between raters for AS was 0.910 (95% CI: 0.834–0.952), QS 0.901 (95% CI: 0.817–0.947) and IS was 0.668 (95% CI: 0.448–0.812). The total duration of each sleep state was recorded, rounded to the nearest minute, and exported to SPSS.

Sleep was described as follows:

- An analysis of the duration of sleep, feeding and wakefulness over a 12-h period (sleep-wake analysis). Wakefulness incorporated periods of quiet wakefulness, time spent unsettled/fussing, $\pm$ crying.
- A total sleep time (TST) analysis, i.e., the total duration of all periods of AS, QS and IS in the 12 h recording period.
- A sleep cycle analysis described in terms of the median time in AS, QS and IS based on two overnight uninterrupted complete sleep cycles at similar time points for all infants. Cycle 1 was between 22:00hrs and 02:00hrs and Cycle 2 was between 03:00hrs and 07:00hrs. Our sleep cycle analysis was based on the median of the values obtained from the two time points.

Statistical analysis

Statistical analysis was performed using IBM SPSS Statistics (version 25.0, IBM Corp, Armonk, NY). Continuous variables were described using the median and interquartile range (IQR) and categorical variables using frequency and percentage. The Mann-Whitney *U* test was used for comparisons of sleep parameters between two groups and the Kruskal-Wallis test was used when there were more than two groups. If statistically significant differences were found between groups for the Kruskal-Wallis test, pairwise comparisons were performed using the Mann-Whitney *U* test, with Bonferroni correction. The groups investigated were GA at birth (32–32 + 6, 33–33 + 6, 34–34 + 6, 35–35 + 6 and 36–36 + 6 weeks), sex, accommodation at the time of monitoring (cot or incubator) and mode of feeding (oral only, mixed mode). The Mann-Whitney *U* test was also used to investigate the relationship between GA and mode of feeding. Relationships between number of interruptions and GA and active sleep duration were investigated using Spearman's correlation coefficient (r_s). All tests were two-sided and a *p* value < 0.05 was considered statistically significant.

RESULTS

Of the 117 infants recruited to this prospective observational study, 13 infants were discharged or transferred to another unit prior to cEEG monitoring, 3 infants were excluded prior to EEG recording due to suspicion of chromosomal abnormality and a further 3 excluded post EEG acquisition due to technical issues. Hence, 98 infants were included in the analysis (Table 2). Infants were from singleton ($n = 51$) and multiple pregnancies ($n = 47$) and nursed in a cot ($n = 80$) or incubator ($n = 18$) at time of monitoring. The majority of infants were exclusively fed orally ($n = 61$) whilst the remainder had either a mixed mode of feeding ($n = 36$) or were fed exclusively via nasogastric tube ($n = 1$).

Table 2. Demographics ($n = 98$).

	Median (IQR)
Maternal age (years)	34.5 (31.0–37.3)
Birth gestation (weeks)	34.0 (33.0–35.0)
Gestation at time of EEG (weeks)	36.1 (35.6–36.6)
Head circumference at birth (cm)	32.00 (30.75–32.90)
Apgar at 5 min	9 (9–10)
Birth weight (kg)	2.10 (1.87–2.40)
Weight at the time of cEEG (kg)	2.26 (2.02–2.47)
Postnatal age at the time of cEEG (days)	10.50 (4.75–14.00)
Sleep cycles per infant per night	6.0 (6.0–7.3)
	<i>n</i> (%)
Ethnicity—Irish	88 (89.8)
Pregnancy	
Multiple	47 (48.0)
Singleton	51 (52.0)
Sex	
Male	52 (53.1)
Female	46 (46.9)
Accommodation at time of monitoring	
Cot	80 (81.6)
Incubator	18 (18.4)
Mode of overnight feeding/infant	
All oral feeds	61 (62.2)
All nasogastric tube feeds (NGT)	1 (1.0)
Mixed feeding (oral and NGT)	36 (36.7)

cEEG continuous EEG.

Sleep-wakefulness analysis

The median (IQR) of TST over the 12 h monitoring period was 7.09 h (6.61–7.76). The remaining overnight hours were spent attending to the infant's feeding orally and periods of wakefulness. The median(IQR) duration of time spent attending to infant cares and feeding (h) was 1.58 h (1.18–2.13) and the duration of wakefulness outside this period was 3.19 h (2.38–3.85) (Table 3). As expected, infants who were feeding orally spent a significantly longer time feeding in comparison to infants who had a mixed mode of feeding ($p = 0.004$). Infants who fed exclusively orally also had a significantly longer duration of wakefulness outside of feeding ($p = 0.033$). There was no difference in feeding and wakefulness based on GA, sex or accommodation at the time of monitoring.

Total sleep time analysis

Time and percentage of time (out of 12 h) in each sleep state overall and by GA, sex, accommodation at time of monitoring and mode of feeding are presented in Table 4. AS was the dominant sleep state with a median (IQR) duration (h) of 4.58 h (3.69–5.09) of TST. The median (IQR) duration (h) of QS and IS were 2.02 h (1.76–2.36) and 0.65 h (0.48–0.89) respectively. There was a statistically significant increase in AS observed with advancing GA ($p = 0.007$). Using pairwise comparisons, we found that this difference was primarily due to the significantly lower duration of AS in the youngest birth GA group (32 weeks) compared to older GA groups of 35 weeks (adjusted $p = 0.019$) and 36 weeks (adjusted p value = 0.017). We investigated if the relationship between GA and AS was confounded by mode of feeding. We did not find a statistically significant relationship between mode of feeding and GA (weeks) ($p = 0.107$) and therefore, the decreased time spent in AS amongst infants born at a younger GA cannot be explained by mode of feeding. No differences were found in durations of QS ($p = 0.825$) or IS ($p = 0.544$) between GA groups.

In a previous study of this MLP group ($n = 98$), we found that 23.3% of overnight sleep cycles in an MLP infant group were interrupted, almost exclusively for the purposes of feeding.²⁶ Therefore, we investigated if this increase in AS with increasing GA could have been due to a difference in the number of sleep interruptions that are followed by a period of wakefulness and then a recommencement of AS. The number of interruptions increased with increasing GA ($r_s = 0.20$, $p = 0.048$) but no association was found between number of interruptions and duration of AS ($r_s = 0.05$, $p = 0.593$). Thus, number of interruptions was not a confounder of the association between GA and AS. The total duration of AS did not significantly differ based on sex or accommodation ($p = 0.457$ and 0.790 respectively).

There were no differences in duration of QS based on sex, accommodation and mode of feeding $p = 0.089$, 0.073 and 0.291, respectively) or IS based on the same groupings $p = 0.845$, 0.989 or 0.852, respectively.

Sleep cycle analysis

All infants displayed sleep cycling on cEEG with a median (IQR) of 6.0 (6.0–7.3) uninterrupted sleep cycles throughout the night. Sleep cycle duration and the duration of AS, QS and IS within, are described by GA, sex, accommodation at time of monitoring and mode of feeding are presented in Table 5. The median (IQR) duration (min) of an overnight uninterrupted sleep cycle was 37.00 min (32.00–45.13) with a median(IQR) of 16.50 min (12.50–22.50) for AS, 16.75 min (14.00–19.50) for QS and 2.75 min (2.00–3.50) for IS.

Within sleep cycles, QS duration differed by GA group ($p = 0.001$). Pairwise comparisons revealed significant differences between the youngest GA group (32 weeks) and the older GA groups of 34 weeks (adjusted $p = 0.001$) and 36 weeks (adjusted $p = 0.010$). The duration of AS, IS or sleep cycle duration did not differ between GA groups ($p = 0.247$, 0.210 and 0.105

Table 3. Total sleep time, time spent feeding and awake over 12-h period ($n = 98$).

	TST(h) median(IQR)	p value ^a	Feeding and cares(h) median(IQR)	p value ^a	Wakefulness(h) median(IQR)	p value ^a
Overall	7.09 (6.61–7.76)		1.58 (1.18–2.13)		3.19 (2.38–3.85)	
Birth gestational age group (weeks)		0.143		0.625		0.128
32 ($n = 19$)	6.96 (6.06–7.34)		1.60 (1.34–2.06)		3.57 (2.63–4.13)	
33 ($n = 12$)	7.09 (6.84–8.01)		1.34 (1.03–1.89)		3.67 (2.45–3.96)	
34 ($n = 36$)	6.96 (6.30–7.84)		1.47 (1.12–2.36)		3.36 (2.22–3.91)	
35 ($n = 15$)	7.50 (6.91–7.82)		1.52 (1.13–2.09)		3.05 (2.07–3.29)	
36 ($n = 16$)	7.17 (6.83–8.16)		1.87 (1.13–2.20)		2.80 (2.35–3.16)	
Sex		0.606		0.177		0.586
Male ($n = 52$)	7.06 (6.59–7.73)		1.75 (1.26–2.17)		3.02 (2.29–3.90)	
Female ($n = 46$)	7.21 (6.70–7.83)		1.46 (1.10–2.13)		3.32 (2.43–3.84)	
Accommodation		0.271		0.319		0.176
Cot ($n = 80$)	7.03 (6.52–7.73)		1.53 (1.16–2.11)		3.27 (2.39–3.90)	
Incubator ($n = 18$)	7.30 (6.83–7.83)		1.87 (1.18–2.28)		2.82 (2.31–3.62)	
Mode of feeding ^b		<0.001		0.004		0.033
Oral ($n = 61$)	6.91 (6.30–7.35)		1.83 (1.32–2.26)		3.37 (2.43–3.99)	
Mixed ($n = 36$)	7.57 (6.94–8.20)		1.28 (1.09–1.71)		2.87 (2.09–3.69)	

TST total sleep time, IQR interquartile range.

^aThe Kruskal–Wallis test was used for gestational age and the Mann–Whitney U test was used for sex, accommodation and mode of feeding.

^bOne infant fed exclusively via NGT tube and eliminated from mode of feeding analysis ($n = 97$).

$p < 0.05$ was statistically significant.

respectively). There was no difference in the sleep cycle duration or the duration of AS, QS, IS within uninterrupted sleep cycle based on sex, accommodation or mode of feeding.

DISCUSSION

In this study, we used cEEG with video to quantify the overnight sleep of a large cohort of healthy MLP infants in the neonatal unit. At the outset, it must be considered that the genetically defined pre-programmed pathway of normal sleep ontogenesis may be altered or supported through physiological and sensory environmental factors.^{27,28} Stressors within the neonatal unit may compromise the quality and quantity of sleep.^{11,29,30}

As the primary activity of the developing brain, sleep plays a crucial role in brain development. As the dominant sleep state of the MLP group, AS provides endogenous stimulation facilitating neural network maturation, synapse formation and synaptic plasticity. AS supports memory, learning, emotional and motor development and has a strong correlation with brain growth.⁶ It is also a prerequisite state for psychosocial and normal sensory system development.³¹ A reduction in AS in the preterm infant may reduce the efficiency of information transmission and decrease overall brain volume.^{32,33} QS is a prerequisite for maturation, supporting connectivity between networks and the preservation of brain plasticity.⁸ QS is also associated with cognitive function and the pre-consolidation phase of learning, memory and the maintenance of physiological homeostasis.^{34,35}

The duration of overnight sleep was shorter than expected whilst the duration of wakefulness was longer in this MLP group. Interestingly, infants born in the lower GA groups had the longest duration of wakefulness which may suggest an increased level of maturation. However, these differences did not reach a level of significance ($p = 0.128$). We have shown that in the MLP infant group the median (IQR) duration of time spent sleeping was 7.09 h (6.61–7.76 h) or 59.1% (55.1–64.7%) of 12 hr monitoring period. It could be considered that 14.18 h extrapolated over a 24-h period falls short of the expected average of 16–18 h of sleep for newborn

infants.^{35,36} Intuitively this might be expected to be an overestimate of TST as an increase in clinical activity and sensory exposure by day may lead to infants sleeping less during daytime hours. However, Ardura et al. suggested the opposite to be true, finding that preterm infants at 35 weeks GA ($n = 11$) slept for 8.88 h during day time hours (08.00–20.00 h) and 8.22 h overnight (20.00–08.00 h). At 36 weeks GA ($n = 14$) infants slept for 8.96 h by day and 7.51 h overnight.³⁷

When investigating the effects of handling on sleep in late preterm low birth weight infants nursed in an incubator ($n = 12$), Maki et al. found no significant difference in duration of AS, QS, IS or wakefulness between day and night time sleep ($p = 0.83$, 0.84, 0.73 and 0.99, respectively).³⁸ These authors also observed that the infant group under study slept for approximately 13.7 h of the 24-h period, i.e., 57.2% of 24 h. As we found no significant difference in TST between infants nursed in a cot or an incubator ($p = 0.271$), these findings are comparable to ours. Sleep duration recommendations have been considered to be based on weak scientific evidence.³⁹ The preterm infant may spend up to 90% of the day sleeping which decreases with advancing GA.⁴⁰ Whilst 14–17 h are identified as appropriate for term newborn any duration between 11 and 19 h has been considered acceptable.³⁹

In our TST analysis, we found that the majority of overnight sleep was AS, representing 63.07% (56.25–67.43%) of the TST. As sleep in the newborn begins with AS it is not surprising that overall, TST was dominated by AS, as following each sleep cycle interruption (or spontaneous awakening) a new phase of AS sleep must begin. It is interesting that at 36 weeks, infants born in the lower GA groups had the longest duration of wakefulness in comparison to those born at a higher GA. Perhaps this suggests an increased maturity amongst infants born at a lower GA. However, these differences did not reach a level of significance ($p = 0.128$).

The overall duration of AS was significantly lower amongst infants born in a lower GA group ($p = 0.007$), whilst the duration of QS was longer differences were not statistically significant (0.825). Consistent with other studies, our results support a trend towards changes in sleep organisation with advancing GA. EEG maturation

Table 4. Total duration of each sleep state expressed in hours and as a percentage of total sleep time ($n=98$).

	AS (h) median (IQR)	P value ^a	% AS median (IQR)	P value ^a	QS (h) median (IQR)	P value ^a	% QS median (IQR)	P value ^a	IS (h) median (IQR)	P value ^a	% IS median (IQR)	P value ^a
Overall	4.58 (0.69–5.09)	0.007	63.07 (56.25–67.43)	0.007	2.02 (0.76–2.36)	0.825	27.88 (24.26–34.24)	0.122	0.65 (0.48–0.89)	0.544	9.13 (6.30–12.24)	0.298
Birth gestational age group (weeks)												
32 ($n=19$)	3.86 (0.38–4.38)		56.22 (49.43–64.79)		2.07 (0.88–2.50)		33.33 (26.03–36.00)		0.68 (0.50–0.92)		9.51 (6.36–13.59)	
33 ($n=12$)	4.76 (4.09–5.15)		63.01 (58.49–68.26)		2.07 (0.70–2.46)		29.14 (24.68–32.77)		0.56 (0.34–0.86)		7.23 (5.31–12.92)	
34 ($n=30$)	4.45 (4.45–5.09)		64.11 (51.20–68.33)		2.04 (0.78–2.28)		27.65 (23.39–34.84)		0.68 (0.48–0.94)		9.86 (6.96–12.61)	
35 ($n=15$)	4.87 (4.60–5.08)		64.94 (60.49–66.86)		1.98 (0.70–2.35)		26.19 (22.43–30.65)		0.65 (0.57–0.87)		8.87 (6.51–11.73)	
36 ($n=16$)	4.95 (4.14–5.48)		65.91 (57.62–69.35)		1.92 (0.43–2.55)		26.15 (21.53–34.21)		0.56 (0.37–0.87)		7.16 (5.44–12.26)	
Sex		0.457		0.200		0.089		0.129		0.845		0.825
Male ($n=52$)	4.59 (0.70–5.24)		64.35 (55.40–69.52)		1.95 (0.71–2.26)		26.55 (23.08–33.65)		0.64 (0.48–0.88)		8.43 (6.06–13.38)	
Female ($n=46$)	4.40 (0.68–5.06)		61.76 (56.25–65.73)		2.14 (0.88–2.49)		29.59 (25.04–35.09)		0.65 (0.50–0.91)		9.23 (6.78–11.69)	
Accommodation		0.790		0.335		0.073		0.263		0.389		0.869
Cot ($n=80$)	4.49 (0.70–5.15)		63.78 (56.23–68.22)		1.99 (0.71–2.31)		27.88 (23.62–34.09)		0.65 (0.48–0.88)		9.23 (6.38–12.26)	
Incubator ($n=18$)	4.75 (0.60–4.98)		60.98 (55.44–65.10)		2.24 (0.93–2.51)		27.88 (26.18–36.19)		0.60 (0.49–0.93)		8.43 (6.03–12.35)	
Mode of feeding ^b		<0.001		0.433		0.291		0.473		0.852		0.229
Oral ($n=61$)	4.29 (0.45–4.86)		63.25 (52.19–68.05)		2.01 (0.75–2.33)		27.90 (24.62–34.66)		0.473 (0.47–0.88)		9.68 (6.42–12.81)	
Mixed ($n=36$)	4.94 (4.47–5.32)		63.09 (59.31–67.01)		2.04 (0.73–2.52)		27.65 (22.76–30.92)		0.59 (0.51–0.93)		8.01 (6.04–11.49)	

The percentage (%) for each infant is the percentage of time spent in each sleep state.

AS active sleep, QS quiet sleep, IS indeterminate sleep, IQR interquartile range.

^aThe Kruskal-Wallis test was used for gestational age and the Mann-Whitney U test was used for sex, accommodation and mode of feeding.

^bOne infant fed exclusively via NGT tube and eliminated from mode of feeding analysis ($n=97$).

$p < 0.05$ was statistically significant.

Table 5. Sleep cycle duration overall by sleep state/groups including feeding and wakefulness ($n = 98$).

	Sleep cycle duration(min) median(IQR)	p value ^a	AS duration median(IQR)	p value ^a	QS duration(min) median(IQR)	p value ^a	IS duration(min) median(IQR)	p value ^a
Overall	37.00 (32.00–45.13)		16.50 (12.50–22.50)		16.75 (14.00–19.50)		2.75 (2.00–3.50)	
Gestational age per group (weeks)		0.105		0.247		0.001		0.210
32 ($n = 19$)	40.50 (31.00–50.00)		15.00 (9.00–23.00)		19.50 (17.00–22.50)		3.00 (2.00–4.00)	
33 ($n = 12$)	39.00 (31.13–43.75)		16.50 (13.00–22.00)		17.75 (16.00–19.00)		2.00 (1.63–3.00)	
34 ($n = 36$)	34.50 (30.00–39.00)		14.25 (12.00–21.38)		15.00 (11.63–18.38)		2.75 (2.00–3.38)	
35 ($n = 15$)	39.00 (35.00–45.00)		19.00 (15.00–23.00)		16.00 (15.00–20.00)		2.00 (1.50–3.00)	
36 ($n = 16$)	41.50 (35.13–48.75)		20.00 (15.00–25.88)		15.00 (13.00–18.00)		2.75 (1.63–3.38)	
Sex		0.706		0.591		0.151		0.294
Male ($n = 52$)	37.00 (32.00–44.50)		16.75 (13.00–23.00)		16.00 (13.00–19.38)		3.00 (2.00–3.50)	
Female ($n = 46$)	38.00 (32.00–46.50)		16.25 (9.88–22.13)		17.25 (15.00–19.63)		2.50 (2.00–3.13)	
Accommodation		0.151		0.451		0.666		0.386
Cot ($n = 80$)	36.75 (32.00–44.25)		15.50 (12.63–22.00)		16.50 (14.00–19.50)		2.50 (2.00–3.00)	
Incubator ($n = 18$)	43.00 (33.50–48.25)		21.25 (11.25–24.63)		18.00 (14.63–19.63)		3.00 (1.88–4.00)	
Mode of feeding ^b		0.098		0.143		0.363		0.820
Oral ($n = 61$)	37.00 (31.50–44.00)		15.00 (11.50–22.25)		17.00 (13.25–20.00)		2.50 (2.00–3.50)	
Mixed ($n = 36$)	39.50 (34.63–46.50)		19.50 (13.25–23.75)		16.25 (14.25–18.00)		2.75 (2.00–3.00)	

AS active sleep, QS quiet sleep, IS indeterminate sleep, IQR interquartile range.

^aThe Kruskal-Wallis test was used for gestational age and the Mann-Whitney *U* test was used for sex, accommodation and mode of feeding.

^bOne infant fed exclusively via NGT tube and eliminated from mode of feeding analysis ($n = 97$).

$p < 0.05$ was statistically significant.

occurs with increased chronological age, i.e., a decrease in the overall time spent in AS and an increase in QS (Table 4).^{19,41,42} However, there is some conflicting evidence suggesting the opposite trajectory may be true. Han et al. utilised cEEG, aEEG, EOG, EMG over a 4-h period and described the changes that occurred with advancing GA. In comparing the percentage of each sleep state between a 34–35 GA group ($n = 20$) and a 36–37 GA group ($n = 23$), results showed an increase in the percentage of AS sleep duration from 39.8 to 46.6%, a decrease in the percentage of QS duration from 36.8 to 32.3%, a decrease in IS from 7.9 to 5.3%, whilst duration of wakefulness differed minimally 15.5–15.8% between groups.⁹ In a review of sleep state measurement, Werth et al. described a plateau in AS duration between 30 and 36 weeks GA at 60–65% of sleep time,⁴³ which is similar to our results of a median of 63.07%. QS was described as increasing until 36 weeks to approximately 38% that is higher than our findings of a median of 27.88%. Thereafter, Werth et al. described both sleep states decrease as wakefulness increases. Similar to Foreman et al. we found no disparities based on sex in our analysis of sleep state duration.⁴⁴

In our analysis of uninterrupted sleep cycles, the median (IQR) duration was 37.00 min (32.00–45.13) that is shorter than previously reported for MLP infants at 36 weeks PMA.^{23,24,45} Learning and memory are dependent on normal cycling.⁴⁶ Our results showed that the duration of QS within an uninterrupted sleep cycle differed by GA age group only ($p = 0.001$) with the younger birth GA group having a significantly longer QS period. Our sleep cycle analysis showed differences in the duration of AS by GA group within an uninterrupted sleep cycle but these differences did not reach statistical significance.

There is extensive variability in the literature pertaining to the duration of a sleep cycle in preterm infants at 35–36 weeks PMA. Tsuchida et al. reported a sleep cycle duration of 50–65 min for infants >35 weeks PMA.²⁴ Curzi-Dascalova et al. described the sleep cycle duration of a small group of participants ($n = 11$ between 35 and 36 weeks GA) by the mean $\pm$ standard error of the mean as 69.4 $\pm$ 6.1 min.⁴⁵ Even for healthy term-born infants, sleep cycles typically last a mean of 50–60 min with a range of 30–70 min described.⁴⁷ Deroymaeker et al. also report extensive variability in cycle duration amongst infants of 35–36 weeks GA that ranged from 6.3 to 109 min with a mean

(SD) of 50.9 min (19.2 min).⁴⁸ In our methodology we defined a sleep cycle and its starting point.²⁴ However, inconsistencies in sleep cycle definitions were noted.^{23,24} Some studies did not define it but described sleep cycling in broad terms such as fluctuations between sleep states.⁴⁹

Heterogeneity in methodology is evident in the literature making it difficult to compare like with like. Many sleep studies carried out have been based on behaviour observation, with or without the inclusion of ECG, EMG and EOG,^{35,37,44,50} or sleep state determined based on activity or actigraphy.¹⁸ Whilst some studies did utilise cEEG with video, differences in inclusion/exclusion criteria, duration and timing of monitoring and environmental conditions were evident.^{9,36,45,51} Whilst environmental factors may impact the duration and intensity of sleep, intra-individual variation in sleep organisation may also be attributed to endogenous factors with genetic links.^{44,52} To date, the majority of studies that used cEEG to monitor preterm sleep have been during daytime hours and recording duration has rarely been longer than 4 h. Shorter duration of monitoring may not be truly representative of sleep cycle duration for an infant group. Inclusion/exclusion criteria also varied with little information provided in relation to mode of feeding and the sleeping environment.^{9,50,51,53} To the best of our knowledge this is the first study to date to utilise cEEG with video over 12 h to determine sleep state organisation of MLP infants at 36 weeks PMA. All infants in this study were cared for under similar environmental conditions and without disturbances for clinical purposes.

Infant feeding in our unit is time-based, generally 3 hourly for infants learning to feed or 4 hourly when larger volumes are tolerated orally. Infants that had a mixed mode of feeding overnight had a longer TST ($p < 0.001$). This is the first study to report these findings. With respect to guidelines for establishing feeding in preterm infants we use passive tube feeds combined with oral feeds offered on an individualised basis. Allowing the infant to sleep and grow through passive feeding supports neurodevelopment and consequently the development of the infants suck swallow coordination. When TST in both mode of feeding groups was broken down by sleep state, the more immature infants who were partially tube fed had a longer AS duration in comparison to infants who fed exclusively orally ($p < 0.001$). Achieving a balance between supporting the infant in establishing feeding and protecting sleep is key.

There is much we do not understand about sleep. Theunissen et al. proposed that the exact role of sleep in brain development is unknown suggesting that perhaps they may be only temporally related, or they are independent, or one may drive the other.⁵⁴ We do not know what factors are particularly destructive to infant sleep architecture and brain development. However, there is a general consensus that an environment supporting the preservation of good quality sleep without disturbance will strengthen sleep architecture and support optimal brain development.⁵⁵

This is the largest study to date focusing on the sleep of the healthy uncomplicated MLP infant through overnight cEEG monitoring with video carried out at a similar PMA under similar environmental conditions. These factors reduced the risk of sleep being compromised by morbidity or environmental exposure during EEG monitoring. The number of male and female participants was reasonably balanced as was the number of multiple and singleton pregnancy's, removing bias related to these factors in the study. We also identified mode of feeding for each infant, a factor that has not been widely reported in previous sleep studies. Previous EEG sleep studies have based percentages of each sleep state on the interpretation of a single sleep cycle or short periods of cEEG.^{23,24,43,56,57}

This study also had some limitations. Our study was conducted in an open-plan neonatal unit where we aimed to minimise environmental stimuli by caring for infants in a quiet low-lit area of the unit during monitoring. However, we did not measure ambient sound or control for potential confounding factors such as environmental temperature, sleeping position or clinical workload. We did not use EOG like many studies but we did use video to assess overall behavioural observations including ocular and facial movements.

As variations in infant sleep play an important role in cognitive development and physical growth,⁵⁸ future sleep research incorporating sequential and prolonged cEEG with video should be considered the gold standard. This will provide an objective robust estimate of TST and a greater insight into sleep architecture unique to GA. It will also provide confirmation of achievement of sleep state organisation appropriate for GA which may be a potential biomarker of future outcome.^{57,59} Alteration in EEG sleep organisation or the maturation trajectory may be an expression of brain dysfunction.⁶⁰ Further research is also required to assess any associations between sleep variables and long-term developmental outcomes.⁶¹

Sleep staging in preterm infants is complex, labour intensive and requires expertise. A growing body of researchers are using machine learning to design algorithms such as seizure detection algorithms.⁶² Sleep detection algorithms, detect specific features of sleep,⁶³ automate sleep staging,⁶⁴ and estimate brain maturity.⁶⁵ Automated approaches have shown great promise in providing robust quantitative data pertaining to sleep organisation and perhaps identifying future biomarkers of developmental outcome. Identifying infants who warrant ongoing surveillance and early intervention will potentially improve outcome, which is the ultimate aim of neonatal care.

CONCLUSION

This is the first study to describe overnight 12-h sleep architecture in a large homogenous group of MLP infants using cEEG with video. Our findings provide robust normative data for each week of GA unique to the healthy MLP infant group. Quantitative changes in sleep state may be used as a reference point in future studies and a potential biomarker of developmental outcome.

DATA AVAILABILITY

For this current study, it is not possible to share data sets. The clinical data are collected under written proxy consent from the participants' guardians/parents that did not include permission for sharing or open data. To be allowed to share these

data under Irish Health Research Regulations we are required to re-consent families or obtain approval from the Health Regulation Consent Declaration Committee.

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AUTHOR CONTRIBUTIONS

M.A.J.R., G.B.B., E.M.D. and S.R.M. made a substantial contribution to the conception and design of the study. M.A.J.R. made a substantial contribution to the acquisition of data and wrote this manuscript. V.L. and M.P.O.S. contributed significantly to the statistical analysis and interpretation. G.B.B., E.M.D., V.L., M.P.O.S. and S.R.M. contributed to the drafting, revising and critically reviewing intellectual content. All authors approve this final version and agree to its submission.

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COMPETING INTERESTS

The authors declare no competing interests.

CONSENT TO PARTICIPATE

Written informed parental consent was obtained for each participating infant.

ADDITIONAL INFORMATION

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Neurodevelopmental outcome of low-risk moderate to late preterm infants at 18 months

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Background: Of the 15 million preterm births that occur worldwide each year, approximately 80% occur between 32 and 36 + 6 weeks gestational age (GA) and are defined as moderate to late preterm (MLP) infants. This percentage substantiates a need for a better understanding of the neurodevelopmental outcome of this group

Aim: To describe neurodevelopmental outcome at 18 months in a cohort of healthy low-risk MLP infants admitted to the neonatal unit at birth and to compare the neurodevelopmental outcome to that of a healthy term-born infant group.

Study design and method: This single-centre observational study compared the neurodevelopmental outcome of healthy MLP infants to a group of healthy term control (TC) infants recruited during the same period using the Griffith's III assessment at 18 months.

Results: Seventy-five MLP infants and 92 TC infants were included. MLP infants scored significantly lower in the subscales: Eye-hand coordination (C), Personal, Social and Emotional Development (D), Gross Motor Development (E) and General Developmental (GD) ($p < 0.001$ for each) and Foundations of Learning (A), ($p = 0.004$) in comparison to the TC infant group with Cohen's d effect sizes ranging from 0.460 to 0.665. There was no statistically significant difference in mean scores achieved in subscale B: Language and Communication between groups ($p = 0.107$).

Conclusion: MLP infants are at risk of suboptimal neurodevelopmental outcomes. Greater surveillance of the neurodevelopmental trajectory of this group of at-risk preterm infants is required.

KEYWORDS

preterm birth, premature infant, moderate to late preterm, developmental outcomes, Griffiths III

Introduction

The World Health Organisation (WHO) has acknowledged that whilst reducing mortality for newborns is the priority there is also a need to prioritise improving health, psychosocial well-being and the learning potential of children, particularly in the early years of life (1). Over the past decades improvement in NICU care and technology has led to increased survival rates of preterm infants. However, despite this success, gains in survival have not been paralleled by improvements in morbidity.

Abbreviations

MLP, moderate to late preterm; TC, term control; GA, gestational age.

Of the 15 million preterm births that occur worldwide each year, approximately 75% to 84% occur between 32 and 36 + 6 weeks gestational age (GA) and these infants are defined as moderate to late preterm (MLP) (2, 3). Current trends have shown that the rise in the rate of preterm birth is primarily attributed to the MLP infant group (4). This may in part reflect the increased use of artificial reproductive technology and its association with multiple pregnancies and preterm delivery and consequently an increased demand for neonatal unit services (5).

In the last six weeks of pregnancy, there is a 35% to 40% increase in brain weight with a five-fold increase in brain volume (6). After birth, the MLP infant is no longer protected by the uterine environment and this critical period of accelerated development takes place externally in the NICU or postnatal wards (7). A challenging external environment may influence neural connectivity (8). Disruption of the typical process of brain development may expose preterm infants to long-term neurodevelopmental impairment. Whilst a more positive outcome is associated with the low-risk uncomplicated preterm infant (9, 10) defining "uncomplicated" may prove problematic as preterm birth itself is atypical and its aetiology may involve deteriorating fetal or maternal health, which in itself may lead to suboptimal outcomes. Maturity at birth has a direct impact on outcome; the lower the GA of the preterm infant, the greater the impact on mortality and morbidity (11–14). Low birth weight associated with preterm birth further increases risk factors for the preterm infant (15).

The availability of neurodevelopmental outcome data for healthy MLP infants is often hindered by the variation in follow-up between institutions internationally (16) and the belief that these infants are low risk, and hence require little developmental surveillance. This limits our ability to assess the prevalence of impairments in this healthy MLP infant group. This study aims to establish the natural neurodevelopmental baseline scores for a healthy homogenous group of MLP infants admitted to the neonatal unit at birth. Secondly, we aim to compare the neurodevelopmental outcome of the MLP group to that of a healthy term-born infant's group at 18 months postmenstrual age, recruited during the same period.

Methods

Cork University Maternity Hospital is the largest Maternity Hospital in the south of the Republic of Ireland, serving the local and surrounding areas, and is a referral centre for high risk pregnancies.

Participants

All participating MLP infants were recruited from the neonatal unit between July 2017 and September 2018 as part of a single-centre observational sleep study. Inclusion criteria required infants to be born between 32 and 36 + 6 weeks GA, admitted to the neonatal unit at birth, a normal physical/neurological exam for GA conducted by the clinical team at birth, clinically stable,

no actual or suspected chromosomal abnormalities and a normal cranial ultrasound if performed.

As this study places a narrow focus on the natural neurodevelopment of a healthy MLP infant group, this study specifically excluded infants with a complicated neonatal stay requiring an escalation in medical care including surgery, treatment of sepsis, requiring prolonged invasive respiratory support, requiring a blood transfusion or persistent hypoglycaemia or hyponatraemia. There is no universally accepted threshold for safe blood glucose concentrations (17) despite the fact that hypoglycaemia has been identified as a risk factor for suboptimal development (18). No MLP infant had a diagnosis of hypoglycaemia based on discharge summary reports. Transient hypoglycaemia may have occurred as part of the normal adaption to extrauterine life (19) in some infants but hypoglycaemia was not a concern for any MLP infant (local protocol defines hypoglycaemia as laboratory blood sugar level <2.8 mmol/L (45 mg/dl)).

Infants requiring treatment for physiological jaundice which resolved with phototherapy were included in this study and were identified in the discharge summary report. No MLP infant required prolonged phototherapy or phototherapy for reasons other than normal physiological jaundice. All infant's enteral feeds progressed in volume as per local feeding regime and were tolerated well, with full oral feeds (breast or bottle) established for a minimum of 48 h prior to discharge. In 2016, the year prior to the commencement of this study, 64,097 infants were born in Ireland and 3.8% were multiple births. Forty six percent of multiple births were born between 32 and 36 weeks GA (20). We therefore included MLP infants from singleton and multiple pregnancies in this study. This percentage has since increased to 49.8% in 2020 (21).

During the same period, a cohort of healthy term-born infants (37–42 weeks birth GA) were recruited on the postnatal wards as part of a randomised clinical trial of a massage intervention (ENRICH study—Clinicaltrials.gov NCT03381027). The non-intervention arm of this clinical trial served as the term control (TC) group for the current study. Parents of infants with no metabolic or genetic anomalies, not requiring specialist ongoing care and not requiring admission to the neonatal unit, were invited to participate. The TC group excluded infants from multiple pregnancies.

Outcome

As per hospital criteria, cranial ultrasounds (CRUS) were routinely performed on MLP infants <1,500 g or if clinically indicated.

Infants from both groups (MLP and TC) returned to the INFANT Early Life Lab for a neurodevelopmental assessment at 18 months PMA (+2 weeks). Identifying, quantifying and predicting suboptimal development requires a valid and reliable standardised neurodevelopmental assessment tool. The Griffiths III mental developmental scale (MDS), is a norm-based validated neurodevelopmental assessment and was used in this study. The Griffiths III was validated on a representative sample of infants born in Ireland and the UK (22). Children were seated at a low table beside their parent/guardian during the assessment which

took on average approximately 45 min. The same group of experienced assessors performed assessments for both study groups. Due to the nature of the study and the assessment used, we were unable to blind assessors to group membership.

The Griffiths III provides an overall general neurodevelopmental score and an individual profile of strengths and weaknesses across five areas or subscales of neurodevelopment (23). Subscales of development include: Foundations of Learning (curiosity, communication creativity, learning and memory), Hearing/speech and communication, Eye-hand coordination coordination, Personal-social performance, and Gross Motor development. Each subscale is equally weighted and is a complete and separate scale in itself. Developmental scores were calculated for each subscale of development based on the mean score obtained for each group (24). Input from primary caregivers is required for this assessment with some subscales requiring more input than others (25). Caregiver input confirms the infant's ability to carry out specific activities in the home that may not be completed on the day of assessment. In calculating scores achieved with Griffiths III, "ratio transformation" is used which divides developmental age in months by psychologists age giving rise to slightly different means and standard deviations for each subscale of development (26).

Using the Irish Central Statistics Office criteria (27) we categorised employment as: professional, lower professional, manager/technical/administration, non-manual or skilled. Data relating to maternal occupation for the TC group data was obtained from a maternal questionnaire completed as part of the original clinical trial. Data relating to maternal occupation of the MLP group was obtained from the maternal electronic health record system if documented. No data was available on paternal occupation.

Research ethics

Ethical approval was granted by the Clinical Research Ethics Committee (CREC) of the Cork Teaching Hospitals. Parents of all infants included gave informed consent for participation and publication of the results. Consent was sought as per good clinical practice (GCP) ICH GCP E6 (R2) guidelines. Data that was recorded followed General Data Protection and Regulations (GDPR 2016/679).

Statistical analysis

Demographic and clinical characteristics of the MLP and TC groups were described using the median and inter-quartile range (IQR) for continuous variables and frequencies and percentages for categorical variables. Comparisons of demographic variables between the MLP and TC groups were made using the Mann-Whitney U-test for continuous variables and using the chi-squared test or Fisher's exact test (in the case of small expected counts) for categorical variables.

Griffiths DQ scores were described using the mean and standard deviation (SD) and the independent samples *t*-test was used to

compare scores between MLP and TC groups. Cohen's *d* formula was used to determine the standardized mean difference between the MLP and TC groups. A *d* of 0.2 was considered a small effect size, 0.5 a medium effect size and 0.8 a large effect size. To adjust for the potential confounding effect of sex, linear regression analysis was performed with sex and group included as independent variables in the model. As the type of pregnancy (singleton vs. multiple) was also a potential confounder and there were no multiples included in the TC group, linear regression analysis, unadjusted and adjusted for sex was also performed using the singleton infants only.

Within the MLP infant group analysis, a comparison of DQ scores between two groups (e.g., sex and pregnancy type) was performed using an independent samples *t*-test and more than two groups (e.g., gestational age groups (week)) using a one-way ANOVA. Infants in the MLP group were defined as having a normal outcome if their general development DQ score was within 1 SD of the mean score of the healthy term control group [mean (SD): 119 (10)].

Statistical analysis was performed using IBM SPSS Statistics (version 25.0, IBM Corp, Armonk, NY, USA). All tests were two-sided and a *p*-value <0.05 was considered statistically significant.

Results

One hundred and one MLP infants and ninety seven term control (TC) infants were recruited at birth and enrolled in this study. The median (IQR) birth GA (weeks) was 34.0 (33.0–35.0) for the MLP group and 39.9(38.9–40.9) for the TC group. There was no difference in maternal age between groups (*p* = 0.785). As expected the TC group had significantly higher birthweight and larger head circumference than the MLP group (*p* < 0.001 for both) (Table 1). A higher percentage of infants in both groups were male (MLP 52.5% and TC 57.7%). Participants from TC and MLP groups were predominantly Irish Caucasian (89.7% and 93.1% respectively) which reflects the local population. Whilst 96.7% of mothers of TC group were employed, maternal occupation was not documented for two participants (2.2%). In the MLP group, 74.7% of mothers were employed with maternal occupation not documented in 18.7% of the group. There was no significant difference in employment category between TC and MLP infant groups (*p* = 0.784).

Within the MLP group, 52.5% (53/101) were from singleton pregnancies. MLP infants from a singleton pregnancy (*n* = 53) had significantly higher birthweight than infants from a multiple pregnancy (*n* = 48) (median (IQR): 2.27(1.95–2.65) kg vs. 1.97 (1.78–2.14) kg, *p* < 0.001); however, this difference in weight between infants from a singleton (*n* = 36 and multiple pregnancy (*n* = 35) did not persist at 18 months (median(IQR): 11.29(10.80–12.49) kg vs. 11.37(10.60–12.24) kg, *p* = 0.666). In the MLP group, nine infants had a birth weight < 10th centile for GA i.e., small for gestational age. Cranial ultrasounds were performed on three infants (all <1,500 g), as per local guidelines, all of which were normal prior to discharge.

TABLE 1 Demographics.

Birth	Term (n = 97)	MLP (n = 101)	p-value ^a
	median (IQR)	median (IQR)	
Maternal age (years)	35.0 (32.0 to 37.0)	35.0 (31.0 to 38.0)	0.785
Gestational age at birth (weeks)	39.9 (38.9 to 40.9)	34.0 (33.0 to 35.0)	
Birth weight (kg)	3.60 (3.29 to 3.83)	2.10 (1.88 to 2.40)	<0.001
Head circumference (cm)	35.20 (34.55 to 36.10)	32.00 (30.80 to 32.90)	<0.001
	n (%)	n (%)	
Sex			0.457
Male	56 (57.7)	53 (52.5)	
Female	41 (42.3)	48 (47.5)	
Nationality			0.435
Irish caucasian	87 (89.7)	94 (93.1)	
Non-Irish caucasian	8 (8.2)	7 (6.9)	
Asian/African	2 (2.1)	0 (0.0)	
Pregnancy			
Singleton	97 (100.0)	53 (52.5)	
Multiple	0 (0.0)	48 (47.5)	
18 months	Term (n = 92)	MLP (n = 75)	
	median (IQR)	median (IQR)	p-value ^a
Weight (kg)	11.50 (10.70 to 12.60)	11.36 (10.75 to 12.40)	0.518
Height (cm)	83.00 (80.00 to 85.00)	81.65 (79.43 to 84.00)	0.079
Head circumference (cm)	48.80 (47.53 to 49.50)	48.00 (47.00 to 48.50)	<0.001
Chronological age ^b	18.46 (18.17 to 18.72)	19.68 (19.32 to 20.50)	<0.001
Adjusted age on assessment ^b	18.00 (18.00 to 18.00)	18.0 (18.0 to 20.0)	<0.001
	n(%)	n(%)	
Sex			0.091
Male	55 (59.8)	35 (46.7)	
Female	37 (40.2)	40 (53.3)	
Nationality			0.010
Irish caucasian	83 (90.2)	75 (100)	
Non-Irish caucasian	7 (7.6)	0 (0.0)	
Asian	2 (2.2)	0 (0.0)	
Pregnancy			
Singleton	92 (100)	38 (50.7)	
Multiple	0 (0.0)	37 (49.3)	
Maternal occupation			0.070 ^c
Unemployed	0 (0.0)	2 (2.7)	
University student	1 (1.1)	3 (4.0)	
Not documented	2 (2.2)	14 (18.7)	
Employed	89 (96.7)	56 (74.7)	0.784 ^d
Professional	57 (64.0)	40 (71.4)	
Lower professional	2 (2.2)	2 (3.6)	
Manager/technical/admin	14 (15.7)	8 (14.3)	
Non-manual	10 (11.2)	5 (8.9)	
Skilled	5 (5.6)	1 (1.8)	
Not documented	1 (1.1)	0 (0.0)	

MLP, moderate to late preterm infants; IQR, interquartile range; kg, kilogram; cm, centimetre.

A p-value <0.05 is considered statistically significant.

Missing data as not recorded in electronic health records or failure to obtain at 18 months.

In MLP group missing data includes: head circumference at birth (n = 2) and at 18 months includes: weight (n = 4), height (n = 1), head circumference (n = 6), and maternal occupation (n = 14).

In term control group missing data includes: head circumference at birth (n = 4) and at 18 months includes weight (n = 4), height (n = 3), head circumference (n = 16), and maternal occupation (n = 2).

^aFrom the Mann-Whitney U-test for continuous variables and from the Chi-squared test or Fisher's exact test for categorical variables.

^bDelay in 18 months assessment for MLP (n = 14) infants due to Covid restriction.

^cFrom Fisher's exact test based on comparisons between groups with 'not documented' group excluded.

^dFrom Fisher's exact test based on comparisons between employment groups with 'not documented' group excluded.

At 18 months (+2 weeks) PMA 74.3% (75/101) of the MLP infant group returned for follow up. The large fall out rate ($n = 26$) may in part have been associated with Covid 19 and some families chose to withdraw and some parents were not contactable or known to have moved overseas. Assessments were delayed for a number of MLP infants ($n = 14$). These infants were assessed at 22 months PMA as soon as restrictions were lifted and Griffiths scores were adjusted according to PMA. There was no significant difference in birth demographics between MLP infants who were lost to follow up and those who returned at 18 months. Follow up of the TC group was completed prior to the global pandemic and hence study retention was not impacted by same. In the TC group 94.8% (92/97) returned for a Griffiths developmental assessment at 18 months(+2 weeks) (Table 1).

Comparison of neurodevelopmental outcome between MLP and TC groups

General development DQ scores and the subscales of development scores for the MLP and TC groups are described and compared in Table 2. The mean(SD) GD DQ score in our healthy TC group was 119.45 (10.78) and the MLP infant group was 112.12(13.67). DQ values for the TC group were significantly higher than published values of 109(19). These higher scores seen in the control group may be due to the fact that these infants were low risk, term infants with normal Apgar scores and did not require admission to the NICU. It may also be due to the fact that this is one of the first studies published using the Griffiths III and may present valuable data on real world use in the Irish population.

The mean DQ score in all subscales of development and overall general development were significantly higher in the TC group compared to the MLP group with the exception of subscale B: Language and Communication. The greatest differences were in Gross Motor development ($p < 0.001$, Cohen's $d = 0.665$) and Eye-hand co-ordination ($p < 0.001$,

Cohen's $d = 0.648$). Being within ± 1 SD from the mean is considered normal for data.

Adjustment for sex

As sex was a potential confounder in the relationship between DQ scores and group, an adjusted analysis was performed (Table 3). Larger differences were found between the two groups in the adjusted analysis compared to the unadjusted analysis.

After adjustment for sex, mean scores were significantly higher in the TC group compared to the MLP group for all subscales, including subscale B: Language and Communication ($p = 0.028$).

Adjustment for pregnancy type (singleton v multiple) and sex

Pregnancy type was a potential confounder in the relationship between DQ scores and group. As the TC group only included singleton infants, we performed an analysis restricted to singleton infants ($n = 92$ in the TC group and $n = 38$ in the MLP group, Table 4). In the unadjusted and adjusted analysis, mean scores were significantly higher in the TC group compared to the MLP group for the general development DQ score and all subscales of development except for subscale B: Language and Communication.

MLP group only—investigation of variables associated with neurodevelopmental outcome

There were no significant differences in outcomes by birth gestational age group. (Table 5) Female infants outperformed male infants subscale B: Language and Communication ($p < 0.001$), subscale C: Eye-hand coordination ($p = 0.003$), subscale D: Personal, Social and Emotional ($p < 0.001$), and overall general development (GD) ($p < 0.001$). (Table 5) There was no difference in overall general developmental (GD) scores between infants born from singleton and multiple pregnancies ($p = 0.319$).

TABLE 2 Neurodevelopmental outcome of term control (TC) and moderate to late preterm (MLP) infant group at 18 months.

	Term control (TC) Group ($n = 92$)	Moderate to late preterm (MLP) Group ($n = 75$)	Difference in means (95% CI) ^a	p -value ^b	Cohen's d^c
	mean (SD)	mean (SD)			
Subscale A DQ Foundations of Learning	112.46 (11.92)	106.45 (14.30)	6.00 (2.00 to 10.01)	0.004	0.460
Subscale B DQ, Language and Communication	107.99 (14.85)	103.97 (17.19)	4.02 (-0.88 to 8.91)	0.107	0.252
Subscale C DQ, Eye - hand coordination	116.16 (10.75)	108.36 (13.45)	7.80 (4.11-11.50)	<0.001	0.648
Subscale D DQ, Personal, Social and Emotional	119.04 (8.17)	113.56 (10.51)	5.48 (2.63-8.34)	<0.001	0.590
Subscale E DQ, Gross Motor	130.30 (13.58)	120.85 (14.94)	9.45 (5.09-13.81)	<0.001	0.665
GD DQ, General Development	119.45 (10.78)	112.12 (13.67)	7.33 (3.59-11.06)	<0.001	0.602

DQ, developmental quotient score; SD, standard deviation; CI, confidence interval.

A p -value <0.05 is considered statistically significant.

^aDifference is TC-MLP.

^bFrom independent samples t -test.

^cCohen's d formula was used to determine the standardized mean difference between the MLP and TC groups.

TABLE 3 Comparison of mean scores at 18 months between TC and MLP infants unadjusted and adjusted for sex.

	Unadjusted analysis	p-value ^b	Analysis adjusted for sex	p-value ^c
	Difference in means(95% CI) ^a		Difference in means(95% CI) ^a	
Subscale A DQ Foundations of Learning	6.00 (2.00 to 10.01)	0.004	6.55 (2.55 to 10.55)	0.001
Subscale B DQ, Language/Communication	4.02(-0.88 to 8.91)	0.107	5.30 (0.58 to 10.02)	0.028
Subscale C DQ, Eye-hand coordination	7.80 (4.11 to 11.50)	<0.001	8.56 (4.93 to 12.19)	<0.001
Subscale D DQ, Personal/Social and Emotional	5.48 (2.63 to 8.34)	<0.001	6.20 (3.44 to 8.96)	<0.001
Subscale E DQ, Gross Motor	9.45 (5.09 to 13.81)	<0.001	9.76 (5.36 to 14.16)	<0.001
GD DQ, General Development	7.33 (3.59 to 11.06)	<0.001	8.25 (4.63 to 11.87)	<0.001

CI, confidence interval.

A p-value < 0.05 is considered statistically significant.

^aDifference is TC-MLP.^bFrom linear regression analysis with group as the independent variable.^cFrom linear regression analysis with group and sex as the independent variables.

TABLE 4 Comparison at 18 months between TC (n = 92) and MLP (n = 38) groups, singleton infants only.

	Unadjusted analysis	p-value ^b	Analysis adjusted for sex	p-value ^b
	Difference in means (95% CI) ^a		Difference in means (95% CI) ^a	
Subscale A DQ Foundations of Learning	6.09 (1.59 to 10.59)	0.008	6.38 (1.86 to 10.91)	0.006
Subscale B DQ, Language and Communication	0.02 (-5.61 to 5.64)	0.996	0.79 (-4.77 to 6.35)	0.779
Subscale C DQ, Eye-hand coordination	5.95 (1.86 to 10.05)	0.005	6.49 (2.43 to 10.54)	0.002
Subscale D DQ, Personal, Social and Emotional	4.23 (1.19 to 7.27)	0.007	4.72 (1.73 to 7.70)	0.002
Subscale E DQ, Gross Motor	11.57 (6.08 to 17.06)	<0.001	11.80 (6.26 to 17.33)	<0.001
GD DQ, General Development	5.76 (1.69 to 9.83)	0.006	6.39 (2.40 to 10.39)	0.002

CI, confidence interval.

A p-value < 0.05 is considered statistically significant.

^aDifference is TC-MLP.^bFrom linear regression analysis with group as the independent variable.^cFrom linear regression analysis with group and sex as the independent variables.

Significant differences by pregnancy type were only evident in subscale B: Language and Communication ($p = 0.040$) in which singleton infants outperformed infants born from a multiple pregnancy. All SGA infants, (four infants from singleton pregnancies and 5 infants from multiple pregnancies) achieved normal Griffiths III General Development DQ score at 18 months.

Discussion

In two carefully categorised prospective healthy cohorts we have shown that MLP infants had significantly lower scores compared to TC infants in the Griffiths III MDS at 18 months. This study shows that MLP infants are vulnerable to suboptimal neurodevelopment. There were no significant differences in scores found in Subscale B (Language and Communication) between the two groups ($p = 0.107$) however, when we controlled for sex, a significant difference was evident between the groups ($p = 0.028$). In a study by Putnick et al. inclusive of very preterm infants ($n = 204$), moderate-late preterm ($n = 276$) and term born infants ($n = 268$), language was assessed at 5 months, 20 months, 4 years, 6 years and 8 years of age (28). Very preterm children consistently performed less well than term-born children, with moderate-late preterm children also identified as being at risk for poorer language performance compared to term-born children. By comparison, Perez-Pereira et al. compared language

development of low risk preterm infants ($n = 150$, mean GA of 32.62 weeks) and term born children ($n = 49$, mean GA of 39.70 weeks) up to 30 months and concluded that low risk preterm children were not at risk of delayed language development (29).

Both MLP and TC infant groups scored high in Subscale E (Gross Motor). However, statistically significant differences were evident between the groups ($p < 0.001$) with the TC group out-performing the MLP infant group. When the MLP group was subdivided by pregnancy type, differences were evident in Subscale B (Language and Communication) in which MLP infants from singleton pregnancies significantly outperformed MLP infants born from a multiple pregnancy ($p = 0.040$). A systematic review comparing the outcome of infants from twin and singleton pregnancies described differences in language and communication but proposed that the likely cause was due to the parental demands of managing two infants (30). Thorpe identified language delay in twins as mild but prevalent especially in male infants (31). It is not certain if low birth weight in multiple pregnancies may be a potential confounder which is associated with 60% of twin pregnancies (32).

Capobianco et al. compared the outcome of preterm infants (average birth GA of 32 weeks and birth weight of 2,200 g) with no neurological sequelae ($n = 20$) and a normal Bayley's III score (>85) at 18 and 24 months with term born infants (average birth GA of 39 weeks and birth weight of 3,500 g) ($n = 20$). The preterm infant group demonstrated significantly slower language acquisition but these differences were only significant at 16 months

TABLE 5 | Relationships between the degree of prematurity (GA week group), sex, pregnancy type and outcome of MLP infants at 18 months, $n = 75$.

Gestational age group (weeks)	Subscale A: Foundations of Learning		p -value ^a	Subscale B: Language and Communication		p -value ^a	Subscale C: Eye-hand coordination		p -value ^a	Subscale D: Personal, Social and Emotional		p -value ^a	Subscale E: Gross motor development		p -value ^a	General development		p -value ^a
	mean(SD)			mean(SD)			mean(SD)			mean(SD)			mean(SD)			mean(SD)		
32 ($n = 12$)	108.58 (12.26)		0.978	109.17 (7.38)		0.450	110.25 (9.72)		0.741	116.50 (6.93)		0.573	120.50 (13.35)		0.713	115.25 (6.09)		0.585
33 ($n = 13$)	106.31 (14.23)			101.54 (20.70)			108.15 (15.09)			114.38 (16.52)			125.08 (14.61)			112.46 (16.86)		
34 ($n = 28$)	105.57 (16.68)			100.14 (18.31)			105.79 (14.30)			111.00 (10.02)			118.29 (18.92)			108.82 (15.14)		
35 ($n = 10$)	105.50 (12.38)			108.70 (20.99)			111.60 (14.86)			115.10 (7.94)			120.20 (8.60)			114.00 (12.98)		
36 ($n = 12$)	107.33 (13.67)			106.42 (13.49)			110.00 (12.02)			114.42 (8.36)			123.17 (10.32)			114.75 (12.69)		
Sex			0.075			<0.001			0.003			<0.001			0.582			<0.001
Male ($n = 35$)	103.31 (16.69)			95.80 (19.38)			103.46 (15.89)			109.26 (12.41)			119.83 (14.49)			106.66 (16.53)		
Female ($n = 40$)	109.20 (11.34)			111.13 (11.00)			112.65 (9.08)			117.33 (6.66)			121.75 (15.44)			116.90 (8.14)		
Pregnancy type			0.959			0.040			0.230			0.298			0.216			0.319
Singleton ($n = 38$)	106.37 (11.46)			107.97 (14.51)			110.21 (10.69)			114.82 (7.48)			118.74 (16.20)			113.68 (10.41)		
Multiple ($n = 37$)	106.54 (16.89)			99.86 (18.89)			106.46 (15.71)			112.27 (12.90)			123.03 (13.39)			110.51 (16.36)		

SD, standard deviation.

A p -value <0.05 is considered statistically significant.^aFrom independent samples t -test when a variable has two categories (sex, pregnancy type) and from one-way ANOVA when the variable has more than two categories (GA group).

($p < 0.001$) and 18 months ($p < 0.005$) but not at 20, 22, 24 months of age (33). However, in the Copabianco study, assessments were based on chronological age rather than corrected age. Using the Bayley's II developmental assessment, Gould et al. described differences in scores achieved by preterm infants ($n = 554$) based on chronological age vs. corrected age as 17.3 points lower on the mental scale (79.5 vs. 96.8 respectively) and 11.8 points lower on the motor scale (84.8 vs. 96.6, respectively) at 18 months (34).

In the MLP group, we did not find any significant differences in outcome related to birth GA. This may be because of low numbers at each gestational age group meaning that we were underpowered to examine this effect. In a similar study investigating the neurodevelopmental outcome and social-emotional competence of MLP infants vs. a term control infant group at 2 years, Cheong et al. also found little evidence of an association with gestational age (7).

As infants in both studies had an uncomplicated neonatal stay / hospital stay on post-natal wards, factors such as suboptimal brain development and/or a high frequency of mild brain injuries (35), maternal well-being, obstetric/fetal complications warrant consideration in explaining the differences in developmental outcome between the groups. Pre-eclampsia, male sex and interventional delivery have been identified as antenatal/perinatal risk factors for poor school performance, which Johnson et al. also identified as independent risk factors for cognitive impairment (14).

Other determinants of neurobehavior and intellectual development include level of parental education, socioeconomic status, cultural background and maternal mental health conditions which may be considered additional risks for suboptimal outcome (36–41).

Socioeconomic metrics may be derived from many different aspects including maternal education (40). Maternal education is the most strongly associated dimension of a child's neurocognitive development (42). Detailed data on maternal education were not available for our MLP cohort. Of those employed similar profiles of maternal occupation were seen, with 71.4% of mothers of MLP infants identified as professionals requiring a minimum of a university degree whilst 64.0% of TC group fell into the same category. These high percentages may in part reflect the Organisation for Economic Co-operation and Development (OECD) findings that 60% of women in Ireland between 25 and 34 years and 51% of women between 25 and 64 years have a tertiary education (43). It also may reflect the local community of 210,000 persons from which the cohort was recruited which is home to world leading information technology and software development organisations, large pharmaceutical companies, the largest acute tertiary hospital outside the country's capital and a high ranking university, all of which have high employment of professionals. Finally, it may also be reflective of the professional mothers who agree to take part in birth cohort studies, which is generally higher than the general population. We have reported similar high rates of tertiary education in a previous cohort study (44).

As 18.7% of mothers in MLP group (vs. 2.2% of TC group) had an undocumented occupation this led to employment analysis of a smaller group ($n = 56$) of MLP infants. This may have impacted the differences in percentage of employed professionals in the MLP

infant group (71.4%) versus TC group 64.0% (57/89). It is reassuring that the differences in neurodevelopmental outcome are not due to an imbalance in maternal employment between the two groups ($p = 0.784$).

The results of our study are consistent with those of Johnson et al. ($n = 638$ MLP infants and $n = 765$ TC infants) (14) describing female infants outperforming male infants in the MLP group with significantly higher scores in overall general development ($p < 0.001$). However, the Johnson study was based on parental questionnaires in comparison to our study which used a well-validated neurodevelopmental assessment tool.

A gender disparity is evident in the literature with male infants having a greater mortality and respiratory morbidity than female infants which is not explained by neonatal factors (45). In a review of outcomes of male and female preterm infants, O'Driscoll et al. described an increased vulnerability of male preterm infants which they suggest is likely to be multifactorial and perhaps includes hormonal and genetic factors and immunological differences between males and females (46). Romeo et al. described significant gender differences in low-risk preterm infant groups ($n = 69$ very preterm and $n = 71$ late preterm infants) and no gender differences amongst term born infants ($n = 48$) based on the scores achieved in the Bayley's scales of Infant Development, 2nd edition at two years (47). However no gender differences were reported by Romeo et al. at 12 and 24 months using the Griffiths II standardised assessment tool which the authors suggested was attributed to a higher ratio of female to male participants (F:M = 1.6:1) (48).

The risk of preterm birth and infant mortality rates are higher in multiple pregnancies (49, 50). Almost half of the MLP infants in this study were from a multiple pregnancy (49.3%). General development DQ scores of MLP infants from singleton ($n = 38$) and multiple pregnancies ($n = 37$) at 18 months were comparable ($p = 0.319$). Restricting the analysis to singletons in both groups did not change our findings (Table 4).

Overall cognitive performance of children born moderately preterm (MPT) and MLP have been described as consistently lower in comparison to the term-born group (51). A study by Cheong et al. (7) of MLP and term-born infants concluded that the greatest difference between the groups was in the language domain at 2 years (-11.4 (95% CI, -15.3 to -7.5). However, our study has not shown a statistically significant difference between the MLP and TC groups in the domain of language and communication ($p = 0.107$). These differences may in part be explained by the differences in the timing of the assessment and the assessment tool used; Cheong et al. used the Bayley Scales of Infant Development assessment (Third Edition) at 2 years corrected age.

Fernald and Marchman cite individual differences in language proficiency which can accelerate and abate at different time points for each infant (52). Our single assessment provides a snapshot of language development at one-time point at 18 months, which may be too early to detect significant language delay and the clinical relevance of our findings on long term outcomes has yet to be established. We accept that neurodevelopmental deficits may be quite subtle and frequently not become evident until preschool or school-age years and may change positively or negatively at an older age (53). All impairments, irrespective of degree, have the

potential to limit professional options, life opportunities and overall quality of life.

In this study, MLP infants had significantly lower scores in the personal, social and emotional subscale of development ($p < 0.001$) with a moderate effect size (Cohen's $d = 0.590$) than the TC group. Research has shown an increased risk of delayed social competence, with social behavioural issues higher amongst MLP infants in comparison to term-born infants (54). MLP infants also have a predisposition to more challenging behavioural issues with an increased risk of hyperactivity and attention deficit hyperactivity disorder (ADHD) (51). However, the association between prematurity and hyperactivity has not been firmly established (55). Children with learning, social, motor and sensory impairments that challenge their performance prior to or during school-going years may be considered to have special educational needs (SEN). Increased SEN are well described in MLP -born groups (56). Odd et al. identified MLP infants as being 50% more likely to have SEN requiring support in comparison to healthy term-born infants (57).

A strong correlation has been shown between prematurity and a diagnosis of cerebral palsy (CP) with the absolute risk of CP increasing with decreasing birth GA. Pierrat et al. reported that 1.0% of infants born between 32 and 34 weeks GA weeks ($n = 1,187$) had a diagnosis of CP at 2 years of age (58). Although more mature preterm infants generally have better outcomes, MLP infants have a six times higher prevalence of CP than children born at term (7 per 1,000 vs. 1.1 per 1,000 live births) (59). This may be related to the incidence of brain lesions in MLP infants described previously on cranial ultrasound (35) the clinical relevance of which warrants further study including long-term follow-up.

Our study has shown that the greatest difference between the MLP and TC groups was in the area of gross motor development ($p < 0.001$, Cohen's $d = 0.665$) and eye-hand co-ordination ($p < 0.001$, Cohen's $d = 0.648$) with a moderate effect size for each subscale. In this study no MLP infants had a diagnosis of CP at 18 months based on neurological exam nor were there any concerns about motor impairment. However, mild motor impairment may only become apparent beyond 18 months. Williams et al. suggest that motor impairment (either CP or non-CP e.g. Developmental Coordination Disorder) may be subtle and not evident until early childhood years and may be the most common form of impairment in the preterm population (60).

Strengths and limitations

This study provides robust data to describe the developmental outcome of a large group of healthy MLP infants. Homogeneity is an important factor for identifying norms for specific groups of preterm children and a major strength of this study. All MLP infants had an uncomplicated journey through the neonatal unit with no neurological concerns and were considered healthy at discharge. Both the MLP and TC groups were recruited over a similar period of time (2017–2018) and infants were assessed by the same group of highly experienced assessors at the same time point of 18 months PMA with the exception of some MLP infants with a delayed assessment due to Covid restrictions.

Assessing infants based on PMA or corrected age decreases the transient developmental gap that MLP infants experience whilst they catch up to infants born at term (61). A failure to correct for GA at birth has implications for intervention programmes. Differences in scores may assist parents/schools in decision-making relating to the age of starting school. Placing the child in the correct year for PMA rather than the school year based on calendar age has led to greater academic success (57). The MLP infant group included infants from singleton and multiple pregnancies. With close to half MLP infants born from a multiple pregnancy, inclusion of multiples in our MLP group ensures a true representative group.

A neurological exam was normal in the MLP group and infants were considered healthy prior to discharge. At the time of this study (2017–2018) healthy preterm infants >32 weeks had a cranial ultrasound performed if <1,500 g ($n=3$) or neurological concerns ($n=0$) as CRUS was not standard practice in the care of MLP in our unit at the time of study recruitment. Boswinkel et al. identified minor brain lesions on CRUS and/or MRI in over 30% of MLP infants (35). As this was not standard practice in our unit at the time of study recruitment, we cannot rule out the presence of minor brain abnormalities in the MLP group, which may have influenced neurodevelopmental outcome. Neurological status was based on clinical neurological examination. Infants did not have a structured neurological exam such as the Hammersmith Infant Neurological Exam at follow up appointments in neonatal clinics. This is a limitation of this study. However, infants did have a clinical neurological examination by a neonatal consultant in outpatients and if any concerns arose, infants would have been referred for further assessment and/or intervention.

Participants were recruited from a single research site reflecting the local population. As a result, there was a lack of diversity in our population which limits the generalisability of our findings.

Follow up of the TC group was completed prior to Covid restrictions. These restrictions may be considered an unavoidable limitation of this study resulting in a delay in the assessment of a small group of MLP infants ($n=14$). Multiple births were an exclusion-criteria for the TC group. All MLP participants were admitted to the neonatal unit to the exclusion of those cared for in postnatal wards. This may have had implications in terms of the level of stress or sensory exposure for the MLP infant in comparison to the TC group (62).

Research has shown that neurodevelopmental delay is more prevalent amongst MLP infants from disadvantaged socioeconomic groups. As this data was not available in our study, we utilised maternal education levels (where documented) as a proxy for socioeconomic status. This data was not recorded for 18.7% of MLP infants. Another possible limitation includes the potential for bias in scoring infants in neurodevelopmental assessments as assessors were not blinded to the study group.

Future research

There is limited data available on long-term neurodevelopmental outcome of MLP infants with an uncomplicated neonatal stay and a normal neurological exam on discharge. Institutional practices

relating to pre-discharge screening including CRUS or MRI and post discharge neurological surveillance vary (16). Standardising screening practice based on findings from multicentre longitudinal research studies with expert input would identify best practice for follow up of this at risk group.

Accuracy in determining the presence and degree of suboptimal neurodevelopment is dependent on the reliability and validity of the assessment tool, the assessment time point and the experience of the assessors. Hence identifying a screening tool(s) and screening intervals appropriate to all infant groups is a priority. Surveillance of preterm infants presents a unique opportunity for early diagnosis of neurodevelopmental delay and intervention with targeted therapies. As neurodevelopmental delay can be transitory or sustained we do not know what these suboptimal scores at 18 months will mean for this MLP infant group in the future. Early assessment is not always predictive of school-age cognitive functioning, long-term learning disabilities or academic achievement (63). Ongoing neurodevelopmental surveillance and sequential screening are especially pertinent to infants who have a previous abnormal assessment.

Conclusion

MLP infants are at higher risk of suboptimal outcome at 18 months in comparison to term-born infants. This study highlights the effect of moderate to late preterm birth on neurodevelopmental outcome. Our findings support the need for a broader effort for longitudinal surveillance of the MLP infant group including those who appear healthy.

Data availability statement

For this current study, it is not possible to share data sets. The clinical data are collected under written proxy consent from the participants' guardians/parents that did not include permission for sharing or open data. To be allowed to share these data under Irish Health Research Regulations we are required to re-consent families or obtain approval from the Health Regulation Consent Declaration Committee.

Ethics statement

The studies involving humans were approved by Clinical Research Ethics Committee (CREC) of Cork Teaching Hospital. The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation in this study was provided by the participants' legal guardians/next of kin.

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Author contributions

MR: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Writing – original draft, Writing – review & editing. DM: Formal analysis, Methodology, Supervision, Writing – review & editing. ED: Formal analysis, Methodology, Supervision, Writing – review & editing. SM: Conceptualization, Formal analysis, Methodology, Supervision, Writing – review & editing. VL: Data curation, Formal analysis, Methodology, Supervision, Writing – review & editing. GB: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Resources, Writing – review & editing.

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Conflict of interest

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

The author(s) declared that they were an editorial board member of Frontiers, at the time of submission. This had no impact on the peer review process and the final decision.

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Publications co-authored

- Wei L, Ventura S, **Ryan MA**, Mathieson S, Boylan G, Lowery M, Mooney C. "Random Forest-based Algorithm for Sleep Spindle Detection in Infant EEG," *2020 42nd Annual International Conference of the IEEE Engineering in Medicine & Biology Society (EMBC)*, Montreal, QC, Canada, 2020, pp. 58-61, doi: 10.1109/EMBC44109.2020.9176339.
- Ventura S, Mathieson SR, O'Toole JM, Livingstone V, **Ryan MA**, Boylan GB. Electroencephalographic sleep macrostructure and sleep spindles in early infancy. *Sleep*. 2022;45(1):zsab262. doi:10.1093/sleep/zsab262
- Garvey, A. A., O'Toole, J. M., Livingstone, V., Walsh, B., Moore, M., Pavel, A. M., Panaite, L., **Ryan, M. A.**, Boylan, G. B., Murray, D. M., & Dempsey, E. M. (2022). Evolution of early cerebral NIRS in hypoxic ischaemic encephalopathy. *Acta paediatrica (Oslo, Norway : 1992)*, 111(10), 1870–1877.
- Smith V, Delaney H, Hunter A, Torgerson D, Treweek S, Gamble C, Mills M, Stanbury K, Dempsey E, Daly M, O'Shea J, Weatherup K, Deshpande S, **Ryan MA**, Lowe J, Black G, Devane D. Training trial recruiters: an educational and training intervention for recruiters to neonatal trials. Final report. Health Research Board Trials Methodology Research Network (HRB-TMRN), University of Galway and Trinity College Dublin: Oct 2022.
- ER de Groot, **MA Ryan**, C Sam, O Verschuren, T Alderliesten T, J Dudink , A van den Hoogen. Evaluation of Sleep Practices and Knowledge in Neonatal Healthcare Advances in Neonatal Care, August 2023 DOI: 10.1097/ANC.0000000000001102

Presentations to date

- UCC Conference on nursing and child health. 2018
Poster Presentation: 'Consent practices in Irish neonatal units'.
- Irish Research Nurses Network (IRNN) Annual meeting 2018
Oral presentation: 'Consent, ethics and the law'
- Joint European neonatal societies (JENS), Maastricht, Netherlands 2019
Oral Presentation: 'Sleep disturbances in the NICU'
- Paediatric Academic Society (PAS) Toronto, Canada
Poster Presentation: 'Nocturnal sleep architecture of preterm infants'
- Fetal Neonatal Neurology- Virtual 2021
Oral presentation: A nurse's perspective on sleep in the NICU
- Joint European Neonatal Society (JENS) 2021 September-
Oral presentation; 'Monitoring **S**leep through **EE**g in **P**reterm infants - The SLEEPi study'
- Neonatal Research Symposium, November 2021,
Poster presentation: Sleep organisation of moderate to late preterm infant. (Best post presentation award)
- European Academy of Paediatric Societies (EAPS), Barcelona, October 2022
Oral presentations x 2

'Developmental Outcome of the Moderate to Late Preterm infant' '

Sleep organisation of the Moderate to Late preterm infant'
- Neonatal Research Symposium, Cork, November 2021

Oral Presentation; Comparing the developmental outcome of the Moderate to Late preterm infant to the term born infant.
- Neonatal Sleep Talks(NEST) 2023

Oral Presentation X 2

Sleep state organisation in healthy moderate to late preterm infant
Quantitative analysis of quiet sleep in moderate to late preterm infants
- Joint European neonatal societies (JENS), Rome September 2023
The role of EEG in sleep protection in the NICU

