Sensory Development in the Newborn Infant: Modulation by early life experience and the impact of brain injury.



Gabriela Schmidt Mellado
St Cross College
Department of Paediatrics

This thesis is submitted for the degree of Doctor of Philosophy (DPhil) in Paediatrics
October 2021

ABSTRACT

Neurological examination in newborn infants is challenging and novel methods of evaluating how multiple sensory systems are affected are needed to compliment developmental care interventions and treatments in the neonatal intensive care (NICU) setting. Infants who are born prematurely and those who are born with a perinatal complication are most vulnerable to debilitating neurocognitive dysfunction. Even though in some cases the problems may arise from clearly identifiable brain lesions routinely captured by brain ultrasound, more subtle changes in brain development may not be identified until school age. Bedside neuroimaging techniques such as the recording of evoked potentials (EP) can provide information regarding the functional integrity of sensory pathways, yet due to the technical challenges of performing them and interpretational issues, they are not widely used in neonatal care. In this thesis, electroencephalography (EEG) was recorded in infants born between 27 – 42 weeks gestational age (GA) and evoked responses to different types of sensory stimulation were analysed using advanced data driven analysis techniques. The spatial and temporal aspects of evoked activity in response to visual, tactile, and noxious stimulation in healthy term infants were characterised, and morphological templates were constructed for each stimulus modality. These templates were designed such that they could be projected onto data collected in independent infant cohorts to evaluate the early maturation of visual and somatosensory processing in prematurely-born infants and to establish how brain activity is altered by brain damage and hypothermia.

Using this data-driven analytical approach, the normative development of stimulus evoked activity was studied longitudinally in infants between 28-36 weeks post menstrual age (PMA). This study demonstrated the refinement of the spatial organisation of the stimulus-evoked activity with age and show how the developmental trajectory of these responses differs with the stimulus modality. Comparisons with cross sectional data recorded from a near-term control group of prematurely-born infants shows how specific components of the tactile and visual evoked response are altered by premature exposure to the ex-utero environment. In addition, similar methods were applied to a cohort of infants with neonatal hypoxic ischaemic encephalopathy (HIE) which were compared to a healthy term control group. Tactile and noxious stimulation in infants with HIE evokes significantly reduced magnitude EP responses compared with healthy term infants.

This thesis demonstrates how novel methods of evoked potential analysis can be used to evaluate evoked brain activity in newborn infants to address clinically relevant questions across various infant cohorts. In this thesis, I show how normal ex-utero sensory development is potentially affected by early exposure to the ex-utero environment. This supports the importance of developmental care interventions in the NICU. I also show the significant impact of neonatal HIE on somatosensory processing, highlighting the important effects of sedation on the interpretation of evoked potentials in this cohort of infants.

ACKNOWLEDGEMENTS

I would like to dedicate this thesis to my grandmother, Maria Luisa Mellado Leon ("Tita") who sadly passed away before I finished writing it and is sorely missed.

My first thanks go to my supervisors Rebeccah Slater and Ana Alarcon Allen for their support and guidance throughout the course of this research degree. Rebeccah, thank you for this opportunity, your positivity and enthusiasm for research in this field has been inspiring. Ana your expertise and feedback has been invaluable.

I would also like to thank my colleagues (past and present) from the Paediatric Neuroimaging group for their assistance during studies and for sharing the highs, lows, and rewards of neonatal research. In particular, I would like to thank Kirubin Pillay for his friendship and positivity for the entire time we have worked together. Without his problem-solving skills and determination much of this work would not have been possible. And a special mention to Luke Baxter for his contribution to the manuscript which includes parts of this thesis work. I feel very privileged to have worked with such a skilled group of people.

I am also very grateful to all the parents of the infants who were involved in this research, and the staff in the maternity and neonatal unit for their help accommodating studies.

Thanks also to my colleagues from the Clinical Neurophysiology department at the John Radcliffe Hospital in Oxford, where I carried out my clinical physiologist training. In particular, I would like to mention Dr Zenobia Zaiwalla, Susan Towle and the paediatric team Deborah Cox, Victoria Stepney, Marie Burke and Claire Bibey who taught me the skills I needed to carry out this research and inspired my passion for paediatric neurophysiology.

Finally, I would like to thank my family who have shaped the person I am today. My mother Gabriela Mellado for her support, good advice and friendship, to my father Dr Enrique Manuel Schmidt for his support, and to my brothers Daniel and Kenny. A special thanks to Daniel Drew who encouraged me to study this degree and for his love and support throughout. And finally, my incredible children Leo and Rafi, for making me happy and taking care of each other so that I could complete this thesis.

CONTENTS

ABSTRACT ..... **II**
ACKNOWLEDGEMENTS ..... **III**
CONTENTS ..... **IV**
LIST OF TABLES ..... **VIII**
LIST OF FIGURES ..... **IX**
LIST OF ABBREVIATIONS AND ACRONYMS ..... **XI**
LIST OF APPENDICES ..... **XIV**
CHAPTER 1 THESIS OVERVIEW **1**
1.1 INTRODUCTION 1
1.2 BRAIN DEVELOPMENT IN EARLY INFANCY 4
1.3 THE NEURODEVELOPMENT OF SENSORY CIRCUITRY 8
1.3.1 Visual Pathway Development 10
1.3.2 Auditory pathway development. 11
1.3.3 Somatosensory pathway development. 13
1.3.4 Descending tracts: Modulation of pain 15
1.4 EFFECTS OF PRETERM BIRTH ON SENSORY DEVELOPMENT 16
1.5 PAIN IN INFANTS 18
1.5.1 Epidemiology and consequences of pain in infants. 20
1.5.2 Measuring pain in infants. 21
1.6 ELECTROENCEPHALOGRAPHY 23
1.6.1 History of Neonatal Encephalography. 24
1.6.2 Maturation of EEG background in preterm and term infants. 25
1.6.3 Sensory evoked potentials in preterm and term infants. 27
CHAPTER 2 GENERAL METHODS **32**
2.1 HOSPITAL SETTING 32
2.2 ETHICS 33
2.3 PARTICIPANTS 33
2.4 RECRUITMENT 34
2.5 RECORDING TECHNIQUES 35
2.5.1 EEG acquisition. 35
2.5.2 EMG recording. 36
2.5.3 Behaviour and physiology. 36
2.6 STIMULATION TECHNIQUES 37

2.6.1 Non-noxious stimulation. 37
2.6.2 Noxious Stimulation. 39
2.7 ANALYSIS 42
2.7.1 EEG Analysis. 42
2.7.2 EMG Analysis. 44
2.7.3 Behaviour and physiology (PIPP-R Score). 45
CHAPTER 3 A FRAMEWORK TO CHARACTERISE EVOKED ACTIVITY IN NEONATES 47
3.1 INTRODUCTION 47
3.2 AIM 51
3.3 PARTICIPATING INFANTS 51
3.4 EXPERIMENTAL DESIGN 52
3.5 RECORDING TECHNIQUES 53
3.6 EEG ANALYSIS OF STIMULUS EVOKED BRAIN ACTIVITY 54
3.6.1 Pre-processing of EEG data. 54
3.6.2 Deriving the temporal templates. 54
3.7 RESULTS 58
3.7.1 Visual inspection of raw amplitude displays stimulus-specific spatial patterns of activity in healthy full-term infants. 58
3.7.2 Cluster analysis reveals stimulus- specific temporal patterns of activity in healthy full-term infants. 59
3.7.3 Principal Component Analysis (PCA) identifies stimulus specific components of activity corresponding to previously identified spatial changes in activity. 61
3.7.4 Derived templates for characterising stimulus- evoked activity. 62
3.8 DISCUSSION 63
3.8.1 Advantages and limitations of acquisition methods. 64
3.8.2 Advantages and limitations of characterising evoked responses using temporal templates. 65
3.9 CONCLUSION 67
CHAPTER 4 SENSORY DEVELOPMENT IN PRETERM INFANTS 69
4.1 INTRODUCTION 69
4.2 STUDY AIMS 76
4.3 METHODS 77
4.3.1 Participating infants. 77
4.3.2 Experimental design. 80
4.3.3 Data Acquisition. 81
4.3.4 Stimulation techniques. 81

4.4 ANALYSIS 81
4.4.1 EEG Analysis. 81
4.4.2 EMG Analysis. 82
4.5 STATISTICAL ANALYSIS 82
4.6 RESULTS 83
4.6.1 Spatial organisation of stimulus-specific activity in preterm infants demonstrates a refinement towards term age. 83
4.6.2 The magnitude of the stimulus- evoked response in preterm infants matures with age. 91
4.6.3 Latency is not a significant factor contributing to the maturity of stimulus evoked activity in premature infants. 92
4.6.4 Visual and tactile evoked activity is differentially affected by premature exposure to the environment. 92
4.6.5 Reflex withdrawal in response to noxious stimulation. 94
4.7 DISCUSSION 95
4.8 STRENGTHS, LIMITATIONS AND FUTURE WORK 106
4.9 CONCLUSION 108
CHAPTER 5 THE IMPACT OF NEONATAL HYPOXIC-ISCHEMIC ENCEPHALOPATHY ON SOMATOSENSORY PROCESSING 109
5.1 WHAT IS HIE 109
5.2 ANIMAL STUDIES OF HIE 111
5.3 PATHOPHYSIOLOGY OF HIE 113
5.4 CONCEPT OF NEUROPROTECTION: THERAPEUTIC HYPOTHERMIA 115
5.5 CLINICAL MANAGEMENT OF INFANTS WITH HIE 119
5.5.1 Clinical Neurologic Evaluation. 119
5.5.2 Biochemical markers of HIE. 121
5.5.3 Phases and Methods of Therapeutic Hypothermia. 122
5.5.4 Supportive care of the infant with HIE undergoing Therapeutic Hypothermia. 122
5.6 PAIN IN HIE 123
5.7 DIAGNOSTIC INVESTIGATION AND NEUROIMAGING IN HIE 126
5.7.1 Cranial ultrasonography (CUS). 127
5.7.2 Magnetic Resonance Imaging (MRI). 127
5.7.3 Electrophysiology in HIE 128
5.8 STUDY AIMS 138
5.9 METHODS 138
5.9.1 Hospital setting. 138

5.9.2 Participating infants. 140
5.9.3 Experimental design. 142
5.9.4 Data acquisition. 143
5.9.5 Stimulation techniques 144
5.10 ANALYSIS 145
5.10.1 EEG Analysis. 145
5.10.2 EMG analysis. 146
5.10.3 Behavioural and physiological analysis. 146
5.11 STATISTICAL ANALYSIS 147
5.12 RESULTS 148
5.12.1 Tactile- evoked responses are reduced in infants with HIE compared to healthy term infants. 148
5.12.2 Compared to healthy controls, the magnitude of noxious evoked activity is significantly reduced in infants with HIE. 150
5.12.3 Peak latencies are delayed in infants with HIE compared to healthy term infants: visual inspection of latency. 152
5.12.4 HIE severity was not associated with the magnitude of tactile or noxious evoked brain activity. 153
5.12.5 Reflex withdrawal to noxious stimulation is reduced in infants with HIE compared to healthy infants. 154
5.12.6 Infants with HIE do not show significant behavioural or physiological changes in response to experimental noxious stimulation. 155
5.13 DISCUSSION 157
5.14 STRENGTHS, LIMITATIONS, AND FUTURE WORK 163
5.15 CONCLUSION 164
CHAPTER 6 DISCUSSION 165
6.1 SUMMARY OF RESULTS 165
6.2 STRENGTHS AND LIMITATIONS OF THIS RESEARCH 168
6.3 FUTURE WORK 170
6.4 CONCLUDING REMARKS 172
CHAPTER 7 REFERENCES 173
CHAPTER 8 APPENDICES 234
APPENDIX 1 PARTICIPANT INFORMATION LEAFLETS 235
APPENDIX 2 PATIENT CONSENT FORMS 245

LIST OF TABLES

Table 2.1 Summary of participant cohorts included in this thesis. 34
Table 3.1 Demographics table: Full-term control cohort. 52
Table 4.1 Infant demographics 79
Table 5.1 Infant demographics 141
Table 5.2 Peak latency values for tactile and noxious evoked brain activity. 152

LIST OF FIGURES

Figure 2.1 Schematic of electrode positions 36

Figure 2.2 Visual stimulation 37

Figure 2.3 Tactile stimulation 38

Figure 2.4 Clinical heel lance 40

Figure 2.5 Experimental noxious stimulation. 41

Figure 2.6 Flow diagram of the EEG pre-processing pipeline. 44

Figure 2.7 PIPP-R scoring form. 45

Figure 3.1 Experimental design 53

Figure 3.2 Schematic of EEG data analysis pipeline 56

Figure 3.3 Spatial distribution and average EEG activity for each stimulus. 59

Figure 3.4. Cluster analysis. 60

Figure 3.5 Principal Component Analysis (PCA). 61

Figure 3.6 Stimulus-evoked response templates. 62

Figure 4.1 Experimental design. 80

Figure 4.2 EEG activity after visual stimulation. 84

Figure 4.3 Spatial localisation of the response magnitudes and corresponding EEG activity for the visual stimulus. 85

Figure 4.4 EEG activity after tactile stimulation. 87

Figure 4.5 Spatial localisation of the response magnitudes and corresponding EEG activity for the tactile stimulus. 88

Figure 4.6 EEG activity after noxious heel lance. 89

Figure 4.7 Spatial localisation of the response magnitudes and corresponding EEG activity for the noxious stimulus. 90

Figure 4.8 Correlations between the size of the response magnitude with PMA, at the spatial location of peak activity. 91

Figure 4.9. Comparison of the stimulus-evoked responses between preterm babies at <32 weeks GA and an older, independent preterm control group at 34–36 weeks GA (both at matched PMA of 34–37 weeks). 94

Figure 4.10 EMG activity in response to noxious stimulation. 95

Figure 5.1 Sarnat scale for neonatal encephalopathy. 121

Figure 5.2 Examples of cerebral function monitoring (CFM) traces. 130

Figure 5.3 Infant with hypothermia blanket. 139

Figure 5.4 Experimental design. 143

Figure 5.5 Illustration of infant showing the three indicators of facial expression used in the PIPP-R. 147

Figure 5.6 Tactile-evoked brain activity. 149

Figure 5.7 Noxious-evoked brain activity. 151

Figure 5.8 Relationship between severity of HIE and the magnitude of evoked response to tactile and noxious stimulation. 153

Figure 5.9 EMG response to noxious stimulation. 155

Figure 5.10 Behavioural and physiological analysis. 156

List of Abbreviations and Acronyms

ACOG	The American College of Obstetricians and Gynecologists
AEP	Auditory Evoked Potential
ANOVA	Analysis of variance
ATP	Adenosine Triphosphate
BAEP	Brainstem Auditory Evoked Potential
BIIP	Behavioural Indicator of Infant Pain
BOLD	Blood Oxygenation Level Dependant imaging
CFM	Cerebral Function Monitoring
CNS	Central Nervous System
CP	Cerebral Palsy
CSF	Cerebrospinal Fluid
CUS	Cranial Ultrasound
DC	Direct Current
DWI	Diffusion- Weighted Imaging
ECG	Electrocardiogram
EEG	Electroencephalography
EMG	Electromyography
EP	Evoked Potential
ET	Endotracheal Tube
fMRI	Functional Magnetic Resonance Imaging
GA	Gestational Age
HFF	High Frequency Filter
HFOV	High Frequency Oscillatory Ventilation
HFT	High Flow Therapy (nasal cannulae)
HIE	Hypoxic Ischaemic Encephalopathy
HL	Heel Lance

IASP	The International Association for the Study of Pain
IEC	International Electrotechnical Commission
ILAE	International League Against Epilepsy
IQ	Intelligence Quotient
ISI	Inter Stimulus Interval
IVH	Intraventricular Haemorrhage
LED	Light-Emitting Diode
LEP	Laser Evoked Potentials
LFF	Low Frequency Filter
LFT	Low Flow Therapy (Oxygen therapy)
LGN	Lateral Geniculate Nucleus
MEG	Magnetoencephalography
MRI	Magnetic Resonance Imaging
MRS	Magnetic Resonance Spectroscopy
NE	Neonatal Encephalopathy
NHS	National Health Service
NICHD	The National Institute of Child Health and Human Development
NICU	Neonatal Intensive Care Unit
NIPS	Neonatal Infant Pain Scale
NIRS	Near-Infrared Spectroscopy
NITU	Neonatal Intensive Therapy Unit
NRM	Nucleus Raphe Magnus
PAG	Periaqueductal Grey
PC	Principal Component
PCA	Principal Component Analysis
PDA	Patent Ductus Arteriosus
PIPP	Premature Infant Pain Profile

PMA	Post Menstrual Age
PNS	Peripheral Nervous System
REM	Rapid Eye Movement
RMS	Root Mean Square
ROP	Retinopathy of Prematurity
SD	Standard Deviation
SEF	Somatosensory Electric Fields
SEP	Sensory Evoked Potential
SIPPV	Synchronised Intermittent Positive Pressure Ventilation
SSEP	Somatosensory Evoked Potential
STOPS	Sharp Theta on the Occipitals of Prematures
SVD	Singular Value Decomposition
TH	Therapeutic Hypothermia
TOBY	Total Body Hypothermia for Neonatal Encephalopathy Trial
VEP	Visual Evoked Potential

LIST OF APPENDICES

Appendix 1 Participant information leaflets..... 235
Appendix 2 Patient consent forms 245

Chapter 1 THESIS OVERVIEW

1.1 Introduction

Over 100,000 infants are admitted to neonatal care units every year across the UK. The majority of infants who require neonatal care are born full term (>37 weeks gestational age (GA)) but are sick, and around 40,000 are admissions due to prematurity. The prevalence of preterm births in the UK is increasing, especially infants born between 33- 36 weeks GA. Only around 1.2% of infants are born extremely preterm (<25 weeks GA), and around 10 % are born between 28-32 weeks' gestation. These percentages are very similar worldwide (1). Advances in neonatal care and technology over the last 50 years have provided neonatologists with the capacity to monitor and support the sickest of infants and in turn survival rates of neonates have increased. However, survivors continue to have a high burden of morbidity and neuromotor or sensory disabilities even in the absence of clear structural damage (2).

The five most common reasons for admissions in full term infants are due to respiratory conditions, infection, hypoglycaemia, jaundice, and hypoxic ischaemic encephalopathy (HIE). 2.5% of full-term admissions are due to HIE,

and it is the major cause of neurodevelopmental impairment at this gestational age. Mild cases of HIE may not result in long term problems, but in moderate and severe cases, infants are particularly at risk of developing cerebral palsy (CP) and other motor, sensory, behavioural, and cognitive delays (3–5). Therapeutic hypothermia has improved the outlook for infants with HIE, with significant reductions in the risk of CP, moderate or severe disability, and improved motor-function scores at 6 to 7 years of age (6). Despite this, many infants go on to develop various neurodevelopmental deficits and more sensitive tools for identifying those who may require more help later in life are needed.

As the number of infants being born early and surviving is increasing (7), there is an increasing need to understand and address the causes of neurodevelopmental impairment in prematurity. Despite the advances in neonatal care, it is not clear if neurodevelopmental outcomes have improved over time and few studies evaluate long-term outcomes at school age and beyond (8). The EPICure study in the UK reported similar rates of severe disability at 2.5 to 3 years of age. Severe disability comprised of non-ambulant CP, deafness not improved with amplification, blindness, or a developmental quotient <3 SD (Bayley III scores) in cohorts of infants born in 1995 and 2006 (7). Prevalence of impairment is inversely related to week of gestation, and the most common neurodevelopmental impairment was cognition, followed by communication and motor disabilities. Furthermore, the risks are higher in those preterm infants who are born with very low birth weight or with an associated infection (9).

The motivation for this thesis was to gain a better understanding of development of sensory function in premature and term born infants and to investigate the

impact of HIE on sensory function, with a view to improving neurodevelopmental outcomes in infants. Electroencephalography (EEG) has played a major role in supporting diagnosis, treatment and prognosis in both preterm and term infants and is widely used in neonatal care. EEG is a passive interrogation of the brain and experienced neurophysiologists are able to evaluate brain maturation and abnormal patterns of activity which can be indicative of poor prognosis. The measurement of evoked potentials in response to external stimuli is a more active investigation of the functional integrity of sensory pathways yet it is not as widely used in clinical diagnostics compared to standard EEG recordings due to the challenges in acquisition and the variation in responses observed with gestational age (10–15), sleep state (16–19), and pathology (20–25). Novel analytical methods developed in this thesis help overcome some of these challenges.

Another important consequence of requiring neonatal care after birth is the high number of painful experiences that occur in this population. Although the notion that newborn infants are incapable of experiencing pain due to an immature nervous system is unfounded, clinical procedures which are deemed painful continue to be performed without optimal pain-relief. Measuring pain in infants is challenging but there is a need to develop suitable measures to appropriately assess the efficacy of analgesic treatments in this population (26,27).

In Chapter 1, the background literature intrinsic to this thesis is discussed. First, a broad description of how the human brain develops in early life is given and the development of the main sensory pathways described. This is followed by a discussion of neonatal pain and the challenges associated with measuring

nociception. Finally, more focus is given to the role of electroencephalography (EEG) during the neonatal period, as this is the technique used in all chapters of this thesis. The EEG maturation of preterm and term infants is detailed, and the main components of sensory evoked activity observed in term-born infants in response to visual, auditory, tactile and noxious stimulation is discussed.

The overarching aims of this thesis are:

- To design a framework to measure and compare sensory evoked responses in infants
- To explore the developmental trajectory of sensory evoked potentials in infants from <32 weeks GA to near term age in a longitudinally study.
- To investigate the impact of prematurity on sensory evoked activity.
- To characterise how HIE impacts somatosensory processing in term infants.

1.2 Brain development in early infancy

The maturation of the human brain continues throughout infancy, adolescence and adulthood. Early brain development is characterised by rapid structural and functional changes, whereby neurons develop, migrate to their final locations, form networks which are fine-tuned by pruning and myelination, and set the framework for sensory, language and then higher-order cognitive functions (28). This section describes some of the critical stages in early brain development and the development of sensory pathways that are relevant to the work carried out in this thesis.

The general architecture of the human brain is formed during the first six months of fetal life. Processes are initially driven by strong ontogenetic influences, but at any stage the developing brain can be modulated by epigenetic and

environmental factors (29). As early as the 2nd week of gestation the ectoderm thickens forming the neural plate, which then creases and folds in a process called neurulation, so that by the end of the fifth week of gestation the neural tube is almost completely fused (30). The neural tube is the precursor to the central nervous system, and also gives rise to specialised tissue along its margins (neural crest) from which the peripheral nervous system including its autonomic components are derived (31).

Neural progenitor cells in the ventricular zone begin symmetrical cell division, resulting in exponential growth during the first 6 weeks of fetal life. Then these cells make a gradual shift to asymmetric division producing neurons that migrate outward initially to a layer called the preplate or plexiform layer (32). Disruption to either of these phases of cell division can alter the thickness or surface area of the cortex (33). The preplate is a transient structure, which splits to form the marginal zone and the subplate shortly before the cortical plate is formed. This sub-compartmentalization of the preplate is aided by Cajal-Retzius cells, which express reelin, and set the upper boundary of the cortical plate (34). The marginal zone and the subplate play a major role in cortical development. The marginal zone becomes cortical layer I, but the subplate is another transient structure which disappears late in gestation.

Early synaptogenesis is facilitated by the subplate, which has expanded during evolution. It consists of migratory and post migratory neurons, growth cones, axons, dendrites, synapses and glial cells (35). The subplate acts as a waiting zone where transient cellular interactions, segregation, and growth of afferents from the brainstem, basal forebrain, thalamus and the two hemispheres occur (35–37). During this stage of development, the subplate is approximately four to five

times thicker than the cortical plate (38). Compared to other species, the subplate zone is larger and is visible for longer in humans than other mammals, to support the increasing number of cortico-cortical fiber systems (35,39–41). As afferent inputs enter the cortical plate directly, the dissolution of the subplate begins at different rates for different regions of the cortex (42–44). For example, the subplate zone disappears faster in the visual cortex compared with the somatosensory areas (28,35,45), even though the somatosensory subplate appears first.

The crucial role of the subplate can be demonstrated in animal studies, whereby deletion of visual (46) subplate neurons results in the formation of aberrant pathways and failed connections with target areas of the cortex. In human preterm infants with a perinatal injury, widespread deficits in visual, motor and cognitive systems supports this observation and suggests that brain development is disrupted (47). As the most vulnerable premature infants are born around the time of peak subplate neuron development, it is possible that subplate neuron injury could be an important component leading to specific neurodevelopmental consequences later in life (48).

The cortical plate is formed by migrating neurons arranged between the outer marginal zone and the inner subplate, with the deepest layers filled in first (38). Peak migration is thought to occur between the third and fifth months of gestation. After 22 weeks' gestation, areal, laminar and cytological differentiation of the cortical plate is most significant (49), so that by the 7th month of gestation the cortex is clearly divided into 6 layers.

The process of wrinkling or folding (gyrification) of the human brain is beginning to be better understood. To begin with, folds along the neural tube define the two hemispheres and later cortical folding increases the brains surface area. Brain surface morphology shows little cortical folding in very preterm infants (50), progressively becoming denser, and by full term age the cortical folds closely resemble the gyral pattern seen in adults. Dendritic arborisation and synaptogenesis accelerate in the third trimester producing a thickening of the developing cortex which coincides with the appearance of the characteristic pattern of sulci and gyri and the increase in total brain volume (44,50,51). Experimental work on fetal monkeys (52) suggests that gyrification is related to axonal connectivity, while more recent work using mathematical and physical models of human brain organoids (53–55) propose that size, shape, placement, and orientation of the folds arise through spatio-temporal differences in mechanical stability of the cortex. Infants with reduced cortical folding, or 'lissencephaly', have severe neurodevelopmental problems, seizures, and reduced life expectancy.

In parallel with the structural development of the brain is the development of functional specialisation, which begins in primary cortical areas first, and later in association areas of the brain. Different regions of the cortex develop at different rates in humans (56), in contrast with findings from the rhesus monkey which suggested that the rate of synaptogenesis is similar across neocortical areas (57–59).

1.3 The neurodevelopment of sensory circuitry

At around 26 weeks' gestation the structural framework of thalamocortical connectivity is established, and sensory driven activity of neuronal circuits begins to develop (60). The olfactory and gustatory systems develop first, followed by the development of other sensory systems including vision, hearing and proprioception (61–63). The basic structures of the eyes, ears and primary receptors for touch develop early in gestation, but the basic neural architecture of each sensory system occurs later between 22- 40 weeks' gestation. Many aspects of sensory processing require the coordination and integration of several sensory systems, therefore although timing of critical events or sensitive periods varies across modality, they develop in close association with each other. Interestingly, this sequence of neurosensory development is not significantly altered or accelerated by preterm birth, even though many of the critical processes that would normally take place in utero occur while the infant is in the ex-utero neonatal intensive care unit (62,64). However, early sensory development can be affected or delayed if infants are exposed to adverse stimulation or if the environment is inappropriate.

Sleep and sleep cycles are essential for the development of the neurosensory and motor systems in the fetus and neonate, and interference with sleep and disruption of sleep cycles can significantly affect early processes of sensory development (65). The youngest preterm infants, 28-32 weeks GA, spend most of their time asleep and around 80% of sleep is active sleep. Quiet sleep increases with gestational age so that by term age infants spend around 50% in active and 50% in quiet sleep and spend around 70 % of their time sleeping (66,67). The decline of active sleep with gestational age is a good indicator of brain maturation

across species (68), and novel methods for assessing infant sleep to predict later neurodevelopment are being developed to this end.

Although established sleep states are not clearly defined until after 36 weeks' gestation/postmenstrual age, sleep state cyclicity is expressed in some neonates between 25-30 weeks postmenstrual age (69–71). The appropriate development of sleep organisation with gestational age is important, as active sleep has been connected to the development of the central nervous system, particularly functional competence of neuronal circuitry, whereas quiet sleep has been associated with synaptogenesis. Endogenous neuronal activation occurs during active sleep, promoting brain development when environmental experiences are usually limited (72,73), and creating permanent circuits and critical relationships (74,75). The development of all of the sensory systems and parts of the cerebral cortex, hippocampus, thalamus, brain stem, spinal cord, and auditory system are activity dependent (62). Preservation of brain plasticity, the capacity to change, adapt, and learn in response to the environment and needs is an essential role of sleep cycles (62,65).

Animal studies show the importance of sleep for plastic processes which underlie the maturation of brain circuits (76,77). Infants in the NICU are exposed to repeated environmental stimuli causing frequent disruptions to the infant's sleep/wake cycles. Preterm birth has been associated with altered sleep architecture, and prematurely-born infants spend less time in active sleep compared to infants born full term, and have shorter arousals and fewer body movements (70,71,78), with the exception of infants with chronic lung disease who show the opposite traits (79). Impaired sleep can affect sleep structure and

can result in delayed or disordered development of sensory networks (65,80) therefore promoting sleep in the NICU environment forms a significant part of developmental care practices (81–83). Sleep problems in children who were born preterm are also more prevalent than in those who were not (84), especially in those premature infants with neurological impairment (84).

1.3.1 Visual Pathway Development

The development of the complex cellular structure of the retina starts at around 24 weeks' gestation. The retina allows the eye to adapt to changing light levels and has two types of photoreceptors: rods and cones. Rods are designed to respond to low light and a wide range of frequencies but not colour. Cones respond to higher levels of light and specific wavelengths. As the eye grows rods migrate to the periphery and cones migrate more centrally in the retina without the need for light or visual stimulation (85).

Light is transduced into neural signals via the activation of photoreceptors which generate action potentials. Second order neurons in the ganglion cell layer and nerve fiber layer of the retina contain the cell bodies and axons of temporal and nasal fibers which bundle at the optic disc to form the optic nerve. Optic nerves from the two eyes emerge from their optic discs and intersect at the optic chiasm which lies in front of the pituitary gland. Nasal fibers decussate in the optic chiasm to allow crossed processing of the visual signals before proceeding along the optic tract. The majority of nerve fibers in the optic tract project to the lateral geniculate nucleus (LGN) in the posterior part of the thalamus which then relays the information to the visual cortex via the optic radiation. The retina also projects to the superior collicus which control eye movements, the hypothalamus

to regulate the circadian rhythm, and the pretectum, which control the pupillary light reflex (61,85).

The processing of visual information begins in the primary visual cortex (or striate cortex, V1) and secondary visual cortex, V2, where a representation of the contralateral visual hemifield is formed. The foveal region is mapped in the most posterior part of the occipital lobe whereas peripheral regions are mapped in progressively more anterior parts. The upper visual field is represented in the lower bank of the calcarine sulcus, the lower visual field on the upper bank. Visual information then continues along the ventral pathway which extends to the temporal lobe, also known as Meyers loop (recognition of objects), and the dorsal pathway which projects to the parietal lobe (location of objects).

The neurons in the visual cortex are formed in the germinal matrix and migrate to form six layers. The cells must be stimulated so that they move and form columns (ocular dominance columns) during the last 8 to 10 weeks of gestation (46,85). REM sleep and stimulation of the pons-geniculate- occipital waves are required to form this framework of columns (86). Disruption of REM sleep or deprivation of visual experience in experimental animals results in major disruption in the topographic arrangement of the cells in the LGN as well as failure to develop ocular dominance columns in the visual cortex (87–90).

1.3.2 Auditory pathway development

Auditory information enters the body via the external ear canal leading to the tympanic membrane (eardrum) which is connected to the cochlea via the middle

ear (containing the malleus, incus and stapes bones) (63). The vibrations of the tympanic membrane create fluid waves in the cochlea where auditory information is initially encoded. Hair cells in the organ of Corti in the cochlea convert the mechanical deflections of their stereocilia bundles into a nerve signal that transmits through the spiral ganglion and the relay nuclei in the pons and midbrain to the auditory cortex in the temporal lobe (91). From the temporal lobe, neurons connect to other parts of the cortex such as the limbic system and hippocampus (63,91).

The structural components of the auditory system develop early in gestation, so that by 20 weeks' gestation the system is structurally functional (92,93). Around 25- 29 weeks GA the ganglion cells of the spiral nucleus in the cochlea connect inner hair cells to the brainstem and temporal lobe (94) and are sufficiently mature to begin tuning for specific sound frequencies. Interestingly, the earliest evidence of an auditory evoked response was recorded in utero at 16 weeks' gestation, when connections between ganglion cells and the brainstem are functional and capable of producing a physiological response to the stimulus (95). Neural connections to the temporal lobe are functional between 28-30 weeks GA with the development of tonotopic columns in the auditory cortex (63,92). Unlike the visual system which does not require visual stimulation until after birth, auditory stimulation is required as part of normal development between 28-40 weeks GA (63,91). In the NICU environment the development of the cochlea and auditory cortex are most affected due to the high levels of unnatural high frequency background noise making it difficult for the infant to differentiate background noise and meaningful sounds like mothers' voice (63,96).

1.3.3 Somatosensory pathway development

Somatosensory pathways consist of a chain of neurons which transmit sensory information from receptors to the cortex via nerve fibers. There are two main fiber types; myelinated A-fibers, which have large diameter axons and fast conduction speeds, and unmyelinated C-fibers, which are slower and smaller in diameter (97). Broadly, Aβ fibres predominantly transmit the sensation of touch whereas nociception is predominantly transmitted via Aδ fibers, responsible for the transmission of sharp acute pain and evoke a reflex. C-fibers are the most abundant sensory neurons and can be activated by weak or dull pain and crude touch (97). In contrast to adults, infants have a higher density of high threshold Aδ and low threshold Aβ mechanoreceptors per cm^2 which respond with lower firing frequency (98,99), which may explain why infant reflexes are larger in magnitude in the youngest of infants (100,101).

The sensation of touch and pain is transmitted to the cortex via separate pathways which will be described briefly. Both pathways begin with the activation of dorsal root ganglion cells, and axons cross the midline before reaching the somatosensory cortex, but the level at which this occurs is different. The medial leminscal pathway (posterior "dorsal" column) carries and processes information regarding discriminative touch, vibration and proprioception. Cutaneous sensory neurons (low threshold mechanoreceptors) activate sensory nerve fibers located in the medial aspect of the dorsal root, enter the dorsal column of the spinal cord and ascend to the level of the medulla (102). In the brainstem, they synapse in the dorsal column nuclei so that axons arriving from the lower part of the body target the nucleus gracilis and axons from the upper

part of the body terminate in the nucleus cuneatus. The axons of second order neurons decussate and cross the medulla as a bundle (medial leminiscus) and terminate in the thalamus. From the thalamus, third order neurons project to the postcentral gyrus of the cortex, where somatosensory stimuli are initially processed, and conscious perception of touch occurs.

Nociceptive information is relayed via receptors called nociceptors, which can be found on the skin, joints, viscera and muscles. Receptors on the skin are further subdivided based on the information they are relaying, such as: high threshold mechanoreceptors, thermal, chemical and polymodal receptors. Receptors innervate primary afferent nerve fibers whose cell bodies are either in the dorsal root ganglia or trigeminal ganglion (for nerves innervating the face) and terminate in the dorsal horn of the spinal cord (97). In the dorsal horn, primary afferent fibers synapse with second-order neurons and ascend to higher centers in the brain via the contralateral spinothalamic and spinoreticular tracts. These tracts are located in the anterolateral white matter of the spinal cord, and terminate in their respective medial and lateral nuclei, before projecting to primary and secondary somatosensory cortices.

Sensory receptors, afferent connections to the spinal cord, and connections to the thalamus are in place by 20 weeks GA (103). Yet, in the earliest stages of development infants are only capable of endogenous spontaneous activity (42,104). Cortical processing of external inputs becomes possible once thalamocortical connections are present, and thalamocortical afferents reach the cortical plate around 23-26 weeks GA, developing at a similar rate to other sensory cortices. By 32 weeks the six layered structure of the cortex is apparent,

and growth of interhemispheric callosal connections and intra hemispheric associative connections mature from 35 weeks onwards (42,104). Although the framework of basic connections is in place by 25 weeks GA, discrimination between sensory inputs appears to develop later from 35-37 weeks GA (105,106).

Unlike other sensory systems, noxious input evokes a diffuse pattern of activity in different brain areas which then results in the sensory, emotional, motivational, and cognitive experience of pain (106). The insula, anterior cingulate cortex and amygdala are traditionally thought to be associated with the affective motivational components of pain, whereas the somatosensory cortices play a role in the sensory discrimination aspect of pain and these areas also interact with other brain regions associated with motor function such as the cerebellum and basal ganglia. An fMRI study by Goksan et al (2015) identified that similar areas of the brain were activated in response to noxious stimulation in infants and adults, with the exception of the orbitofrontal cortex and amygdala (107). The findings support the idea that infants are capable of cerebral processing of nociceptive input by term age but may be too immature to contextualise the experience or attach emotional significance to the sensation (107,108).

1.3.4 Descending tracts: Modulation of pain

The descending pain modulatory system is a neuronal network which includes pathways that run from the midbrain to the medulla and then to the spinal cord. In the dorsal horn, complex interactions between excitatory and inhibitory interneurons occur, and it is the site where descending inhibitory tracts from higher centers exert their effect. The periaqueductal grey (PAG) region surrounds

the aqueduct in the midbrain and receives inputs from the thalamus, hypothalamus and cortex, as well as collaterals from the spinothalamic tract (97). It plays an important role in the control of pain and sensory integration. PAG neurons also excite cells in the nucleus raphe magnus (NRM) which projects down the spinal cord to block pain transmission by dorsal horn cells (97). Endogenous pain modulation may explain one mechanism that contributes to why pain perception varies across individuals.

Although ascending nociceptive pathways are functional by 25 weeks GA, the ability to manage pain is immature in premature infants due to the increased excitation and decreased inhibition in the dorsal horns (109). This likely contributes to the exaggerated, often inappropriate, and generalised responses seen in preterm infants in response to a variety of sensory inputs (99). Indeed, research in newborn rats has demonstrated that inhibitory pathways are not functional until P10 and that proposed descending inhibitory mechanisms are not effective until P21 (109). In term infants some modulation of pain may occur in brain regions within the descending modulation system (110). Other evidence suggestive of endogenous modulation over the preterm period is demonstrated by the refinement of spinally mediated withdrawal reflexes with GA (101,111), and the influence of stress (112) and affective touch (113) on noxious evoked brain activity.

1.4 Effects of preterm birth on sensory development

Advances in perinatal and neonatal intensive care have led to a marked increase in survival rates in premature infants, but this reduction in mortality is associated with an increase in morbidity and prevalence of neurodevelopmental problems

(114). The infant brain develops rapidly over the third trimester, and a major concern for neonatologists is that in preterm infants, this rapid period of development is occurring outside the protective and optimal environment of the uterus. Preterm infants are exposed to loud sounds and bright lights which are no longer softened by the maternal abdominal wall and amniotic fluid (115,116). In addition, routine monitoring and care of infants can involve major procedural pain such as surgery or repeated minor acute pain including heel lances, venipunctures and lumbar punctures. Infants are also exposed to chronic pain from necrotizing enterocolitis or prolonged ventilation. Even routine cares such as nappy changes, weight checks, adhesive tape removal and rectal stimulation are all gestationally inappropriate experiences in the preterm infant that can cause a behavioral response that is similar to that observed when noxious stimuli are applied to the body, which may alter the development of neuronal circuits (117).

During the infant's admission to NICU, it is unclear whether the infants' experience of severe and intense stimuli is more detrimental to development than mild repetitive or inappropriate stimuli during critical periods of development (118). The neurological process of sensory integration refers to the organization of sensations from the body and the environment, and somatosensory, vestibular and visual experiences are fundamental to this process (119). Sensory processing is essential for the development of body schema, motor coordination and planning, which promotes adaptive behavioural responses and facilitates learning and participation (119). It is also important to note, that sensory integration becomes even more complex in infants with other risk factors that can contribute to poor neurodevelopmental and cognitive outcomes such as prenatal

or perinatal events or conditions, immunologic vulnerability and genetics (120,121), and socio-economic status (122).

In this context, there is a growing body of evidence that more focus on neurodevelopmental care practices, monitoring of physical environmental elements and family involvement philosophies that may favourably impact neurodevelopment of the premature newborn should be adopted in NICU (118,123,124). Developmental care refers to strategies which adjust the environment to reduce stress, support the behavioural organization and improve physiological stability of the newborn, respect sleep patterns, and generally promote the growth and maturation of the preterm infant. Some interventions such as non- nutritive sucking (125,126), massage (127), kangaroo care (128) are encouraged and form part of clinical guidelines and have been shown to reduce hospital stay by promoting self-regulation. Nevertheless, there is limited evidence supporting its benefits for behavioural, cognitive and motor development later in life (129).

All aspects of neurologic development can be affected by preterm birth (118,130). In the next section more focus is given to the effects of neonatal pain on development and the recent advances in our understanding of nociception in early life.

1.5 Pain in infants

The International Association for the Study of Pain (IASP) recently updated its definition of pain to ‘an unpleasant sensory and emotional experience associated with, or resembling that associated with, actual or potential tissue damage’’ (131)

with six key notes expanding this definition. Pain is a subjective perception influenced by biological, psychological and social factors. Pain is innately an adaptive process, but it can also cause adverse effects on an individual, and life experiences help shape a person's concept of pain. In the context of neonatal pain, a key note is that 'the inability to communicate verbally does not negate the possibility that an individual experiences pain'. Nociception is 'the neural process of encoding noxious stimuli' which refers to the transmission of nociceptive information from the periphery, along the spinal cord, to regions of the brain, and resulting in an autonomic or behavioural response. Pain and nociception are different phenomena, so it is possible to feel pain even in the absence of a noxious input, and nociception does not necessarily result in the experience of pain.

In the clinic, the gold standard for assessing pain is verbal report, as pain is a subjective experience. However, pre-verbal infants and individuals with no speech cannot express how they feel, posing a challenge for clinicians who want to minimise neonatal pain experience. Regardless of the philosophical conundrum concerning the phenomenological experience of pain, or lack of, there is evidence that nociceptive information is conveyed to and represented in the cortex from approximately 25 weeks' gestation (132,133). Thus, in the clinical setting there is a moral imperative to assume pain is, or can be, experienced in association with nociceptive stimulation even in the absence of verbal or behavioural affirmation (134).

In the next sections, the epidemiology of pain in the neonatal setting, the consequences of a high burden of nociceptive input in early life and available methods of assessing pain are described.

1.5.1 Epidemiology and consequences of pain in infants

The consequences of pain can be seen in the short-term and in the long-term. Immediately following a painful procedure, such as a blood test or cannulation, infants mount a clear biological response. For example, in preterm infants an increase in heart rate is often observed, as well as a drop in oxygen saturation and sustained facial grimacing (135). Infants have also been shown to be more clinically unstable in the 24- 48-hour period after a painful procedure with increased periods of desaturation and apnoeas (136). This has been observed after procedures such as retinopathy of prematurity (ROP) screening (136). In the long-term, cumulative exposure to pain and stress in early life has been directly correlated with a reduction in cortical thickness (137–139), detrimental changes to white matter microstructure (140,141), altered cognitive and motor function and development in toddlers (142) and school-age children (141,143) and increased pain sensitivity (123,144).

The number of painful procedures infants undergo as part of their routine clinical care is surprisingly high, and only a few studies have described the frequency and analgesic approaches for the procedures carried out in the NICU (145–147) . Infants born prematurely or those who are born sick, require the most procedures and are most at risk for developing neurodevelopmental impairment (148,149). Carbajal et al (2008) reported that in the first 14 days of admission infants undergo numerous procedures associated with pain and stress (mean of 12-16

painful procedures a day and as many as 62 in the sickest of infants). The frequency of these procedures remains relatively high throughout the infants stay in NICU, and on average, some procedures such as intravenous cannula insertion, central catheter insertion and peripheral arterial line insertion can require 4 or more attempts before completion. Importantly, the study also reported that these procedures were not being adequately medicated with analgesia (145).

1.5.2 Measuring pain in infants

1.5.2.1 Behavioural and Physiological measures

Measuring pain in infants is not simple. In infants it is not possible to get a verbal pain report, therefore surrogate measures of pain are the best indicators available for clinicians and researchers to determine if an infant requires pain relief. These surrogate measures include physiological changes such as reflex withdrawal, increase in heart rate, or decrease in oxygen saturation, and behavioural observations in facial expression and vocalisation. Variations of these measures have been incorporated into multidimensional pain assessment tools (150). The most commonly used in infancy are the Behavioural Indicator of Infant Pain (BIIP) scale (151), the Neonatal Infant Pain Scale (NIPS) (152) and the Premature Infant Pain Profile (PIPP) which was recently revised (PIPP-R) (135), to name a few, as there are many. In this thesis the PIPP-R is used to assess the physiological and behavioural responses to noxious stimulation. The PIPP-R is the best validated pain assessment tool to evaluate acute pain in the research setting in neonates of all gestational ages and reliable in infants who are ventilated (153,154).

Although multimodal pain scores are the most widely used tools for pain assessment in the neonatal setting, they have several limitations. Perhaps most importantly is the fact that pain scores are not specific enough to differentiate pain from stress. Some events, which are deemed not painful but distressing, like a nappy change, may evoke a similar behavioural and physiological response in a newborn as a heel lance (155,156). This example alone demonstrates the need for a more robust and objective measurement of pain.

1.5.2.2 Brain-derived measures of neonatal pain

Ultimately, the experience of pain is a perception generated by the brain, therefore it is feasible that in non-verbal populations brain-derived approaches may provide the most specific indication of pain processing and perception. A brief description of how non-invasive brain imaging techniques such as near-infrared spectroscopy (NIRS) and functional magnetic resonance imaging (fMRI) have advanced our understanding of pain in infants is given here, but electroencephalography and evoked potentials are described in more detail in Section 1.6 as they are the techniques used in this thesis.

Near-infrared spectroscopy (NIRS) can be used in newborn infants in the clinical setting and it is portable so it can be used cot-side. It measures regional changes in cerebral oxygenation by determining the level of absorption or near-infrared light. Several studies have demonstrated an increase in total haemoglobin concentration in the somatosensory cortex contralateral to the side of the heel of the foot being lanced, and bilaterally following venipuncture of the hand (132,133,157). These studies included preterm and term infants and provided evidence that even premature infants are able to transmit signals evoked by

noxious stimulation to the cortical level, and that this activity increases with PMA.

Functional Magnetic Resonance Imaging (fMRI) also measures changes in blood oxygenation (BOLD imaging) to infer changes in brain activity that are evoked by nociceptive input. fMRI studies suggest that the pattern of brain activity evoked by nociceptive input in the term infant is similar to that which is observed in adults when they report that they are experiencing mild pain (107). Nevertheless, there are key differences between how the neonatal and adult brain process nociceptive input (107,108,158) and this work is an active area of research.

Although behavioural and physiological measures have limited nociceptive specificity compared with other equally-arousing sensory input, brain derived measures, which primarily record the afferent nociceptive input to the brain within the first few seconds following stimulation, also do not provide a direct measure of pain, and therefore a combination of all of these approaches is most appropriate to investigate, measure and treat pain in the neonatal population.

1.6 Electroencephalography

Electroencephalography (EEG) is widely used clinically to record electrical activity to support diagnosis of pathology and to assess brain maturation. It measures changes in electrical field potentials with a high temporal resolution, and limited spatial resolution (dependent on the number of electrodes placed on the head). The activity represents a spatio-temporal average of excitatory and inhibitory post-synaptic potentials arising from synchronous activity of neurons

arranged at right angles to the surface of the cerebral cortex. Compared with other neuroimaging techniques such as near-infrared spectroscopy (NIRS) and magnetic resonance imaging (MRI), it has the best temporal resolution (435, 436). Like NIRS, it is portable and can be performed with minimal discomfort to the infant at the cot side (159). In the next sections, a brief summary of the history of neonatal encephalopathy is followed by a detail account of the development of background EEG activity in preterm and term infants and evoked potentials in term infants.

1.6.1 History of Neonatal Encephalography

In his search for 'psychic energy', the German neurologist and psychiatrist, Hans Berger, was the first to record EEG activity from humans and pioneered the field of electroencephalography (EEG). His work covered a wide range of topics (160), and in 1933 he published his first attempts to record neonatal EEG which were not successful and led him to wrongly conclude that no cerebral electrical activity was apparent before the age of 35 days (161).

In 1938 Smith first described the EEG of infants under 3 months of age (162,163) and reported the increased presence of slower frequency activity during sleep and over the sensori motor cortex, and the lower activity observed during wakefulness. In the 1940s and 1950s major contributions to our understanding of neonatal EEG were made, together with advances in electrophysiological equipment (164). The first EEG atlas was published in 1941 illustrating well developed cerebral activity in three infants at 2, 6 and 9 days of age (165). Lindsley (1942) first recorded heart and brain activity from the fetus in utero (166) and Hughes et al (1948) recorded EEG in infants over the first few days of life

(167). In 1953, Dreyfus-Brisac began to study the EEG of premature infants, and her work along with other colleagues in France (Fischgold, Sureau, Samson-Dollfus, Monod and Pichot) provided detailed accounts of the EEG of both full term and preterm newborn infants, the effect of maternal drugs on the infant's EEG, and abnormal patterns (168). By 1955 the basic EEG background patterns were described and basic terminology we are familiar with today, such as "activité moyenne" primarily occurring during wakefulness, and "tracé alternant" appearing in deeper sleep, was introduced (169). In 1999, a team of 12 physicians compiled a neonatal glossary to harmonize French and English techniques and terminology (161,170).

1.6.2 Maturation of EEG background in preterm and term infants

The EEG of preterm and term newborns develops in parallel with extensive anatomical, functional and chemical processes including neuronal and glial multiplication, structural and neurochemical synaptogenesis, and myelination (171). Afferent inputs particularly from the thalamus and brainstem drive the progressive maturation of the EEG (172).

Background EEG activity is dynamic and changes rapidly during the preterm period and after birth. In extremely premature infants the EEG appears discontinuous, with short bursts of activity interrupted by long periods of relative EEG inactivity known as inter-burst intervals (tracé discontinu). In preterm infants as young as 24 weeks gestation the inter-burst interval can last for up to 60 seconds (173,174). Periods of inactivity gradually decrease in length as the infant matures, giving rise to a more continuous EEG by 35 weeks' gestation. Bursts of activity are dominated by high amplitude (around 400μV in

infants 34 weeks GA) slow activity, which decreases in amplitude with gestation (to around 200μV by term age) (161,175). Other aspects of EEG background which evolve with gestation include inter-hemispheric synchrony and sleep/wake cycling. In preterm infants between 24-27 weeks GA the EEG shows hyper-synchronous bursts of activity (172), then becomes relatively asynchronous up to around 32 weeks, before gradually becoming more synchronous by term age. As early as 25 weeks GA cyclical patterns of activity can be detected (67) but these do not correlate with specific behavioural states until around 32-35 weeks GA, when periods of quiet sleep increase (66).

Characteristic transients of the neonatal EEG can be seen to increase/decrease in abundance with gestation, some of which have been associated with the rapid structural and maturational changes occurring in the brain. In particular, delta brush activity, described as a slow delta wave with superimposed alpha/ beta fast activity, has been a feature of great interest. Delta brushes are seen diffusely in the record of preterm infants (from 24 weeks GA), reaching a peak incidence around 33-34 weeks' gestation, and becoming more prominent over temporal and occipital regions before disappearing by full term age (161,176–178). Animal and human studies have revealed that delta brushes share many features of spindle bursts recorded from rodents providing new insights into the mechanisms and physiological roles of these early EEG features (179–182). Delta brushes are thought to play a role in the formation and strengthening of thalamocortical connectivity in the developing brain as their generation is driven by the insula and subplate (176,183). Other transient patterns include premature temporal theta (saw-tooth waveform) seen in infants between 27- 32 weeks' gestation and sharp theta on the occipitals of prematures (STOPS) mostly

dominant in younger infants between 23- 28 weeks GA. In near term and full-term infants (34-40 weeks GA) transients have a more anterior topography (frontales encoche and anterior slow dysrhythmia) (161,172).

In neonates, evaluation of developmental features, sleep–wake cycling and reactivity to external stimuli are important indicators of brain maturation. In addition, EEG is a useful diagnostic tool to support doctors' decisions in infants at risk of neurodevelopmental delay due to pathology. In the neonatal setting, EEG is mostly used to evaluate brain activity in term infants with Hypoxic Ischaemic Encephalopathy (HIE). EEG is highly predictive of outcome in this group of infants and is a focus of this thesis, therefore a more detailed section on the role of EEG in HIE is given in chapter 5.

1.6.3 Sensory evoked potentials in preterm and term infants

Although EEG is widely used to look at background activity, evoked potentials or event related potentials are not routinely assessed in neonatal intensive care units and instead neuroimaging using fMRI or cranial ultrasound (CUS) are more readily requested. Whereas neuroimaging techniques are useful to look at structure, evoked potentials provide important information regarding the integrity of sensory function. It is possible that more comprehensive functional assessments could be important for optimising care in the NICU in the future.

Three kinds of evoked potentials are in widespread clinical use; visual evoked potentials, auditory evoked potentials (recorded from the scalp but originating at brainstem level) and somatosensory evoked potentials. Other types of auditory evoked potentials such as cortical auditory evoked potentials are not routine

neurophysiological tests as they are not as diagnostically useful (more information on the different types of auditory evoked potential is given later in section 1.6.3.2). Evoked potentials are distinct from spontaneous EEG activity as they are specifically generated in response to a stimulus of known modality, and can be characterised in terms of their polarity, latency and topography. Clinically, characteristics such as latency and amplitude are usually measured after averaging many trials (~100-200 trials). In adults evoked potentials are small in amplitude, therefore averaging high numbers of trials is essential to improve the quality of the signal of interest compared to background noise (i.e. the signal to noise ratio). Due to the immaturity of the preterm nervous system, it is possible that several aspects of conventional clinical evoked potential recording and reporting could potentially distort or abolish the physiological activity of interest. This point is discussed further in chapter 3. In this section, the responses to visual, auditory, tactile and noxious stimulation seen in term infants are briefly described.

1.6.3.1 Visual evoked potentials

Visual evoked potentials (VEPs) are probably the most exhaustively studied type of sensory evoked potential. In the late 1950s and 1960s several studies investigated the normal maturation of VEPs in term and preterm infants, and the effects of perinatal disorders and asphyxia (11,184,185). VEPs can be elicited in response to luminance (flash) and contrast (pattern) changes in the field of vision. In adults, pattern VEPs are more useful clinically as they produce a simple waveform composed of 3 reproducible peaks (N70, P100 and N145). The morphology of adult flash VEPs is more variable and shows 6 major peaks (186).

In neonates, pattern VEPs cannot be obtained, but flash VEPs evoke reproducible components, which change with maturity (12,16,184,187–190).

In full-term healthy infants, components of flash VEPs include a positive deflection around 200 ms after the flash stimulus (P2), preceded by a negative deflection N1 and often followed by a later negative peak N3. Although latency of these components within an individual infant does not vary significantly, the amplitude does. Latency of the components and the amplitude of P2 is affected by sleep state, so in awake infants the latencies are shorter and amplitudes higher (191). The shorter latency components represent cortical activity evoked via the direct retino-geniculo- cortical pathway, however, the late potential is thought to originate from subcortical regions and represent afferents running via nuclei in the mesencephalic and non-specific thalamic structures (192,193).

1.6.3.2 Auditory evoked potentials

Auditory evoked potentials have also been well characterised in infants and are used as part of the UK newborn hearing screening programme. Acoustic stimulation can evoke a variety of potentials that reflect sequential activation of the auditory system; from the cochlear receptor through to the primary and secondary projection areas in the cortex. Short latency (<10ms) auditory evoked potentials (AEP) originating from the brainstem are of most clinical use as they are consistent and are unaffected by sedation and only minimally affected by anaesthesia. In contrast, middle latency and long latency (>50ms) potentials are greatly affected by state of alertness and by sedation (194) and are not commonly used clinically. Nevertheless, newborn cortical auditory evoked potentials have

been evoked by high and low frequency tones and words over fronto-central regions of the head and are characterised by a prominent positive deflection around 250ms followed by a negative peak around 400-500 ms, with earlier peaks appearing more variably. Words evoke higher amplitude responses than tones, but the morphology is very similar (195–197).

1.6.3.3 Somatosensory evoked potentials: tactile and noxious

Somatosensory evoked potentials have conventionally been recorded using electrical stimulation of peripheral nerves. Although this method is advantageous for assessing the integrity of the nervous system, electrical stimulation non-selectively activates a wide range of peripheral afferent fibers associated with various sensations. This is a limiting factor if you want to assess tactile and noxious processing separately.

The neonatal somatosensory response to tactile stimulation consists of a sequence of 4 deflections in healthy term infants: P1, N2, P2 and N3, occurring within 800ms post stimulation (199). The earlier components (P1 and N2) can be seen over the midline central region when the foot is stimulated and over the contralateral central area when the hand is stimulated. The later components (P2 and N3) are distributed more diffusely around the midcentral area for both hands and feet (198,199). The sequence of peaks are thought to represent different levels of processing, and therefore the emergence of each deflection changes and matures with gestational age (199), and amplitude is reduced during periods of active sleep (18).

Noxious evoked brain activity in adults was first identified in the 1960s (200) and since then a wide range of different stimulation types have been used. Activation of A-δ and C nociceptive afferent inputs produces a vertex positive deflection, reported in response to electrical (201,202), mechanical (201), contact heat (203,204), laser (205), and lances (206).

In 2010, Slater and colleagues first reported the evoked response generated by noxious clinical heel lancing in infants (208). The activity was characterised over the midline central electrodes (Cz and CPz) with a dominant positive peak at around 560ms post-stimulus, which was not influenced by sleep state and could be observed in single trials. This pattern of activity has been reproduced in more recent studies using clinical heel lances and mild experimental noxious stimulation (described in Chapter 2) (27,111,158,209–212).

Chapter 2 GENERAL METHODS

In this thesis, the evoked brain responses to different types of stimulation are investigated in newborn infants. The methods used to recruit infants, record their brain activity following sensory stimulation, and analysis methods are consistent across chapters. In this chapter, these common processes regarding recruitment, acquisition of EEG and EMG data, behaviour, stimulation techniques and analysis methods are outlined.

2.1 Hospital setting

This research was conducted in the Neonatal Unit and Maternity wards at the Women's Centre at the John Radcliffe Hospital, Oxford University Hospitals NHS Trust. The Women's Centre has on average 5000-6000 deliveries a year and is a regional referral unit for women with high-risk pregnancies. The neonatal unit is a Level 3 Unit with approximately 42 cots (16 intensive care, 16 high dependency and 10 low dependency). It provides intensive care for infants born in Oxfordshire and across the region. The unit admits around 800 infants a year, with around 450 of these being infants born at less than 37 weeks, and around 50 being born at less than 27 weeks' gestation.

2.2 Ethics

Ethical approval was obtained from the National Research Ethics Service. Studies were conducted as part of the ethics application titled 'Investigating pain in the developing human brain' (reference 12/SC/0447), and studies were carried out in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines.

2.3 Participants

Sixty-seven infants were recruited for the purpose of this thesis. All infants were recruited at the John Radcliffe Hospital in Oxford. In order to design a framework for analysing evoked responses in neonates, 15 healthy full-term infants were recruited (Chapter 3). In Chapter 4, n=10 healthy very preterm infants (<32 weeks gestational age (GA)) were recruited to take part in a longitudinal study and n= 8 near-term infants (34-37 weeks GA) were recruited to act as a control group. In Chapter 5, n=20 infants diagnosed with Hypoxic Ischaemic Encephalopathy (HIE) were recruited to take part in a two-session study. In addition, n=14 healthy term infants who had been recruited prior to starting this thesis were used to act as a control group in Chapter 5.

In Chapters 3 and 4, healthy infants of different gestational ages were eligible for inclusion if they were clinically stable, had no documented neurological problems including any grade of intraventricular haemorrhage (IVH), and no maternal substance abuse. In addition, none of the infants received invasive mechanical ventilation (via endotracheal tube) or analgesic or sedative medication at least 24 hours prior to the study taking place. In Chapter 5, infants were eligible to take part if they were diagnosed with Hypoxic Ischaemic

Encephalopathy (HIE) and had commenced hypothermia treatment. No infant with a metabolic disorder or a genetic abnormality was recruited. More details on inclusion criteria for each study are outlined in each chapter. A summary of infant characteristics is shown in table 2.1.

Table 2.1 Summary of participant cohorts included in this thesis.
Mean and (range) is displayed for continuous variables. Discrete variables are expressed as number or range.

	Healthy full-term cohort	Longitudinal preterm cohort	Preterm control cohort	Healthy full-term cohort 2	HIE cohort
Number of infants	15	10	8	14	20
GA at birth/ weeks	38.1 (36.1-41)	29 (27.1-31.4)	34.7 (34- 35.7)	39.2 (37-42.1)	39.4 (35.9-42)
PMA at study/weeks	38.7 (36.6-41.7)	30.4 (28 -31.9)	35.5 (34.7-36.4)	39.5 (37.3- 42.3)	39.7 (36.3- 42.3)
Number of females	6	5	3	11	6
Number of recording sessions	1	2 - 4	1	1	1 - 2
Applied stimulation	Visual, auditory, tactile and experimental noxious	Visual, auditory, tactile and clinical heel lance	Visual, auditory, tactile and clinical heel lance	Tactile and experimental noxious	Tactile and experimental noxious

2.4 Recruitment

Eligible infants were identified by screening the daily handover sheets in the neonatal unit and post-natal wards of the hospital. Parents were approached if the clinical team caring for the infant deemed it appropriate, and for infants with HIE the consultant in charge was also consulted before approaching. The study was explained verbally, and if parents wanted to think about taking part, they were given a patient information leaflet. If the study involved the use of the experimental noxious stimulus, the stimulator was shown to the parents and they were encouraged to test it on themselves. A reasonable amount of time was given for parents (at least a couple of hours, more often a couple of days) to consider

the study and allow time for questions to be answered. If parents were happy to take part, informed, written consent was then obtained. Parents were informed that participation was voluntary, would not affect any on-going care of their child, and that they were able to withdraw their baby from the study at any time. Patient Information Leaflet and consent form can be found in the Appendix 1 and 2.

2.5 Recording Techniques

2.5.1 EEG acquisition

CURRYscan7 neuroimaging suite (Compumedics Neuroscan) was used to record electrophysiological activity. The equipment has a 65-channel headbox with SynAmps RT amplifiers, and a bandwidth from DC- 400 Hz. EEG activity was recorded using a 2 kHz sampling rate. This equipment and all the patient interface attachments conformed to the electrical safety standard for medical devices, IEC 60601-1.

In Chapters 3 and 4, all recordings were performed using 25 recording scalp electrodes (Ambu Neuroline disposable Ag/AgCl cup electrodes) placed according to the international 10-20 system of electrode placement (Fp1, Fp2, F7, F8, F3, F4, A1, T3, C3, Cz, C4, T4, A2, P3, Pz, P4, T5, O1, O2, T6) and additional electrodes in positions FCz, CP3, CPz, CP4 and Oz (figure 2.1 A). A reference electrode was placed in electrode position Fz, and the ground electrode on the forehead (Fpz). In Chapter 5, a more limited number of scalp electrodes was used (8 recording electrodes in positions FCz, T3, C3, Cz, C4, T4, CPz, Oz, and the same reference and ground electrode positions) (figure 2.1 B). The skin was

rubbed with an abrasive gel (NuPrep gel, D.O. Weaver and Co.) to optimise contact with the skin and reduce resistance prior to attaching the electrode using EEG conductive paste (Elefix EEG paste, Nihon Kohden).

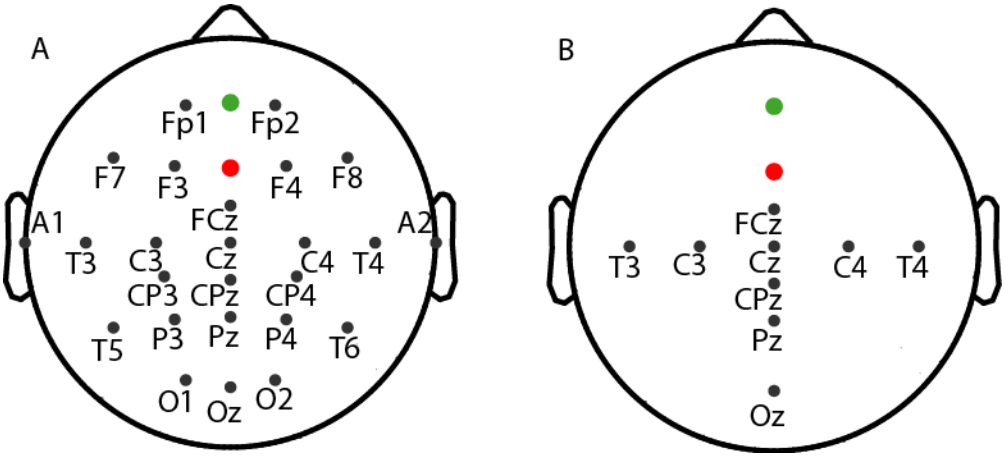


Figure 2.1 Schematic of electrode positions

A) 25 recording electrode set up and B) reduced 8 recording electrode set up. The reference electrode was placed in electrode position Fz (red dot) and the ground electrode on the forehead (green dot).

2.5.2 EMG recording

Bipolar EMG gel electrodes (Ambu Neuroline 700 solid gel surface electrodes) were placed over the biceps femoris muscle of both legs with approximately 2 cm distance between the two electrodes. Reflexes were recorded in all infants and were analysed after experimental noxious stimulation in Chapters 4 and 5.

2.5.3 Behaviour and physiology

Behavioural pain scores were calculated for infants with HIE (Chapter 5) using a validated pain scale, the PIPP-R scale. For this, facial expression, heart rate and oxygen saturation were recorded. Facial expression was recorded using a

handheld camera which was manually time-locked to the noxious stimulus using a foot pedal that turned an LED light on.

Physiology was recorded using ixTrend software (ixellence GmbH) on a Philips neonatal monitor (IntelliVue MX800 patient monitor, Philips). Oxygen saturation was recorded using a pulse oximeter probe on the infant's foot, and heart rate was recorded using electrocardiogram (ECG) leads on the chest. The point of noxious stimulation was manually marked on the monitor.

2.6 Stimulation techniques

The responses to a variety of stimuli (non-noxious and noxious) were studied in this work. Presentation of stimuli was randomised.

2.6.1 Non-noxious stimulation

2.6.1.1 Visual Stimulation

Visual stimulation was performed using the Lifelines Photic Stimulator (Lifelines Ltd). The light was placed 30 cm in front of the infant's eyes (due to limited space inside the incubator or cot this distance was reduced to 20 cm in some cases). Single flashes, with an intensity of approximately 514 lumens, were presented in a block of 20-30 trials. When the light flashed, this triggered an event mark on the EEG recording.

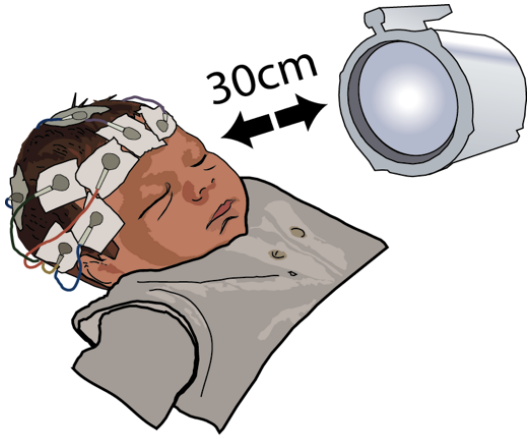


Figure 2.2 Visual stimulation

2.6.1.2 Tactile stimulation

Tactile stimulation was performed using a modified tendon hammer with a built-in force transducer (Brüel & Kjær) that triggered an event mark on the EEG at the point of stimulation. The weight of the hammer was allowed to drop and lightly touch the infant's heel, but no force was applied. The infant's foot was held loosely to allow the infant to withdraw the foot after the stimulus, if desired.

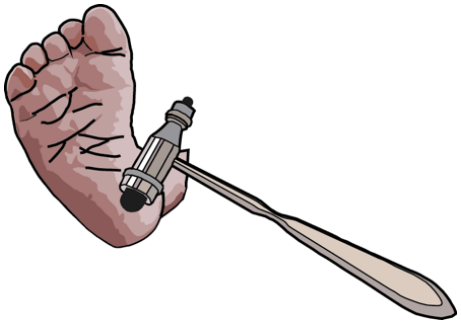


Figure 2.3 Tactile stimulation

2.6.1.3 Auditory Stimulation

Auditory stimulation was performed using two different equipment devices, however, both devices delivered a single tone with a frequency of 1000 Hz and presented 20-30 times. In n=8 infants, the tone was delivered with a duration of 100 ms using an MP3 player (ZEN StyleM300, Creative) and speakers (X-mini MAX II Portable Speakers, Xmi). The speakers were placed at either side of the infant's head approximately 10-15 cm away. The EEG was event-marked using an output trigger pulse from the MP3 player.

In n=23 infants, auditory stimulation was performed using the Surpass Ltd (EMS Biomed), which automatically triggered an event mark on the EEG. This system delivered the tone using headphones at a volume of 70 dB nHL (normalised hearing level) with a duration of 4 ms.



Figure 2.4 Auditory stimulation using headphones.

2.6.2 Noxious Stimulation

2.6.2.1 Clinical heel lance

All infants in this study were inpatients in the neonatal unit or post-natal wards during all the test occasions. These infants required blood tests as part of their routine clinical care. In Chapter 4, the test occasion was always performed on days when the infants required a heel lance to collect blood. Heel lances were not performed solely for the purpose of this study.

Heel lances are widely used in neonatal care to collect blood samples from infants who do not require a central venous or arterial line in place. The lancet triggers the release of a retractable blade, which is applied to the infant's heel, and the drops of blood that form on the skin are then collected. The release of the blade

triggered an event mark on the EEG using an event detection interface and accelerometer (211).

Heel lancing was always performed in accordance with local unit protocols by a neonatal nurse/clinical staff trained in heel lancing. The foot chosen for heel lancing was not controlled for in this study but was chosen based on clinical judgement. Depending on the age and weight of the infant, lancets with varying penetration depths were used (preterm infants: BD Quikheel Preemie Lancet, depth of 0.85 mm. Term infants: BD Microtainer Quikheel Infant Lancet, depth 1.0 mm. Becton, Dickinson and Company). Heel lances are applied to either the medial or lateral plantar surface of the heel.

The heel lance was the last stimulus to be performed, and on a few occasions, when insufficient blood was obtained after a single lance, a second one was performed and recorded when necessary. As the study was particularly focused on the acute noxious response to the release of the blade into the skin rather than the squeezing of the foot, the clinical member of staff performing the heel lance waited for 30 seconds before squeezing the foot and only collected droplets of blood that were on the skin during this time. Comfort measures such as using a dummy, swaddling or parental stroking were used where appropriate if the infant became distressed after the heel lance. At parent's request, the procedure was performed while being held in their arms.



Figure 2.5 Clinical heel lance

2.6.2.2 Experimental noxious stimulation

Experimental noxious stimulation was performed using a PinPrick™ (MRC-systems, Germany) stimulator with a force of 128 mN. These stimulators have a flat contact area of 0.25 mm in diameter with controlled edge curvature so that when the pin touches the skin, the pin retracts into the tube, and a predefined force is applied. They were originally developed for quantitative sensory testing of pain and perception thresholds in adults as they allow for reproducible measurement of A- and C- fibre activation (214,215). Pinprick stimulation has been used in infants in previous work relating to neonatal pain. It is well tolerated and does not cause behavioural distress to the infants (216).

In this thesis, the Pinprick stimulator was applied up to a maximum of 30 times on the heel of the foot of healthy full-term infants (Chapters 3 and 5) and in the cohort of term infants with HIE (Chapter 5). These infants did not require a clinical heel lance either because they were healthy or because they had a central venous or arterial line in place, as is the case of infants with HIE. In some of the earlier recordings (Chapter 5) the point where the barrel of the stimulator begins to drop was manually event-marked on the EEG using a high-speed camera (220 frames per second; Firefly MV, Point Grey Research Inc.) that was directly linked to the EEG recording. However, most recordings used a Pinprick stimulator with an in-built contact trigger device (MRC Systems) that automatically time-locked the event to the EEG recording.

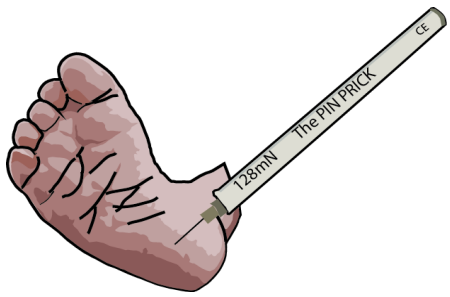


Figure 2.6 Experimental noxious stimulation.
Pinprick stimulator with a force of 128 mN.

2.7 Analysis

2.7.1 EEG Analysis

Analysis of the acquired EEG data was performed using MATLAB (R2018a) scripts. Epochs of 10 seconds (5 seconds before and after each stimulation took place) were filtered with standard filter settings (Low frequency filter (LFF)= 0.5 Hz, High frequency filter (HFF)= 70Hz and Notch= 50Hz-including harmonics) and then average referenced. In studies where the full set of 25 electrodes (figure 2.1 A) was used (chapters 3 and 4), all electrodes except behind the ears (A1 and A2), prefrontal (Fp1 and Fp2) and electrodes that were in 10/10 locations (CPz, CP3, CP4 ad FCz) were included in the calculated average. This was to reduce unwanted noise in the average from muscle and eye movements and so that the electrodes used were equally distant from each other. In Chapter 5 when a limited number of electrodes were attached (figure 2.1 B), all electrodes were included in the average. Average referencing is commonly used to derive EEG activity as it reduces the effects of using a single reference, giving a better indication of the distribution of activity in the brain.

Next, the trials were inspected visually (GSM) and were rejected if any channel contained gross artefacts such as movement and/or excessive muscle activity, or if the baseline of the signal was not stable prior to stimulation taking place. The remaining trials for each stimulus was averaged, and further removal of artefacts, such as blink and electrocardiogram breakthrough, was carried out using automated spherical interpolation.

Spherical interpolation is a method of artefact removal achieved by 1) projecting all the electrode activity onto a unit sphere, 2) creating a mapping matrix and 3) using the matrix to interpolate the data (434). The mapping matrix was created using a Laplacian montage arrangement, whereby each channel was considered adjacent to up to four neighbouring electrodes. Using this matrix, the EEG data from an artefactual channel can be interpolated based on the neighbouring clean EEG channels. For each trial average, a maximum of four channels (not adjacent to each other) were interpolated per recording.

The trial-averaged clean EEG activity was also baseline-corrected. Baseline correction is used to correct activity over the post stimulus period by subtracting the mean of the period 500ms immediately prior to stimulation (pre-stimulus mean) to each time point in the epoch.

In this thesis, a template approach for measuring evoked potentials is adopted. This approach has been used previously to calculate the magnitude of noxious-evoked brain activity in infants after a noxious stimulus (27,217). This method of evoked potential analysis uses cluster analysis to identify periods where a response is present, and Principal Component Analysis (PCA) to derive templates to measure a magnitude. These methods are described in more detail in Chapter 3, as they were developed as part of this thesis to create stimulus-specific temporal templates from a cohort of 15 healthy term infants. These modality-specific templates were then projected onto independent cohort of infants in the studies described in Chapters 4 and 5.

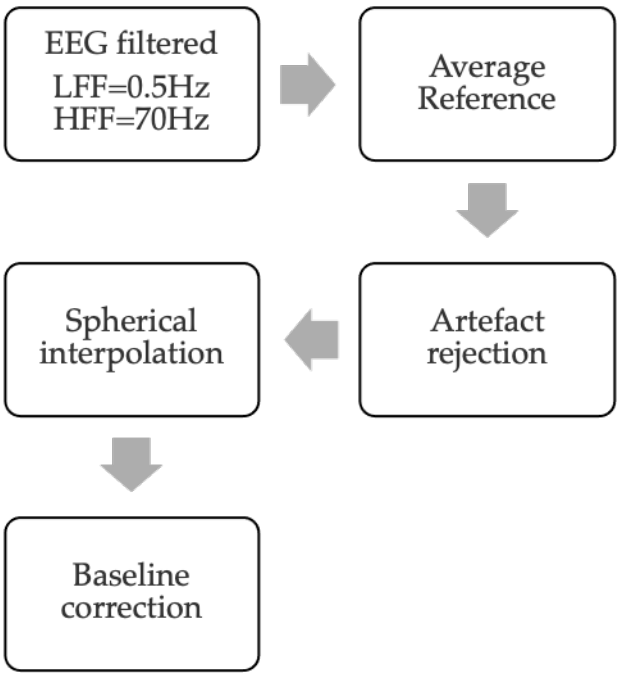


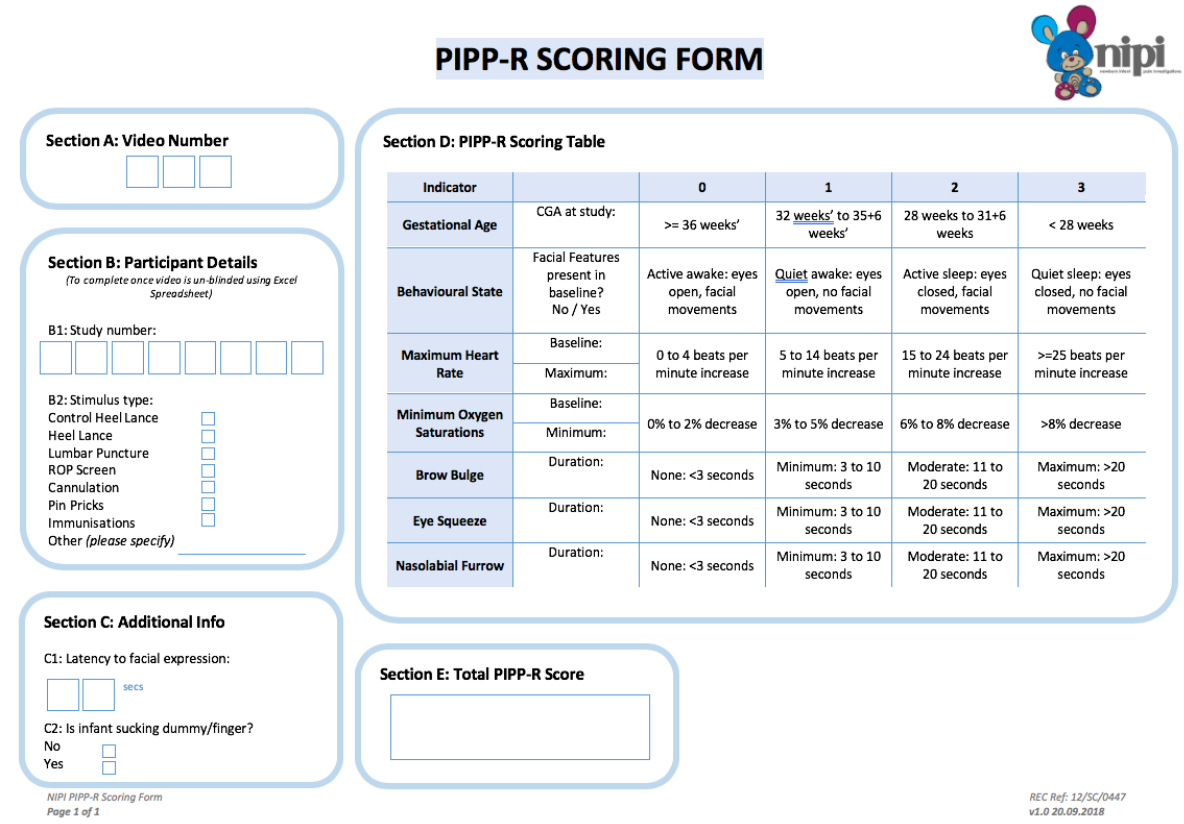
Figure 2.7 Flow diagram of the EEG pre-processing pipeline.

2.7.2 EMG Analysis

EMG activity from the ipsilateral (side being stimulated) biceps femoris was analysed when experimental noxious stimulation was applied in infants in Chapter 5. EMG signals were extracted 500 ms prior to and 1000 ms after the stimulus was applied. The signal was filtered (LFF=10 Hz, HFF=500 Hz, Notch=50Hz) and rectified before visual inspection and artefact rejection took place. Trials were rejected if the baseline was not stable prior to stimulation. The data was divided into 250 ms windows and in each window the root mean square (RMS) was calculated. The post stimulus mean was calculated as the mean across all windows (0-1000 ms).

2.7.3 Behaviour and physiology (PIPP-R Score)

Behaviour and physiology were scored using the Premature Infant Pain Profile-Revised (PIPP-R) when noxious stimulation (experimental noxious) was performed in the cohort of infants with HIE. A PIPP-R score was calculated for the first and the last trial using the criteria shown in the form below (figure 2.8).



PIPP-R SCORING FORM

Section A: Video Number

Section B: Participant Details

(To complete once video is un-blinded using Excel Spreadsheet)

B1: Study number:

B2: Stimulus type:
Control Heel Lance
Heel Lance
Lumbar Puncture
ROP Screen
Cannulation
Pin Pricks
Immunisations
Other *(please specify)*

Section C: Additional Info

C1: Latency to facial expression:

secs

C2: Is infant sucking dummy/finger?
No
Yes

Section D: PIPP-R Scoring Table

Indicator		0	1	2	3
Gestational Age	CGA at study:	>= 36 weeks'	32 weeks' to 35+6 weeks'	28 weeks to 31+6 weeks	< 28 weeks
Behavioural State	Facial Features present in baseline? No / Yes	Active awake: eyes open, facial movements	Quiet awake: eyes open, no facial movements	Active sleep: eyes closed, facial movements	Quiet sleep: eyes closed, no facial movements
Maximum Heart Rate	Baseline: / Maximum:	0 to 4 beats per minute increase	5 to 14 beats per minute increase	15 to 24 beats per minute increase	>=25 beats per minute increase
Minimum Oxygen Saturations	Baseline: / Minimum:	0% to 2% decrease	3% to 5% decrease	6% to 8% decrease	>8% decrease
Brow Bulge	Duration:	None: <3 seconds	Minimum: 3 to 10 seconds	Moderate: 11 to 20 seconds	Maximum: >20 seconds
Eye Squeeze	Duration:	None: <3 seconds	Minimum: 3 to 10 seconds	Moderate: 11 to 20 seconds	Maximum: >20 seconds
Nasolabial Furrow	Duration:	None: <3 seconds	Minimum: 3 to 10 seconds	Moderate: 11 to 20 seconds	Maximum: >20 seconds

Section E: Total PIPP-R Score

NIPI PIPP-R Scoring Form
Page 1 of 1

REC Ref: 12/SC/0447
v1.0 20.09.2018

Figure 2.8 PIPP-R scoring form.

Behaviour was scored retrospectively using the video recordings. Two experienced observers watched the videos and scored the 15 seconds prior to the stimulus to assess the infant's behavioural state (i.e active and awake, quiet and awake, active and asleep, and asleep), and 30 seconds following the event to determine the presence of three facial expressions (brow bulge, eye squeeze, and nasolabial furrow). The duration of all three facial expression changes was timed,

and a score was calculated between 0 and 3 for each one. Observers were blinded to the stimulus type when scoring the videos.

Physiological data was downloaded from ixTrend and analysed using an in-house script in MATLAB. The script calculated the baseline average heart rate and oxygen saturation 15 seconds preceding the event, and the maximum heart rate and minimum oxygen saturation 30 seconds following the event; the difference between the two determined the score between 0 and 3 for each indicator.

Chapter 3 A FRAMEWORK TO CHARACTERISE EVOKED ACTIVITY IN NEONATES

This chapter focuses on the EEG analysis methods used throughout this thesis. In order to characterise the EEG evoked activity to different types of stimulation in infants born prematurely (Chapter 4) and infants with a pathology (Chapter 5), stimulus-specific temporal templates were designed using a control group of 15 healthy term infants. In this introduction I briefly discuss current clinical and research methods of evoked potential acquisition and analysis.

3.1 Introduction

The current clinical methods most often used in the acquisition and analysis of evoked potential data have been taken directly from studies performed in adults and are not bespoke to the neonatal population. These methods rely on averaging many trials, use short interstimulus intervals (ISI), narrow filter settings, and high intensities above sensory thresholds. While these methods have been used successfully in neonatal assessment to record the early components of cortical evoked responses and predict neurocognitive impairment or cerebral palsy (CP) in infants with brain injuries (20,23,218), these methods may not be optimal to record evoked potentials in the neonatal period, in particular when we are considering responses to nociceptive events that cannot be easily repeated without clinical need. The neonatal period is characterised by rapid changes in

the structure and function of sensory pathways and recent studies have demonstrated how the immature nervous system differs from the adult (105,219). For example, preterm infants have prolonged refractory periods implying that the stimulus rates or ISI used in clinical practice should be longer than the current 0.5-1Hz. This also suggests that longer time windows should be used (>1 second). Recent evidence also highlights the effects of filter settings on the EEG and somatosensory studies which distort and omit aspects of the evoked response (220,221). In addition, evoked potential studies are usually performed with few electrodes which can affect the amplitude of the signal (if placed away from where the signal is originating) (222,223), and overlooks the influence of vigilance states on evoked responses (16,18).

In adults, evoked potentials are small in amplitude and the averaging process is important to increase the signal-to-noise ratio in order to successfully record and analyse the evoked activity. Using the standard stimulating methods described above ensures that a detectable response is evoked and can be recorded in individual participants. This approach ensures that variability in the waveform is reduced between participants so that it is appropriate to assess the integrity of the pathway under investigation. For example, by stimulating three or four times above the sensory threshold in SEP studies, and producing a twitch, the investigator can be confident that an absent response is a result of pathology rather than suboptimal stimulation of the sensory system. However recent studies on infants have challenged this convention (224–226), demonstrating that the variability and multicomponent nature of infant evoked potentials are important aspects of the response and may be distorted or abolished by the averaging process. In the context of pain, there is also the ethical issues

concerning how much stimulation is appropriate, which often and appropriately limits investigations to single events.

Analysis of infant evoked potentials and brain activity data has substantially progressed in recent times. While many clinical studies evaluating evoked potentials continue to rely on visual inspection to manually measure latency, duration and amplitude of consecutive peaks, newer research tools have been developed. Although this method of analysis is quick and intuitive when a response is well defined and the peaks are well formed, in practice infant evoked responses reflect the maturation of the pathway and therefore change in morphology, latency, amplitude and topography with gestational age. The emergence and disappearance of components and morphological changes are important features of development, which can yield more information about the state of sensory maturity than latency and amplitude alone, and this detailed information is only beginning to be exploited in research investigations.

Other methods of EEG evoked response analysis include measures which decompose the activity and can quantify activity that is not phase-locked to the stimulus. Several methods have been used and some examples are described here. The most attractive methods for event related activity create a representation of time, frequency, spatial distribution and connectivity or cohesion of the signal. For example, the neural dynamics of the visual evoked response has been investigated using metrics such as high frequency (40Hz) synchrony, nonlinear phase coupling between frequency bands, volume conduction and coherence (227). These measures showed significant group effects during the time window when the visual evoked response is observed.

Similarly, the spectral analysis of auditory evoked potentials has been investigated in preterm infants, with a view to describing the temporal dynamics of evoked delta brush activity (179,181). Time-frequency analysis of noxious evoked activity has shown differences between the responses of adults and infants, as well as similarities in evoking gamma oscillations (158). Evoked activity in response to tactile stimulation has been quantified using the root mean square (RMS), and the time frequency components were analysed using wavelet transform across GA (228).

In this thesis evoked potentials are characterised using a combination of visual inspection and data- driven template approach of analysis. Characterising evoked potentials using a template is a fairly unique analysis method, developed to measure noxious brain activity in newborn infants and used in clinical trials to investigate the analgesic efficacy of pharmacological interventions. Our group has previously identified and validated a template based on Principal Component Analysis (PCA) applied to a specific time window (400-700ms post-stimulus) at the midline central electrode (Cz), where noxious activity is known to be present (27). The template acts as a measure of magnitude and is scaled so that a magnitude of 1 represents the average response evoked by a stimulus of equivalent intensity to a heel lance performed in a term infant (27). This method of analysis has been validated across a range of studies in newborns >34 weeks GA (27), and has been used as a measure of analgesic efficacy in a recent morphine trial in neonates (217).

A core aspect of this thesis was to adapt the template approach so that it could be used to measure evoked responses not just to noxious stimulation but also other types of non-noxious stimulation. In this way, the dominant activity

observed in term infants in response to a specific stimulus could be compared to other cohorts of infants. There are several advantages of using temporal templates to quantify the magnitude of an evoked response, particularly in premature and term infants, and these are discussed later in this chapter. The methods and approaches used to design these temporal templates are described in detail here.

3.2 Aim

- To design a framework for characterising evoked potentials in neonates

3.3 Participating infants

Fifteen healthy term (>36 weeks gestational age (GA)) infants were used in this chapter. The demographic characteristics of these infants are described briefly below and in Table 3.1.

Full term healthy cohort: To derive temporal templates to quantify stimulus-evoked response amplitudes, a cohort of 15 healthy full-term or near-term infants were recruited with a mean gestational age (GA) at birth of 38.1 weeks (range 36.1-41.0 weeks) and studied with a mean postmenstrual age (PMA) at study of 38.7 weeks (range 36.6-41.7 weeks).

Infants had no documented neurological conditions or maternal drug abuse and were not receiving any sedative or analgesic medications at the time of study. A single infant was on a course of antibiotics and was briefly admitted to the neonatal unit. All infants were clinically stable and background EEG activity was appropriate for GA.

Table 3.1 Demographics table: Full-term control cohort.

Mean and (range) is displayed for continuous variables. Discrete variables are expressed as number.

	Healthy full term cohort
Number of participants	15
Female	6
Gestational age at birth (weeks)	38.1 (36.1- 41.0)
Birthweight (g)	3166 (2130 -4590)
Antenatal factors:	
Prolonged rupture of membranes	1
Intrauterine growth restriction	1
Abnormal Doppler	0
Maternal infection (Chorioamnionitis)	0
Delivery:	
Vaginal (assisted/unassisted)	8
Caesarean	7
Apgar score at 10 mins	10
Conditions during admission in NICU:	
Neurological conditions	0
Cardiac conditions	0
Respiratory distress syndrome	1
Invasive mechanical ventilation <24 hrs	0
Invasive mechanical ventilation >24 hrs	0
Infection suspected	1
Previous surgery	0
Test occasion:	
Postmenstrual age at study (weeks)	38.7 (36.6 -41.7)
Respiratory support (LFT/HFT)	0
Medications at study:	
Non-standard drugs[1]	0
Analgesics < 24 hrs prior to study	0
Antibiotics	1
Prior pain	2 (0- 23)

[1]Standard drugs include: Caffeine citrate, Vitamins, Probiotics, Breast milk fortifier, Iron, Sodium and Phosphate supplements.
LFF= oxygen therapy HFT= high flow nasal cannulae
Prior pain= number of skin breaking procedures, endotracheal tube or oral suction.

3.4 Experimental design

Each participant was studied on a single test occasion. Infants were stimulated with all stimulus modalities (visual, tactile, auditory and noxious) as described in Chapter 2: Section 2.6. Auditory stimulation was performed using headphones

only. The PinPrick stimulator with a force of 128 mN was used as the noxious stimulus. All stimuli were presented in blocks of 20-30 trials with at least 10 seconds between each stimulus. The experimental design is illustrated in figure 3.1.

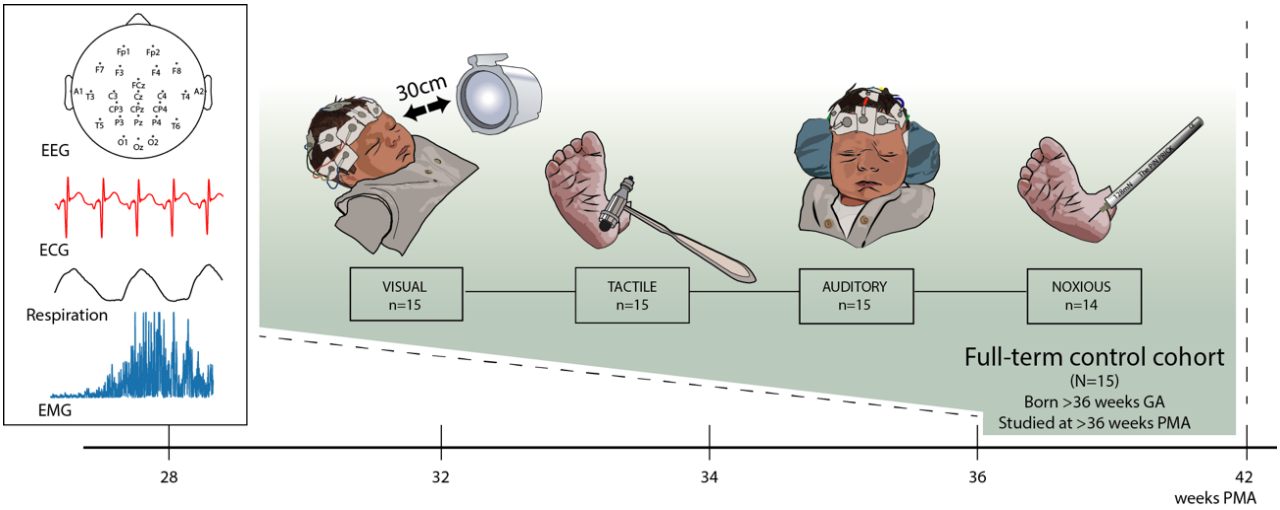


Figure 3.1 Experimental design
For each test occasion a set of 25 recording electrodes were applied and electrocardiograpm (ECG), respiration and electromyography (EMG) were also recorded (left). Infants were presented with 20-30 trials of each stimulus (presentation of stimuli was randomised) (right). N denotes the total numer of infants in the study and n denotes the number of recordings included in the analysis for each stimulus.

3.5 Recording Techniques

EEG activity was recorded in response to visual, tactile, auditory, and noxious stimulation. All stimuli were presented, and time-locked to the EEG recording as detailed in Chapter 2: Section 2.6. A 25-recording electrode set up (illustrated in chapter 2: figure 2.1A) was used for all infants.

3.6 EEG Analysis of stimulus evoked brain activity

3.6.1 Pre-processing of EEG data

The EEG data was first epoched using CURRY7 and then exported to MATLAB (R2018a). As detailed in Chapter 2, EEG data was filtered and average-referenced. The average reference was calculated using a subset of electrodes, excluding mastoid and frontal electrode positions, as these sites are more sensitive to muscle and blink artefacts. Each trial was visually inspected for artefacts before averaging, and when the artefact was limited to a few channels, spherical interpolation was used. The trial averages for each stimulus were baseline-corrected prior to deriving the stimulus- specific templates of activity (see Chapter 2: Section 2.7.1 for details).

3.6.2 Deriving the temporal templates

3.6.2.1 Spatial EEG analysis (visual inspection of raw amplitude)

To evaluate the brain region where maximal amplitude change occurs after application of a stimulus, the evolution of the spatial distribution of amplitude changes immediately after the stimulus was visually assessed. This was done using a grand average of the trial averages per infant. The pre-processed EEG amplitude changes were visually evaluated and depicted in successive 200ms intervals using heatmaps. The electrode locations where the change in activity was maximal post-stimulus determined the channel of interest for further analysis of the signal.

3.6.2.2 Cluster analysis

In order to identify periods of salient activity after a stimulus, non-parametric cluster analysis as defined by Maris and Oostenveld (2007) was performed (229). Cluster analysis was performed on a single channel of interest, determined by the visual analysis of amplitude changes from section 3.6.2.1. Each time point in the period following the stimulus was compared using a paired t-test to the pre-stimulus period. All time points exceeding the threshold for the t-statistic where 97.5% of the area under the t- distribution was covered (two- tailed test $p<0.025$), were considered significant and time-adjacent values were grouped into clusters. This was done for all trial-averaged EEG activity of each infant. Additionally, the trial averages were baseline normalised when this analysis was performed (by dividing by the pre-stimulus standard deviation 500ms prior to stimulation). The cluster masses (sum of the t-statistics within each cluster) were compared against a permutation distribution of cluster masses derived after repeating this procedure with the post-stimulus and pre-stimulus labels randomly re-assigned each time for 1000 iterations. Remaining clusters whose mass exceeded the permutation distribution in a two-tailed test ($p<0.025$) were considered significant. The significant period of salient activity was defined as the time range of the first and last timepoint in the significant cluster.

3.6.2.3 Principal Component Analysis (PCA)

Principal Component Analysis (PCA) was applied within the stimulus- specific latency identified by the cluster analysis to obtain waveform components (principal components, PCs) to explain the variance in the data (201,230). The EEG data from all channels was combined before PCA was performed to ensure

that the dominant components were not biased towards a single channel or spatial location on the head. This approach was performed separately for each stimulus and the top three PCs were re-projected onto the data and visually assessed to identify which PC best represented the evoked response. The PC which best fitted the data was determined based on two criteria: 1) the spatial distribution of the component was comparable with the amplitude changes observed in section 3.6.2.1, and 2) the PC fit to the temporal activity at the dominant electrode had the highest cross correlation.

3.6.2.4 Applying the temporal templates to new data

Each derived stimulus-specific temporal template was projected and rescaled onto the EEG using Singular Value Decomposition (SVD). SVD is a robust numerical method for decomposing a matrix of multichannel EEG or evoked potential data into a sharply reduced set of features with corresponding waveform, amplitude, and spatial vectors (231). This decomposition allows the template to rescale to the EEG activity via a process of least squares optimization.

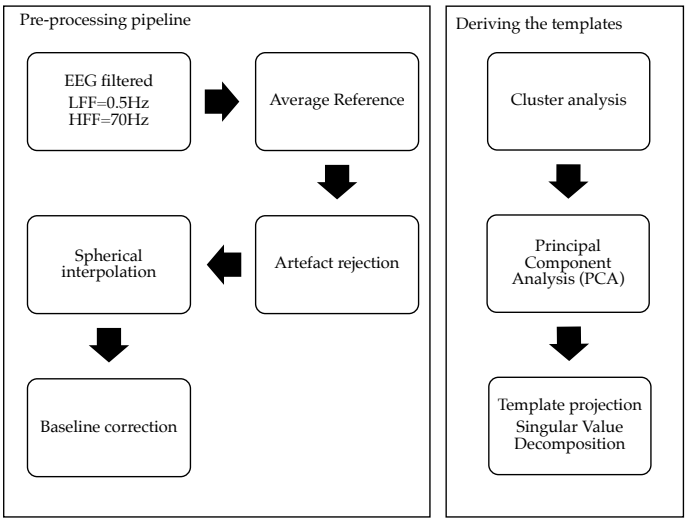


Figure 3.2 Schematic of EEG data analysis pipeline

3.6.2.5 Latency and Woody filtering

Woody filtering was used to allow the template to correct for inter-individual differences in latency of the evoked activity. Woody filtering adjusts or 'jitters' the signal relative to the template to achieve a maximum cross correlation between the two and has shown great success when applying a noxious-evoked template in previous studies (27,113,217). It ensures that the template is projected optimally onto the evoked potential and that the most accurate stimulus response magnitude is calculated (irrespective of any observed trends in latency).

A maximum jitter of ±50ms was selected for visual and noxious stimuli and ±100ms for the tactile stimulus. Woody filtering was performed at the channel of interest with the obtained adjusted latency applied to the remaining channels. This approach was used as Woody filtering always assumes that the expected activity is present (232). Applying Woody filtering to each channel separately would have resulted in poor estimates of stimulus response magnitude due to the absence of responses at certain channels. Furthermore, the fixed latencies due to spatial propagation of the response from the peak location would be lost.

3.6.2.6 Background correction

In order to reduce the effects of underlying background activity (such as sleep state differences) on the stimulus-evoked response magnitudes, a background correction approach was used. This was also done to correct for possible bias caused by woody filtering all channels relative to the channel of interest.
The temporal template was applied to successive windows of stable pre-stimulus baseline activity across the one second period prior to the stimulus taking place.

The median of these values resulted in a robust pre-stimulus weight which could then be used as a surrogate for the background and was subtracted from the stimulus-evoked response magnitude to obtain a 'corrected' value. This was performed independently for each channel, per stimulus for each individual.

3.7 Results

3.7.1 Visual inspection of raw amplitude displays stimulus-specific spatial patterns of activity in healthy full-term infants

The spatial evolution of amplitude changes for each stimulus is represented in figure 3.3 using heatmaps and the pre-processed EEG. Visual stimuli evoked strong positive deflections over the occipital regions (maximal over the midline occipital electrode- Oz) within the first 200 ms, later becoming more negative in that region (from around 400 ms). Tactile stimulation evoked a negative deflection focused over the midline central electrode Cz, also within the first 200 ms post-stimulation. Auditory stimulation evoked positive activity over temporal regions within the first 200 ms. Noxious evoked activity was identified later than non -noxious stimulation, from around 400 ms onwards, when a distinct positive deflection could be seen centrally maximal.

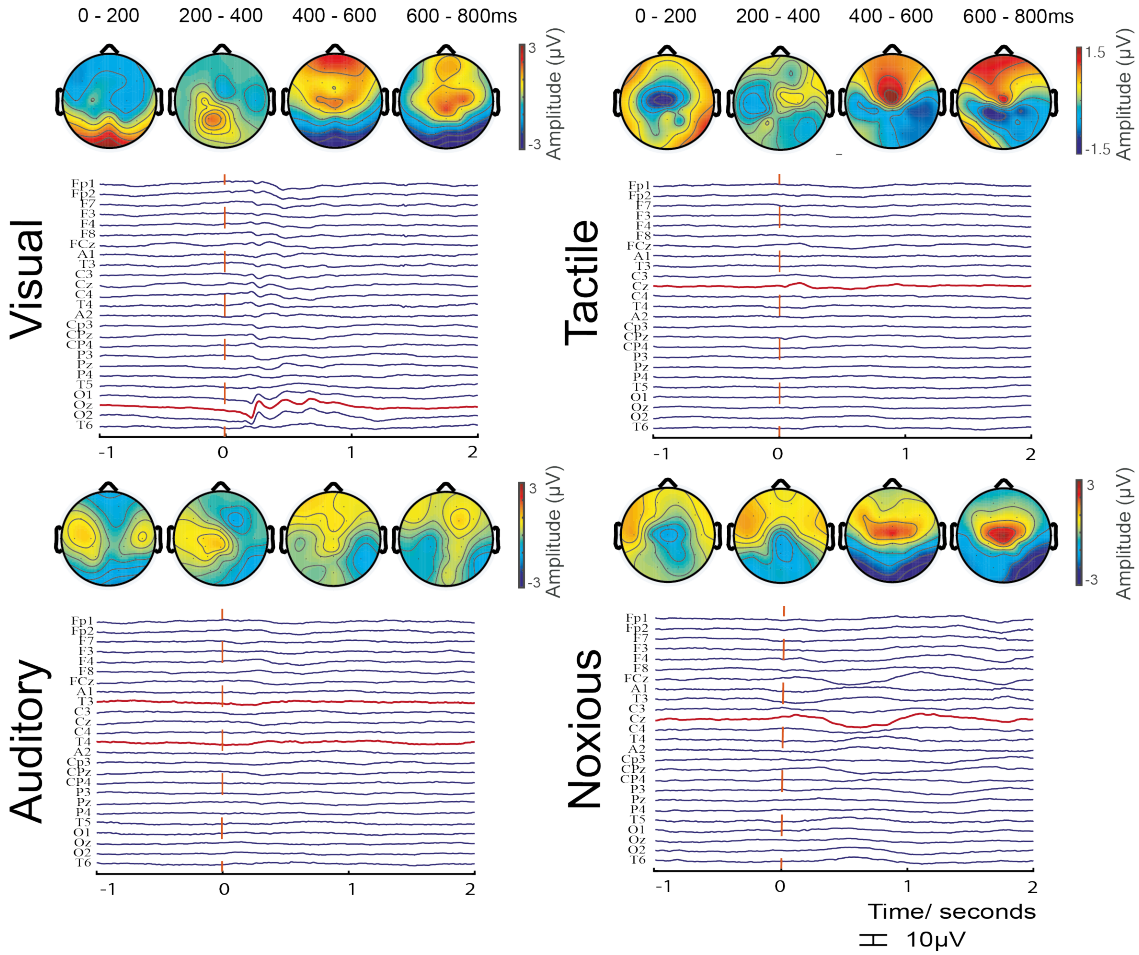


Figure 3.3 Spatial distribution and average EEG activity for each stimulus. Heatmaps illustrate the amplitude changes over the first 800ms post-stimulus. Changes in the spatial distribution is presented in 200 ms intervals. Average EEG activity is shown one second prior to stimulation and two seconds after the stimulus (red dotted line marks the point of stimulus). EEG signal highlighted in red shows the electrode where maximum amplitude change was observed. Maximal activity was recorded at different electrode sites dependent on stimulus modality.

3.7.2 Cluster analysis reveals stimulus- specific temporal patterns of activity in healthy full-term infants

Cluster analysis identified significant clusters which spanned a single positive or negative deflection. As shown in figure 3.4, visual stimuli evoked a significant response between 0-231 ms (temporal cluster analysis, p=0.0010), with a clear positive deflection maximal at Oz. For tactile stimuli, peak activity at Cz had a statistically significant response between 76-218 ms (p=0.0150), and a clear negative deflection. Auditory stimuli did not exhibit any significant clusters and

therefore did not entail any statistically significant salient responses when either midline temporal electrode position was assessed independently and combined. For completion, cluster analysis was also performed over the midline central electrode but again, this did not yield any significant clusters. Lastly, noxious stimuli evoked a statistically significant salient response at Cz in the period 426-800 ms (p=0.0010), with a clear positive deflection.

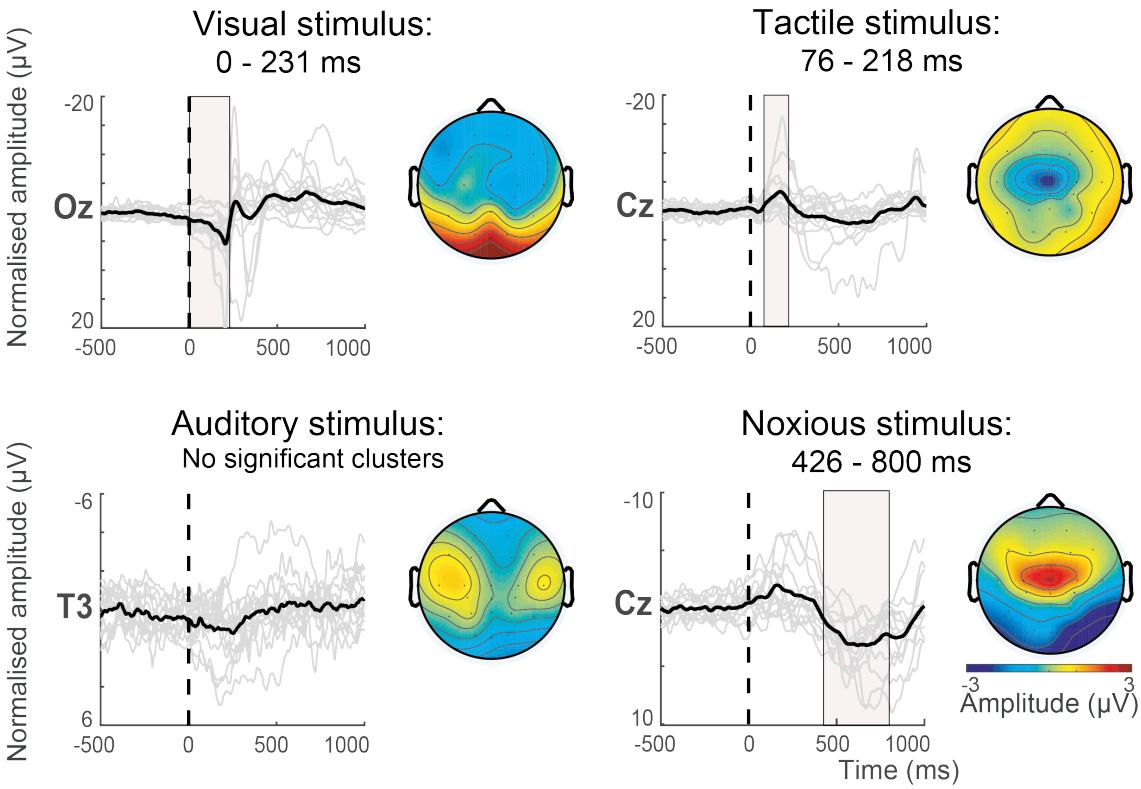


Figure 3.4. Cluster analysis.
Average evoked responses 500 ms pre-stimulus and 1000 ms post-stimulus at the spatial location of peak activity. Dotted lines denote the point of stimulus and grey lines show individual infant (trial-averaged) responses, with the global average superimposed in black. Shaded regions denote the significantly salient period of stimulus-evoked activity derived from temporal cluster analysis. Visual–evoked responses at Oz have a significant cluster in the period 0-231 ms (top left), tactile-evoked responses at peak location Cz have a significant cluster in the period 76-218ms (top right), auditory-evoked responses have no significant clusters at peak location T3 (bottom left). Noxious evoked responses at peak location Cz have a significant cluster in the period 426-800 ms (bottom right). For each stimulus modality, heat maps display the corresponding spatial distribution of the average EEG activity for each period showing a significant cluster. The heatmap for the auditory stimulus is repeated from figure 3.3 as no significant clusters were identified.

3.7.3 Principal Component Analysis (PCA) identifies stimulus specific components of activity corresponding to previously identified spatial changes in activity

In each case, the second Principal Component (PC2) best represented the stimulus-evoked response in morphology and spatial distribution. The first Principal component likely reflects generalized EEG activity that is not dependent on stimulation. For visual stimuli, the first three PCs explained 95.7% of the variance (scaled cross-correlation, PC1: 19.6, PC2: 21.5, PC3: 16.8). For tactile stimuli, the first three PCs explained 87.5% of the variance, (PC1: 2.94, PC2: 2.99, PC3: 1.67). For noxious stimuli, the first three PCs explained 88.8% of the variance, (PC1: 43.1, PC2: 59.8, PC3: 42.9). The temporal and spatial fits of the first three PCs for each stimulus are presented in figure 3.5.

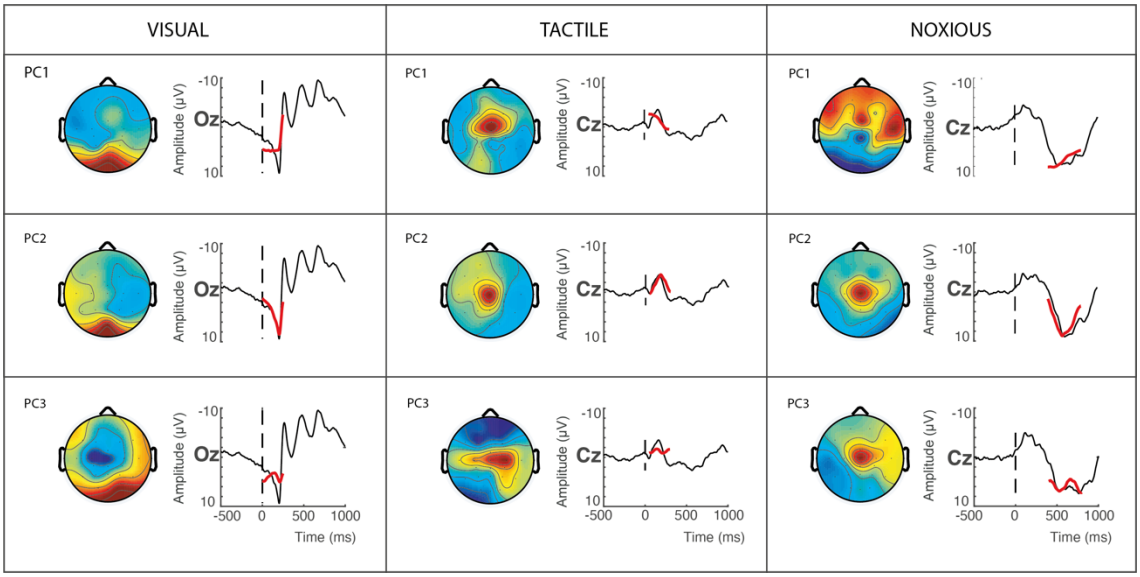


Figure 3.5 Principal Component Analysis (PCA).
For each stimulus, the first three Principal Components (PC) are shown. The shape of the component can be seen overlaid in red over the average evoked responses 500ms pre-stimulus and 1000ms post-stimulus at the spatial location of peak activity. The corresponding spatial distribution for each component is also shown. The second PC (PC2) best represented the evoked response in all cases.

3.7.4 Derived templates for characterising stimulus- evoked activity

The stimulus- specific temporal templates were derived with a view to investigate these patterns of activity in preterm infants (chapter 4) and in a cohort of infants with HIE (chapter 5). For each stimulus, the template identified the dominant morphology within a specific time window. As explained in Section 3.6.2.2 the time interval was determined from the cluster analysis and widened slightly to incorporate the full temporal morphology of the evoked potential. Figure 3.6 shows the spatial distribution and specific temporal window of salient activity for each template when the template was re-projected onto the full-term data.

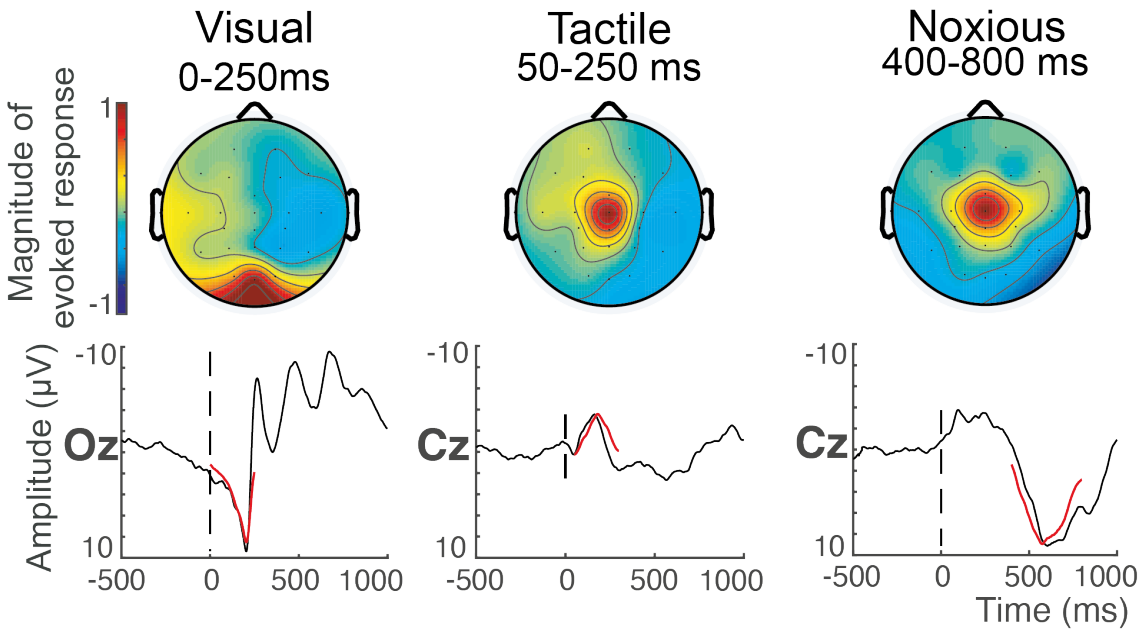


Figure 3.6 Stimulus-evoked response templates.
(Top row) Spatial distribution of the average stimulus-evoked response magnitudes across all spatial locations after (re-) applying the derived stimulus-specific templates. (Bottom row) The derived templates (in red) superimposed onto the average EEG response 500ms pre-stimulus and 1000ms post-stimulus.

3.8 Discussion

The aim was to design a framework of analysis to objectively quantify stimulus-evoked responses so that they could be compared between groups and across modality. Using a combination of visual inspection and data analysis techniques, temporal templates were designed to capture the dominant activity observed in response to visual, tactile and noxious types of sensory input in healthy term infants. The templates were each designed using a consistent framework across stimulus modality, using similar methods to those used to create a validated template of noxious evoked brain activity (27). Similar methods of analysis have been employed in other areas of research such as cognitive neuroscience and other neuroimaging modalities such as fMRI (233) and NIRS (157).

By term age (>36 weeks GA) the infant brain can discriminate between different sensory stimuli in a spatially distinct, stimulus-specific manner. This is shown in figure 3.3 before any data- driven analysis techniques were applied. This discrimination between stimuli allowed us to select the electrode location where activity appeared maximal so that temporal cluster analysis and PCA could be applied to a single electrode. These methods have been used previously to describe evoked related potentials in terms of components rather than a peak, deflection or waveform (230,232).

Here I have shown that temporal templates can be used to characterise evoked responses to different types of stimulation, allowing cross-modality comparison. Previous work using a template approach to quantify amplitude of noxious activity has shown to be of high value, given that it has been able to track development (111,209), correlate with intensity of stimulus (216), and show

differences between interventions (113,234). Here I will discuss the advantages and disadvantages of the acquisition and analysis methods used in this study as they are similar for chapters 4 and 5.

3.8.1 Advantages and limitations of acquisition methods

There are significant differences in the acquisition methods used in this study compared to those used to record standard evoked potentials in clinical studies. We have used a set of 25 recording electrodes covering the whole head to gain better visualisation of the spatial distribution of the brain activity. This has huge advantages in that the ongoing EEG activity was visible during recordings to optimise timing of stimulation, assess background activity and sleep state, and to evaluate spatial distribution of the response (also aided by using an average reference rather than a referential montage). The number of trials was reduced to a maximum of 30 and the ISI was increased to at least 10 seconds. These alterations are more respectful of the unique properties of developing sensory pathways in the neonatal period, with the disadvantage that the signal-to-noise ratio is low and stimulation time is increased slightly.

It was not possible to design an auditory temporal template because the raw EEG activity evoked by the auditory tone selected for this study did not show any convincing evoked activity and cluster analysis did not identify any significant clusters to guide the PCA analysis. The absence of a response was likely due to suboptimal presentation of the auditory stimulus in terms of the duration and intensity and perhaps also in the precise choice of tone. For example, the use of phonetic sounds may have yielded better results (197). Further piloting of the stimulus is necessary for future studies.

3.8.2 Advantages and limitations of characterising evoked responses using temporal templates

Observationally, stimulus-evoked activity is defined by the sequence, polarity, and latency of the deflections. This method is intuitively appealing as it is simple, and amplitudes can be measured independent of latency (232). However, in practice, visual peak measurement can be unreliable when the waveform does not stand out from the background activity clearly or if the baseline or reference peak is difficult to define, particularly when there are few trials in the average or when higher frequency activity is present. Similar problems are encountered when using area measures to quantify the waveform, and both types of analysis do not yield information regarding the shape of the wave (235,236). In contrast, components are a theoretical construct rather than an observed waveform and most of the variance in evoked related potentials is accounted for by six to eight components (235,236).

Applying PCA to the data, has several advantages over manual measurement. It is more objective as the data handling is automated and therefore also less time consuming and less biased. The magnitude weights which are calculated by projecting the template are based on time coordinates instead of a timepoint on a peak which could be noisy and not always well defined.

There are two main disadvantages of using PCA. Firstly, PCA struggles to extract components with temporal overlap (232), a disadvantage which is also reported when measuring peak amplitudes or using area measures. Secondly, PCA extracts components based on changes in amplitude and not latency. This limitation can be circumvented by techniques that deal with latency variability

or latency jitter such as woody filtering (232,237). This method is advantageous in that it reduces trial-to-trial variability of peak latencies which can result in average waveforms of lower amplitude and altered shape. However, a major problem with latency alignment techniques is that the template can be aligned erroneously to background noise instead of the activity of interest. This was a particular problem when applying the template to all channels and was circumvented by Woody filtering only at the channel of interest and applying the obtained adjusted latency to the remaining channels.

The advantages of using Woody filtering probably outweigh the disadvantages of overfitting, but the technique does introduce background noise when there is no observable response. Of note, in this study, a visual evoked response was identified in all infants. Four infants (n=4/15) did not show a response after tactile stimulation and noxious stimulation. Woody filtering was introduced to reduce the variability between infants rather than trials to limit the amount of noise that may be introduced by the woody filtering, and in addition, all template fits were visually inspected. Another possible disadvantage of Woody filtering is that because the shape of the template is rigid, individual variability in the response morphology is not accounted for and may cause a poor fit of the template to the raw EEG data, resulting in a lower magnitude. However, striking the right balance between the different metrics of latency, amplitude and shape is difficult, and on balance this approach has been demonstrated to be highly robust and optimal for this study population.

A limitation to this study was the number of infants in the cohort. This limited our ability to perform further analysis using the spatial dimension and other types of more complex analysis. Small numbers of infants are a general limitation

of infant research and the challenges of recruiting large cohorts of infants are also discussed in Chapter 6.

It is evident that using analysis methods that rely solely on visual inspection has several disadvantages compared to the approaches developed in this Chapter, particularly in neonatal investigations. Although data driven approaches tend to be more robust, they have several disadvantages too. A similar combination of visual inspection and data analysis techniques was successfully used by Leikos and colleagues (228) to measure evoked responses to touch. Similar to the previous literature, analysis in this study was initially guided by visual inspection to allow for more sophisticated data analysis techniques to be applied and in this thesis, ultimately to design templates that could quantify the dominant activity evoked by a visual, tactile and noxious stimulus. However, Leikos et al (228) only performed data analysis techniques on carefully pre-selected responses whereas in this study, a more clinical approach was taken in that trials were rejected based on artefact and not the absence/presence of a response.

3.9 Conclusion

Stimulus-evoked brain activity can be evaluated using temporal templates derived using EEG activity from healthy full-term infants. This method of analysis offers researchers a more objective and robust means of measuring evoked brain activity in infants. It allows for comparisons across modalities and age. It provides an objective metric to quantify the amplitude of the evoked activity, which is not influenced by bias that is implicit when visual analysis inspection approaches are used. The tools developed in this Chapter allow us to

investigate the development of sensory responses throughout the period of prematurity and to assess how these evoked patterns of activity are impacted by pathology in term-aged infants.

Chapter 4 SENSORY DEVELOPMENT IN PRETERM INFANTS

4.1 Introduction

Advances in neonatology have significantly reduced the mortality rate of prematurely born infants in higher-income countries. However, preterm birth remains the leading cause of death in children under five worldwide (238). Also, despite the abovementioned advances in the care of premature infants which has reduced mortality rates, the number of patients with neurological morbidities remain high. Moreover, even in healthy preterm infants, premature birth is likely to have an impact on critical stages of brain and neurosensory development, typically occurring during the third trimester of pregnancy and early postnatal life. The consequences of prolonged exposure to the Neonatal Intensive Care Unit (NICU) environment are only beginning to be understood (239). Robust methods of early neurosensory assessment are key in improving our understanding of how preterm ex-utero development impacts brain function and subsequently impacts long-term development. A better understanding of neurological development is needed to guide developmentally supportive care and neuroprotective interventional strategies.

Neurosensory development during late gestation and early life is described in Chapter 1 of this thesis. Evoked potentials are complimentary to the clinical

neurologic examination of the newborn infant and correlate with critical steps in the organisation of neural circuits. In this chapter, the development of specific characteristics extracted from stimulus- evoked brain activity is investigated sequentially in infants 28-37 weeks post menstrual age (PMA), and then compared to a near term control group of infants. Although this is the first study that looks at the longitudinal development of visual, tactile, and noxious evoked brain activity in the same infants, each of these sensory responses has been studied independently, as summarised below. By term-age, evoked responses to sensory stimulation are similar in morphology to adult responses, but during the preterm period precursors of these components change with maturity, and it is not fully understood how preterm birth affects the development of the neural origins of these components.

The development of the visual evoked response has been reported by several authors in preterm and term infants. Longitudinal and cross-sectional studies (12,190,240,241) have demonstrated how visual evoked responses to flash stimulation increase in complexity with age, from a basic negative peak (around 300ms) in the preterm infant <30weeks gestational age (GA), to the appearance of an earlier positive peak (P200) around 32-36 weeks GA, and the three main components (P200- N300-P400) being invariably present by term age (>36 weeks GA). The emergence/ increase in amplitude of the early positive peak (P200) from 30-36 weeks GA, was found to be a potential measure of maturity in some studies (190,191), but this component was more variable in amplitude after 36 weeks' gestation, when no consistent relationship with maturity of the evoked activity was observed. The appearance of the negative deflection observed at around 300ms (N300) is consistently seen throughout the preterm period and its

latency gradually decreases by 4.2- 5.5 ms/week between 30 weeks PMA and term age (242,243)

There are contradicting reports regarding the relationship between the latency of the visual evoked response and gestational age. Some authors report a progressive decrease in latency of each peak (241), while other authors did not find significant decreases in latency across the preterm period until term age (190). The maturation of the components of the waveform are thought to reflect the integration of the afferent terminals in specific layers of the visual cortex, so the observation that there are no significant changes in latency before 36 weeks GA suggests that myelination of the visual pathways starts at near term age and that this process is not altered by preterm extrauterine birth experience (244). In dark-reared cats no difference in the myelination process of the optic tract and maturation of glial cells was observed even in the absence of visual input (245).

Later components of the visual evoked response have not been studied in such detail. Early studies on visual responses noted slow waves with a prolonged latency and rhythmic activity overlaid (11,12,184). Hrbek (1969) (17) suggested that this non-specific response may be associated with cognitive processes. In 2010, Colonnese and colleagues (180) studied preterm infants and non-anaesthetised postnatal rats under comparable conditions and made the observation that like in the rat, preterm infants experience a rapid switch of activity near term age (>36 weeks GA) characterised by the disappearance of delta brush activity, originally present at around 800 ms after a visual stimulus. The disappearance of delta brush activity was associated with the development of cortical vigilance states and suggests that although retinal maturation largely

contributes to the development of the early components of the visual evoked response, the disappearance of delta brush activity involved changes in the thalamus or cortex, and therefore potentially reflects the maturation and strengthening of thalamocortical connections in the brain.

The development of median and posterior tibial somatosensory evoked potentials in response to rapidly repeated electrical or tactile stimulation have primarily focused on the maturation of early components of the response. The early negative deflection (N1) which is seen maximally over the contralateral region following hand stimulation, and the positive deflection (P1) at the midline central after foot stimulation, are thought to represent the afferent volley arriving at the cortex from the periphery (224,246–248). Fewer studies have been reported in preterm infants compared to full term infants (12,13) due to the challenges involved. Median nerve stimulation is less technically challenging than recording from the posterior tibial nerve, but the procedure can be lengthy. In addition, preterm somatosensory evoked responses are variable, longer in latency and duration, and smaller in amplitude (12,225), thought to reflect immature myelination and synaptic connectivity of the central nervous system.

Myelination begins in the peripheral nervous system (PNS) before myelin appears in the central nervous system (CNS). Motor roots myelinate before sensory neurons in the PNS, but in contrast, sensory pathways tend to myelinate before motor pathways in the CNS (249). Myelination starts in the medial lemniscus around 23 – 25 weeks GA following a caudal- rostral order (250,251) and continues until the age of 12 months (252). Longitudinal studies of posterior tibial nerve stimulation report a rapid decrease in the latency of the main 4

components with increasing GA, accompanied by an increase in nerve conduction velocities (225,253–255). Topographically, the spatial distribution of the early components of the somatosensory response are stable over the central area. The N1 and P1 components of the response can be seen frontally maximal in the youngest preterm infants, becoming more central with age. The N2 and P2 components remain centrally maximal during the preterm period up to full term age. Interestingly, the N2 component reduces in amplitude with GA whereas the P2 component increases with GA (253).

Fewer studies have investigated the maturation of higher-level somatosensory processing. Pihko and colleagues first conducted nonelectrical tactile stimulation using inflatable membranes to lightly tap the fingertips of newborns (18). These studies using EEG and MEG describe a late negative deflection in the contralateral hemisphere with a peak latency of 200- 250 ms (256,257). This component may correspond to the N2 component observed around the same latency in newborn somatosensory evoked potential studies and thought to be arising from the secondary somatosensory cortex. Rahkonen et al (2013) (256) recorded somatosensory electric fields (SEFs) to tactile stimulation of the fingertip from extremely preterm infants (born < 28 weeks) at term-equivalent age (range: 37–44 weeks gestational age) and found that although the first cortical component was present in all infants, the M200 response was observed in 21 out of the 30 infants. The absence of the M200 response was associated with poor neurodevelopmental outcomes at 2 years of age (examined using the Griffiths Mental Development Scales). This study and others (258) demonstrate the potential clinical importance of the late component of the response in predicting neurodevelopmental outcomes.

Although no longitudinal work has been reported on noxious-evoked activity during the preterm period, the maturation of this response has been explored. The shift from non-specific delta brush activity to modality- specific evoked activity observed around 33-35 weeks GA in response to noxious and tactile stimulation has been demonstrated in cross sectional studies (111,209). The magnitude of noxious evoked activity in response to noxious heel lancing increases from around 34 weeks GA, and this is also associated with a decrease in amplitude and duration in reflex withdrawal (101,111), reflecting changes in spinal cord excitability (259). Additionally, the emergence of discriminative facial expressions coincides with the switch from non-specific noxious evoked activity to more refined responses around 33 weeks GA (211). Delta brush activity is a dominant feature of neonatal EEG. Their presence in background EEG is a good indicator of brain maturation and are associated with involuntary hand and foot movements (182). The emergence of nociceptive specific brain activity during the preterm period coincides with the disappearance of the subplate and the formation of thalamocortical connections (104). Therefore, the increase in magnitude may reflect the strengthening of connections during this time.

The importance of sleep in driving endogenous brain activity crucial for survival and guidance of brain networks (65,73), and its relationship with cognitive, psychomotor and behavioural development (260) in infants is summarised in Chapter 1. The influence of vigilance states on visual and somatosensory evoked potentials are also a key factor in the observed variability of responses. In term infants, sleep increases the latency of the components and the visual evoked P200 disappears in both quiet and active sleep. While the N300 and P400 components are seen more variably in sleep, wakefulness is the optimal state to reliably

observe all components (16). Somatosensory evoked responses are best recorded when the infant is asleep (13), as N2 is enhanced in quiet sleep, and the N1 response is better formed in active sleep (254).

Cross-sectional studies looking at the effects of ex-utero experience on visual evoked activity suggest that the emergence of the positive P200 component is accelerated in preterm infants, and the authors attribute this to early exposure of visual input (16,240). Unlike visual evoked potentials which become increasingly more complex with maturity, components of somatosensory evoked responses are fairly consistent in morphology even in the youngest preterm infants and the maturation of the pathway occurs at a similar rate independent of extrauterine experience (14,226). However, although decreases in latency are unaffected by prematurity (13,14,225,226,255,261,262) the amplitude of the N1 response is smaller in premature infants studied at term age compared to term aged infants, which suggests prematurity may influence sensory fiber maturity (255). Slater et al (2010) (263) compared the noxious activity in preterm infants at term equivalent age with term born infants and found a significantly greater response in the infants that had been born prematurely. However, this finding is more likely to demonstrate the effects of a high burden of painful procedures and pathology rather than an effect of normal ex-utero maturation.

Stimulus evoked responses recorded from preterm and term infants are substantially different from those in older children and adults. Traditional methods of analysis may not be suitable to assess the dynamic evolution of the different components of the evoked response and stimulation techniques are impractical in the neonatal setting. It is possible that novel methods of analysis

can overcome some of these challenges and provide insight into the maturation and disruption of normal developmental trajectories, which in turn could be prognostically useful. Importantly, understanding the maturity of sensory pathways during this sensitive period of brain development and the resulting impact of the environment is key to determining what developmental interventions may be effective in intensive care units. Investigating the developmental trajectory of evoked responses in premature infants using temporal templates could be a first step to identifying novel methods for neurological assessment.

4.2 Study Aims

- To characterise the developmental trajectory of specific components of stimulus-evoked brain activity in response to different sensory stimuli in preterm infants studied longitudinally.
- To evaluate the impact of preterm birth on the characteristics of stimulus-specific brain activity in response to different stimuli.

4.3 Methods

4.3.1 Participating infants

Three independent cohorts of infants were included in this chapter. The healthy full-term cohort was described in detail in Chapter 3, and a summary of their characteristics is again shown in Table 4.1. The preterm longitudinal cohort and the preterm control cohort is described in detail in this chapter.

Preterm longitudinal cohort: Ten infants were recruited from the neonatal unit at the John Radcliffe Hospital (Oxford University Hospitals NHS Trust) to take part in this study. Infants were born between 27.1- and 31.1-weeks' GA (mean of 29 weeks GA).

The infants in this cohort were remarkably healthy preterm infants given their young age at birth. Except for one infant who had a choroid plexus cyst on their cranial ultrasound scan (a normal variant of no pathological significance), no infants had documented neurological findings during their admission in the neonatal unit. Only 2 infants required mechanical ventilation for over 24 hours, but none were intubated at the time of study. Apart from a single infant who was given budesonide during admission, the rest of the infants were given standard medications, and in all cases no analgesic or sedative medication was given at least 24 hours prior to each study taking place. Study test sessions did not disrupt any ongoing routine treatments such as phototherapy and incubator temperature and humidity, and infants were clinically stable when recordings took place. Infant recordings were later grouped according to their PMA at the time of study.

Preterm control cohort: To evaluate the effects of prematurity on sensory evoked responses, a group of n=8 preterm infants with mean GA of 34.7 weeks (range 34.0 - 35.7 weeks), were recruited and studied once within the first 15 days of birth (mean PMA of 35.5 weeks, range 34.7 - 36.4 weeks). Infants had no known neurological diseases or conditions other than prematurity at the time of study, and respiratory support, when given, was not for a prolonged period of time (<24 hours). All infants required a clinical heel lance for their routine care. Reasons for heel lancing included: blood gas analysis, serum bilirubin, Guthrie test, and urea and electrolytes (U&E).

Table 4.1 Infant demographics

The information was taken from the infants' medical notes. Mean and (range) is displayed for continuous variables. Discrete variables are expressed as a number or range.

	Healthy full-term cohort	Preterm longitudinal cohort			Preterm control cohort
Number of participants	15	10			8
Female	6	5			3
Gestational age at birth (weeks)	38.1 (36.1- 41.0)	29.0 (27.1- 31.4)			34.7 (34- 35.7)
Birthweight (g)	3166 (2130 -4590)	1173 (760-1900)			2449 (1550- 4525)
Antenatal factors					
Prolonged rupture of membranes	1	5			2
Intrauterine growth restriction	1	3			2
Abnormal Doppler	0	1			1
Maternal infection (Chorioamnionitis)	0	4			0
Delivery					
Vaginal (assisted/unassisted)	8	2			5
Caesarean	7	8			3
Apgar score at 10 mins	10	9 (8-10)			9.75 (8- 10)
Conditions during admission in NICU:					
Neurological conditions	0	1*			0
Cardiac conditions	0	1**			0
Respiratory distress syndrome	1	7			1
Invasive mechanical ventilation <24 hrs	0	2			3
Invasive mechanical ventilation >24 hrs	0	2***			0
Infection suspected	1	4			2
Previous surgery	0	0			0
Test occasion:		<32	32-34	34-37	
Postmenstrual age at study (weeks)	38.7 (36.6 -41.7)	30.4 (28 -31.9)	32.7 (32 - 33.3)	35.1 (34.3 -36.3)	35.5 (34.7 -36.4)
Respiratory support (LFT/HFT)	0	5	5	4	0
Medications at study:					
Non standard drugs[1]	0	0	1	1	0
Analgesics < 24 hrs prior to study	0	0	0	0	0
Antibiotics	1	1	0	0	0
Prior pain	2 (0- 23)	21.3 (11- 46)	33.9 (22- 59)	37.5 (25 -65)	10 (3- 21)

* choroid plexus cyst

** Patent Ductus Arteriosus (PDA)

[1]Standard drugs include: Caffeine citrate, Vitamins, Probiotics, Breast milk fortifier, Iron, Sodium and Phosphate supplements.

***Infants were ventilated for a maximum of 4 days and did not require further periods of ventilation

LFT= Oxygen therapy HFT= High flow nasal cannulae

Prior pain= number of skin breaking procedures & Endotracheal tube/oral suction- accumulative mean and (range)

4.3.2 Experimental design

Infants in the longitudinal preterm cohort were recruited to take part in a longitudinal study. Only those who lived locally and would remain at the John Radcliffe Hospital for the duration of their care were eligible. Once the parents consented to the study, the first test occasion was always performed within 15 days from birth (mean= 9.8 days, range= 5-15 days). Serial test occasions were then performed every two weeks until the infant was discharged. Test occasions were dependent on routine bloods being required by the clinical team, therefore in some cases the test occasion was postponed until the next set of bloods were requested. On average infants were tested on 3 occasions before discharge (range between 2-4 test occasions). Figure 4.1 illustrates the experimental design.

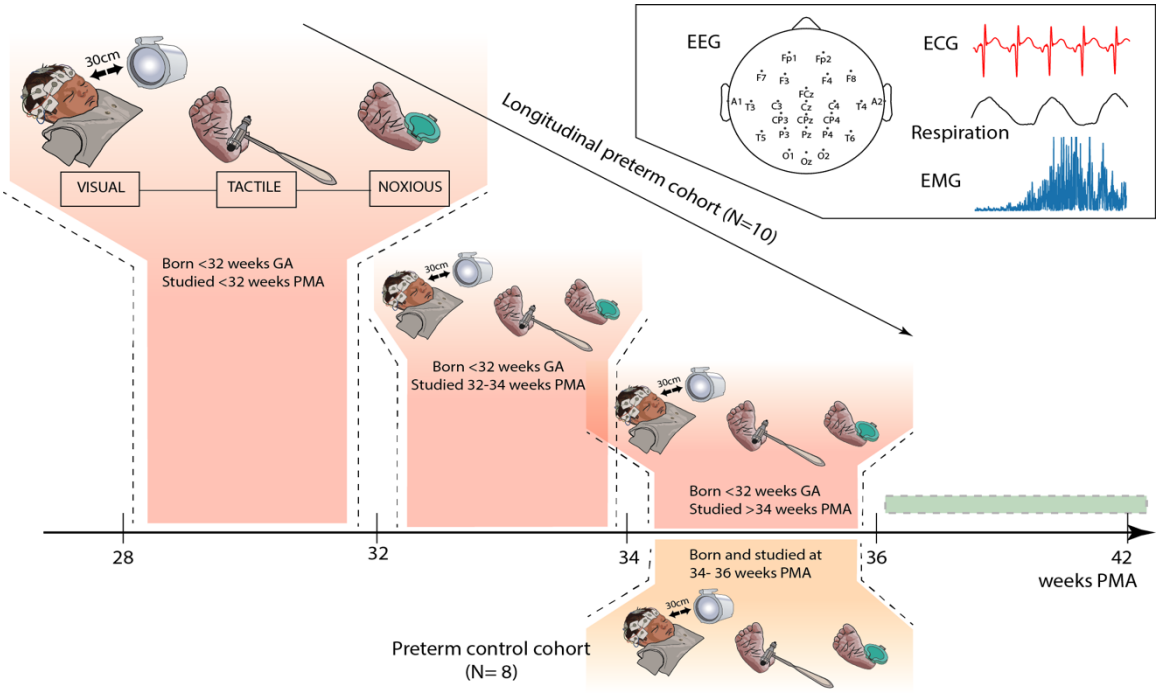


Figure 4.1 Experimental design.
Infant recordings included a 25 recording electrode EEG set up with simultaneous recording of electrocardiogram (ECG), respiration and electromyography (EMG) (top right). Infants in the longitudinal preterm cohort were tested on several occasions whereas the preterm control cohort was tested on a single occasion.

4.3.3 Data Acquisition

All recordings were performed using 25 recording scalp electrodes as described in Chapter 2: Section 2.5.1 and illustrated in figure 2.1 A.

4.3.4 Stimulation techniques

Wherever possible, all four different types of stimulation were performed per test occasion. This was not always possible due to technical equipment faults. Non-noxious stimulation consisted of visual and tactile stimulation as detailed in Chapter 2: Section 2.5.4.1. Noxious stimulation consisted of a single clinical heel lance (described in Chapter 2: Section 2.5.4.2.1).

4.4 Analysis

4.4.1 EEG Analysis

The EEG data was processed using the methods outlined in Chapter 2 section 2.7.1. The temporal templates designed for each stimulus (Chapter 3) using healthy full-term infants were applied to each recording from the longitudinal preterm cohort and the preterm control cohort of infants. The templates were rescaled onto the EEG data using the singular value decomposition (SVD) and least squares estimate as outlined in Chapter 3 section 3.6.2.4. Each template was applied to the trial-averaged activity for each stimulus per recording to obtain weights (or scaling factors). These weights quantified the stimulus-evoked response in terms of magnitude.

4.4.2 EMG Analysis

EMG data from the ipsilateral side (foot being stimulated) was processed using the methods described in Chapter 2 Section 2.7.2 for all infants after noxious stimulation.

4.5 Statistical analysis

Regression analysis using a linear random effects model was selected to correlate the stimulus-evoked response magnitudes with PMA in the longitudinal cohort (significance threshold $p< 0.05$). This was done using the weights calculated at the dominant channels for each stimulus (i.e visual stimulus- midline occipital electrode, Oz). Random intercepts were introduced to group repeated recordings from the same infant. To assess if GA and number of trials in each trial-average was a potential confound, these were separately correlated with PMA (Pearsons correlation).

For the comparison between the preterm control cohort and the last group of the longitudinal preterm cohort, the stimulus response magnitudes were regressed using a linear random intercepts regression model. Repeated measures were accounted for by applying random intercepts. As with the regression for the longitudinal data, PMA and trial number could have been potential confounds so these were regressed between the two cohorts to confirm they were appropriately matched for PMA and trial number. Additionally, to determine the strength of the comparisons, Cohen's d effect size was calculated to compare the two groups. Typically, values >1 denote a high effect size.

In addition, visual labelling of the evoked activity for each stimulus-evoked response was done to identify if latency correlated with PMA and to confirm the choice of jitter used for Woody filtering. The presence of delta brush activity was also noted after visual inspection.

4.6 Results

4.6.1 Spatial organisation of stimulus-specific activity in preterm infants demonstrates a refinement towards term age.

The stimulus-evoked temporal templates of activity that were characterised in a population of term-aged infants were projected onto the ten infants who were born prematurely and studied longitudinally. Across the preterm period, from 28 weeks PMA to near-term age, distinct patterns of activity can be seen between the different types of stimulation. Each modality is described in more detail below.

4.6.1.1 Visual

From 28 weeks PMA to near-term age, components of the visual evoked response were visually identified in all infants. Figure 4.2 shows the EEG trial-averages (prior to Woody filtering) for each infant and the group average activity for each of the groups separated by PMA. The evoked activity was initially maximal over parieto- occipital areas, but with increasing PMA responses became markedly better localised to the occipital region of the brain. The most premature infants (<32 weeks PMA) showed high amplitude, widespread responses, becoming more focused and stronger by near term age. In addition, 9/15 infants < 34 weeks PMA also evoked delta brush activity following the visual evoked response at around 800 ms.

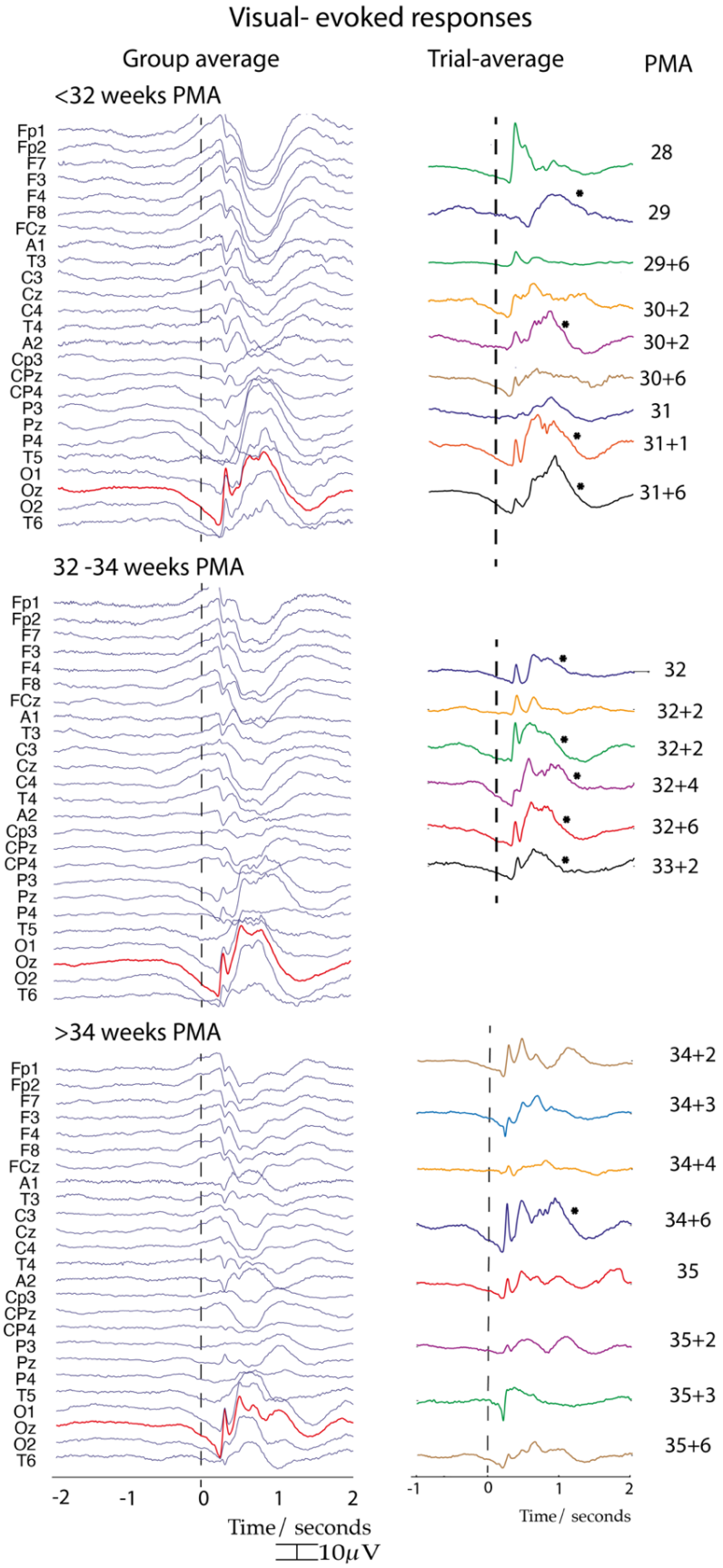


Figure 4.2 EEG activity after visual stimulation.
The grand trial average for each group separated by PMA and the individual trial averages for each infant is shown. Dotted lines mark the point of stimulation. Note the presence of delta brush activity marked with *.

When the visual temporal template was projected onto the EEG trial averages, spatially the activity became more focused to the midline occipital region with age, and the early positive component increased in magnitude. The dominant activity observed in term infants was only present in some infants, and the emergence of this activity became more and more apparent with age (figure 4.3).

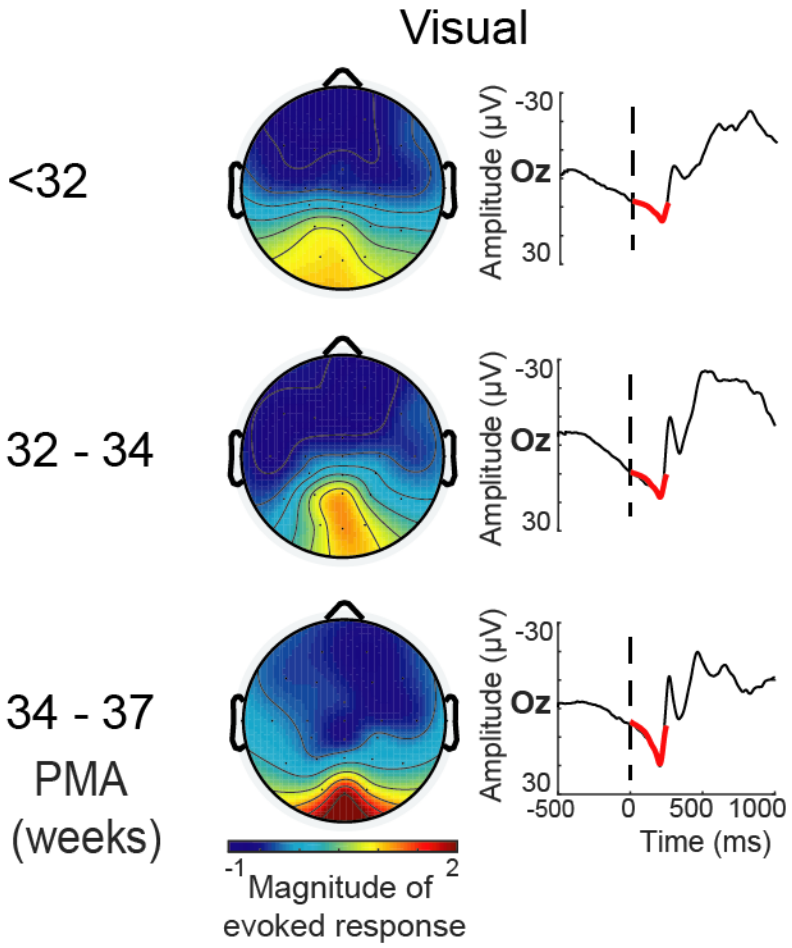


Figure 4.3 Spatial localisation of the response magnitudes and corresponding EEG activity for the visual stimulus.
Spatial localisation of the response magnitudes and corresponding EEG activity for the visual stimulus, averaged across three age groups, based on postmenstrual age (PMA = GA + postnatal age).: <32 weeks, 32-34 weeks and 34-37 weeks PMA. The group average evoked activity is shown with the template overlaid in red.

4.6.1.2 Tactile

In contrast to visual evoked activity, responses to tactile stimulation were centrally focused as early as 32 weeks PMA, and little change in the spatial distribution of the activity is observed. The EEG trial averages (figure 4.4) clearly show negative deflections consistently over the midline central and midline centro-parietal region in the youngest of infants, decreasing in amplitude with PMA. A clear response was not identified in all infants, especially in infants >34 weeks PMA. When the tactile temporal template was projected onto the EEG trial averages (figure 4.5), the spatial focus over the mid central region of the brain can be seen. The amplitude of the waveform can also be seen to decrease with PMA.

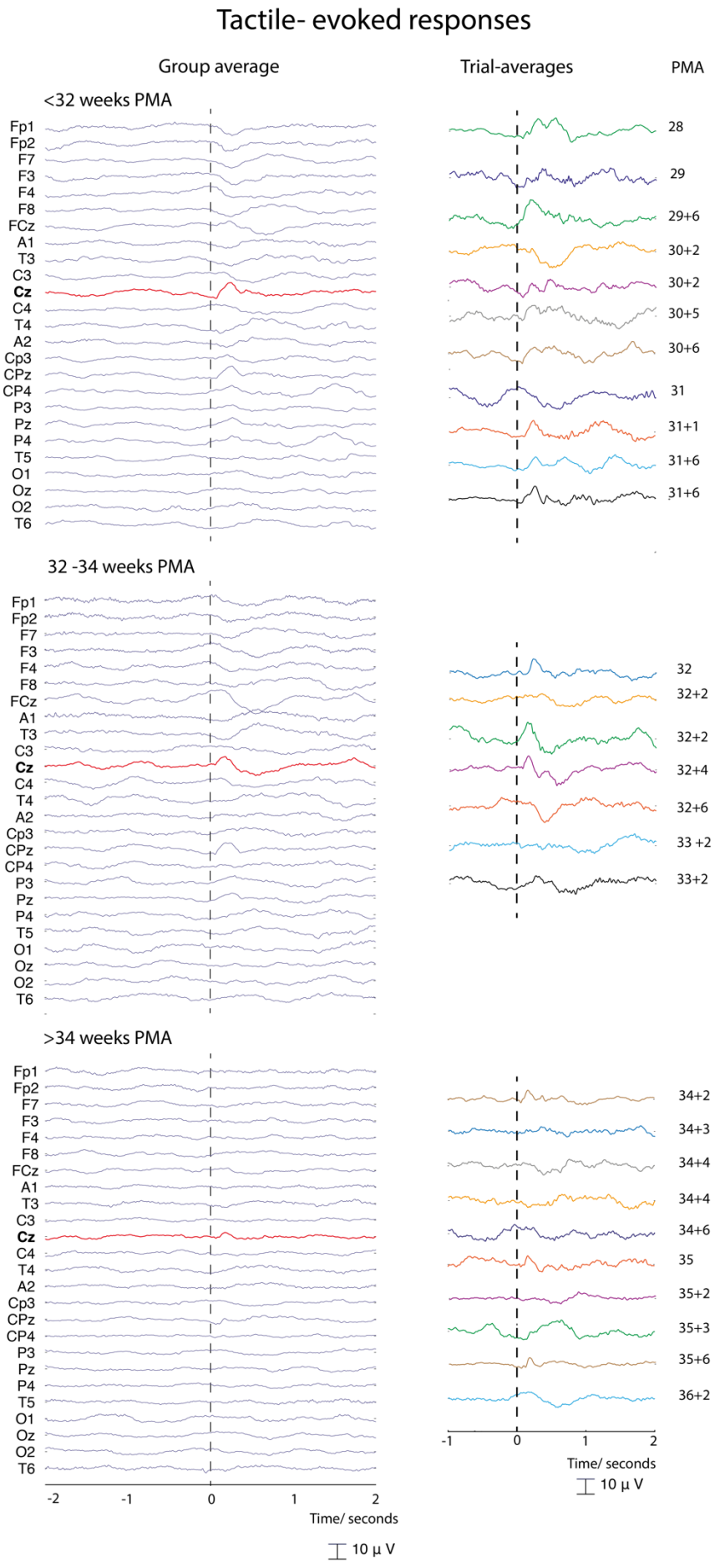


Figure 4.4 EEG activity after tactile stimulation. For each group the grand trial average and the individual trial averages for each infant is shown. Dotted lines mark the point of stimulation.

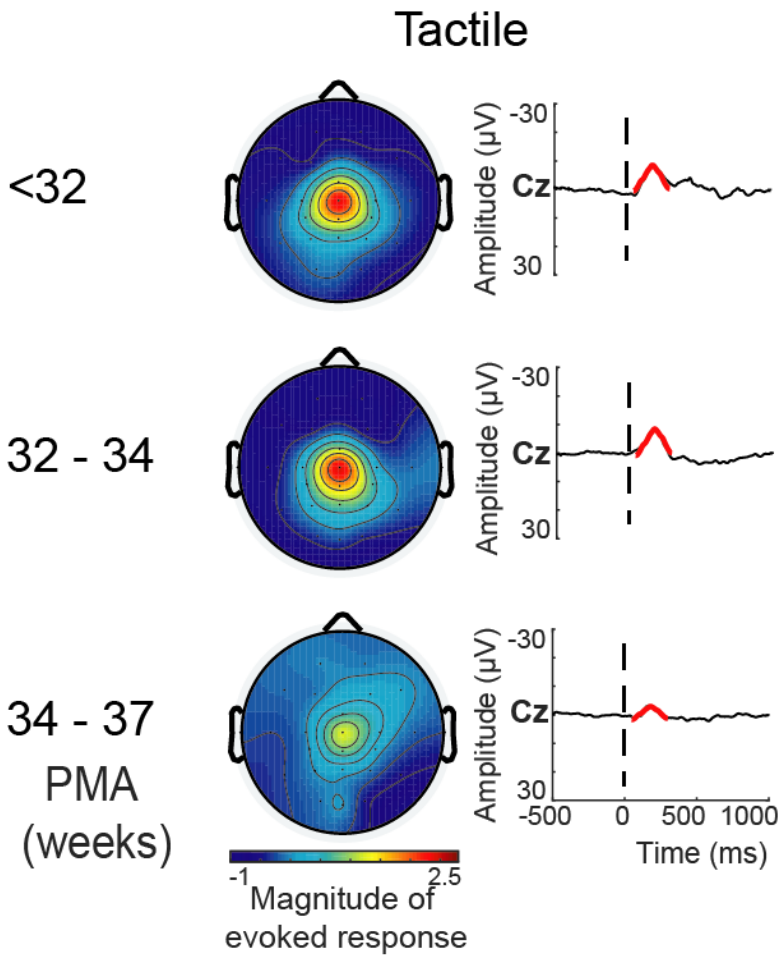


Figure 4.5 Spatial localisation of the response magnitudes and corresponding EEG activity for the tactile stimulus.
Spatial localisation of the response magnitudes and corresponding EEG activity for the tactile stimulus, averaged across three age groups, based on postmenstrual age (PMA = GA + postnatal age).: <32 weeks, 32-34 weeks and 34-37 weeks PMA. The group average evoked activity is shown with the template overlaid in red.

4.6.1.3 Noxious

The evoked brain activity in response to noxious stimulation was strikingly different in the youngest preterm infants, showing disorganised and widespread activity, later becoming localised to the midline central region by 34 weeks PMA (figures 4.6 and 4.7).

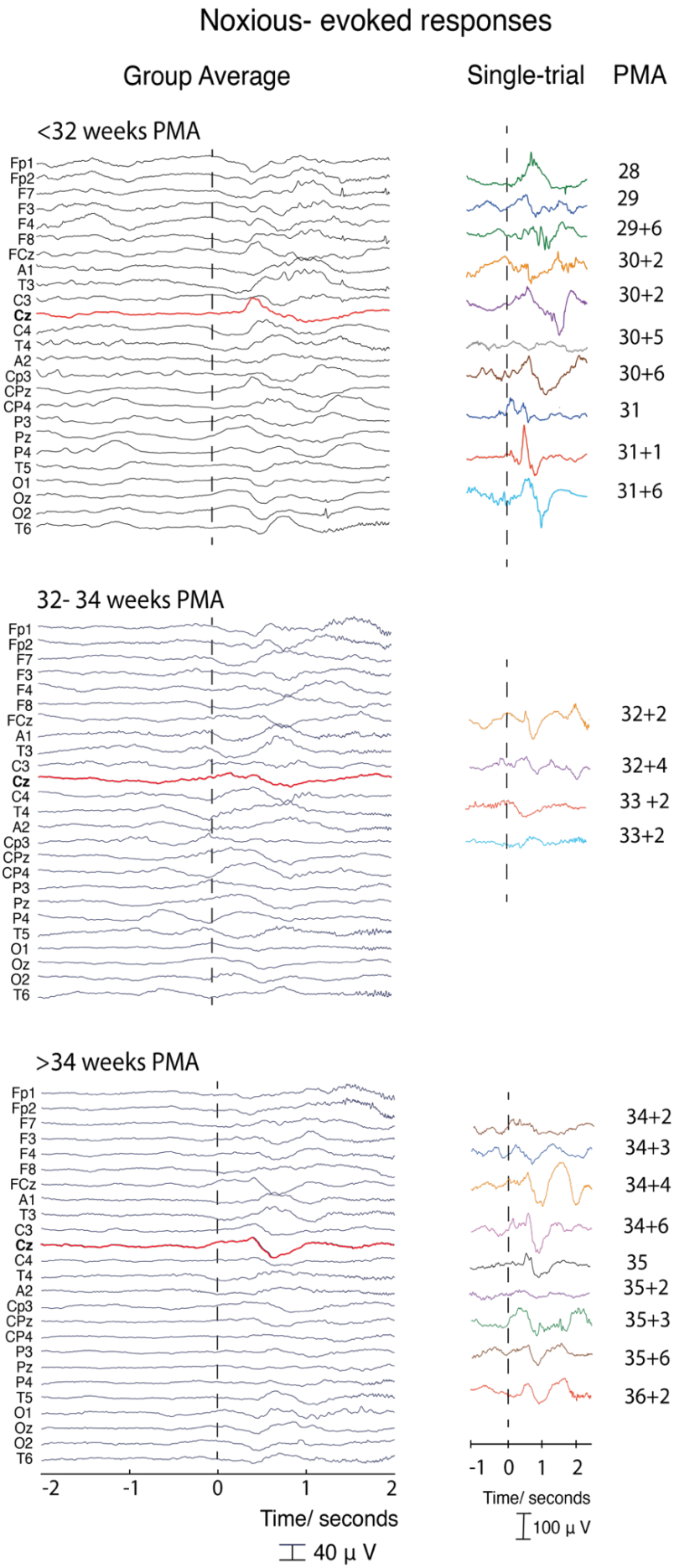


Figure 4.6 EEG activity after noxious heel lance.

For each group the grand group average and the individual single trials for each infant is shown. Dotted lines mark the point of stimulation.

In preterm infants <34 weeks PMA, noxious stimulation is known to evoke delta brushes, and these were visually labelled by GSM. The presence of delta brush activity resulted in poor fit of the temporal template and magnitudes below zero. In this data set n=4 recordings showed delta brush activity in response to the heel lance. In some cases, no response was observed, and this also resulted in low magnitudes.

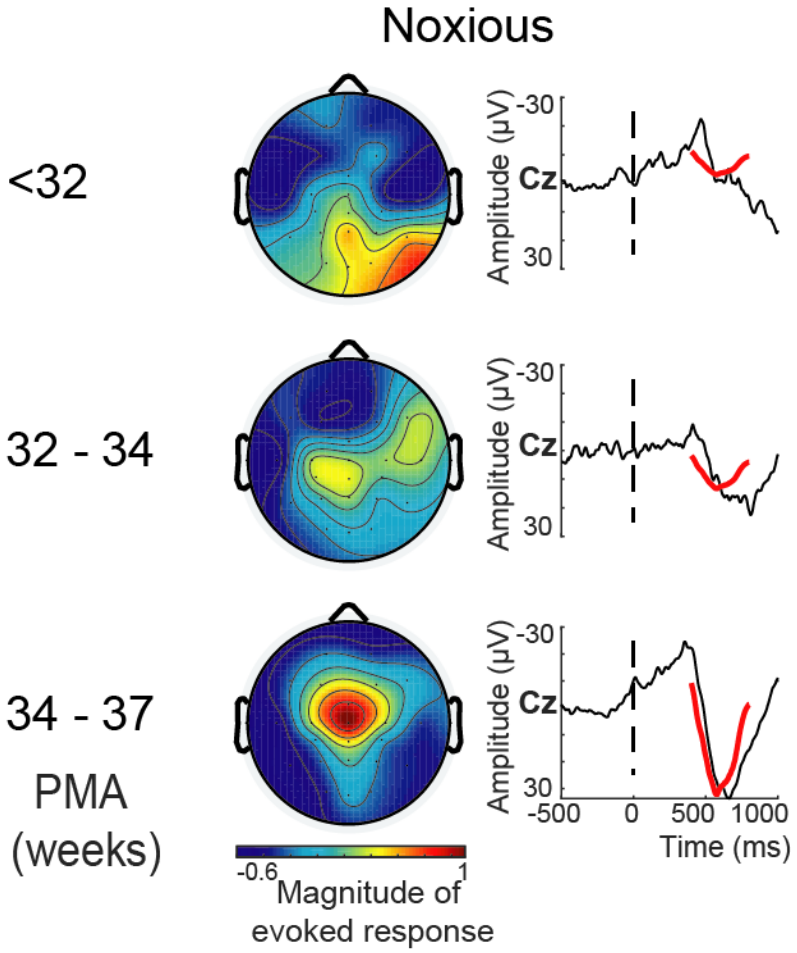


Figure 4.7 Spatial localisation of the response magnitudes and corresponding EEG activity for the noxious stimulus.

Spatial localisation of the response magnitudes and corresponding EEG activity for the noxious stimulus, averaged across three age groups, based on postmenstrual age (PMA = GA + postnatal age).: <32 weeks, 32-34 weeks and 34-37 weeks PMA. The group average evoked activity is shown with the template overlaid in red.

4.6.2 The magnitude of the stimulus- evoked response in preterm infants matures with age.

The change in magnitude of stimulus-evoked brain activity with age was also investigated. The magnitude of response correlated significantly with PMA for each stimulus, but the direction of the correlation differed between stimulus modalities. There was a significant positive correlation in the magnitude of the positive deflection measured by the visual temporal template with PMA (linear random intercepts model, R^2=0.234 and p=0.015) and with noxious stimulation (R^2=0.498 and p=0.009). Conversely, the negative deflection measured by the tactile temporal template showed a significant negative correlation (R^2=0.168 and p=0.031) with PMA. Magnitudes below zero represented a poor fit of the template. This occurred when there was no response or in the presence of delta brush activity.

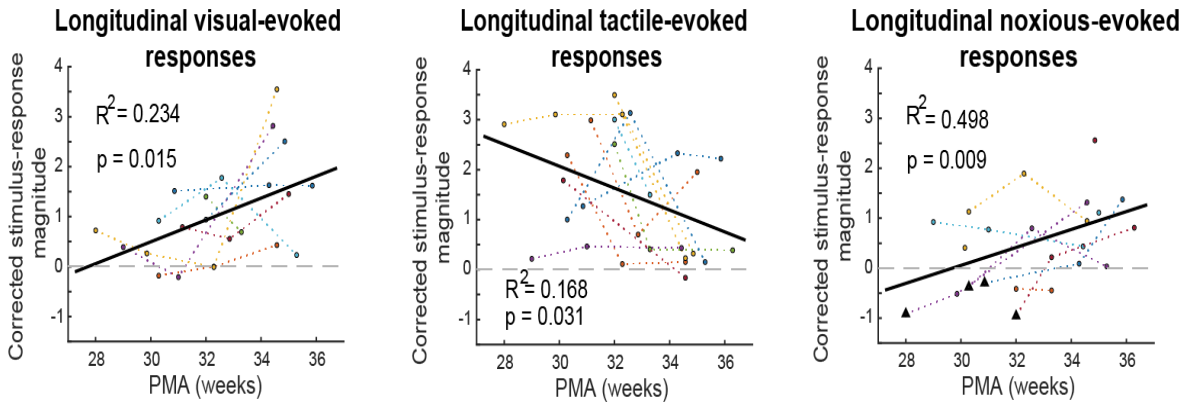


Figure 4.8 Correlations between the size of the response magnitude with PMA, at the spatial location of peak activity.
Linear random intercepts models are fitted between the response magnitudes and PMA (accounting for potential confounds due to baseline changes in pre-stimulus activity, GA and trial number). Visual-evoked responses are shown for channel Oz, tactile-evoked responses at Cz, and noxious-evoked responses at Cz (triangles represent delta brush activity). Each patient's trajectory is plotted in a different colour, with the overall (fixed effects) regression line in black. Dashed lines denote the point of zero magnitude and the p-values and R^2 from models are also shown.

4.6.3 Latency is not a significant factor contributing to the maturity of stimulus evoked activity in premature infants.

Peak latencies for each stimulus per recording were visually inspected and measured. The early visual component was identifiable in all infant recordings (24/24) across gestation with mean peak latencies (± standard deviation) of 199.8 ± 36.1 ms in infants <32 PMA, 218.8 ± 9.9 ms in infants between 32-34 weeks PMA, and 209.4 ± 16 ms in infants >34 weeks PMA. In 7/29 recordings, no discernible tactile evoked response could be identified or measured, and they all belonged to the last age group from the longitudinal cohort. Mean peak latencies were 223.8 ± 44.7 ms, 240.8 ± 73.6 ms and 184.8 ± 32.3 ms for each age group respectively. Noxious evoked activity was identified as either a prominent positive waveform around 400- 800 ms post stimulus or a negative waveform around the same latency window. In 9/29 recordings a negative peak was most dominant and of those 9, 6 of them show characteristics of delta brush activity (ie. slow delta wave with superimposed alpha/beta activity). Four recording sessions evoked no clear positive or negative activity around this PMA. Mean peak latencies including either a positive of a negative deflection were 524.3 ± 55 ms for infants <32 weeks PMA, 522.8 ± 107.1 ms for infants between 32-34 weeks PMA and 601.8 ± 77.6 ms in infants >34 weeks PMA. No significant correlation with PMA was found across modality.

4.6.4 Visual and tactile evoked activity is differentially affected by premature exposure to the environment.

To investigate the possible impact of early exposure to the environment in preterm infants, the stimulus-evoked magnitudes of the preterm infants who

were studied longitudinally were compared with the responses of an independent, age-matched near-term cohort of preterm infants. To this end, the last group of the longitudinal cohort (born <32 weeks GA and studied at 34-37 weeks PMA) was directly compared to the preterm control cohort (born at >34 weeks GA and studied between 34-37 weeks GA).

Visual comparison of the spatial organisation of each stimulus between the two groups, shows that regardless of the degree of prematurity, the topography of responses to each stimulus is similar to those seen in healthy full-term infants by near term age, but the intensity of the response differed between the two cohorts (figure 4.9).

When the magnitude of the stimulus-evoked response was compared between the two cohorts, stimulus-specific differences were apparent. The infants from the longitudinal cohort, who had been exposed to the environment early, had significantly greater visual-evoked responses than the PMA-matched control group (linear random effects model, $p=0.020$). In contrast, the very premature infants born < 32 weeks GA had significantly lower tactile-evoked response magnitudes ($p=0.026$) than the control group. No significant difference was observed for noxious evoked responses ($p=0.930$).

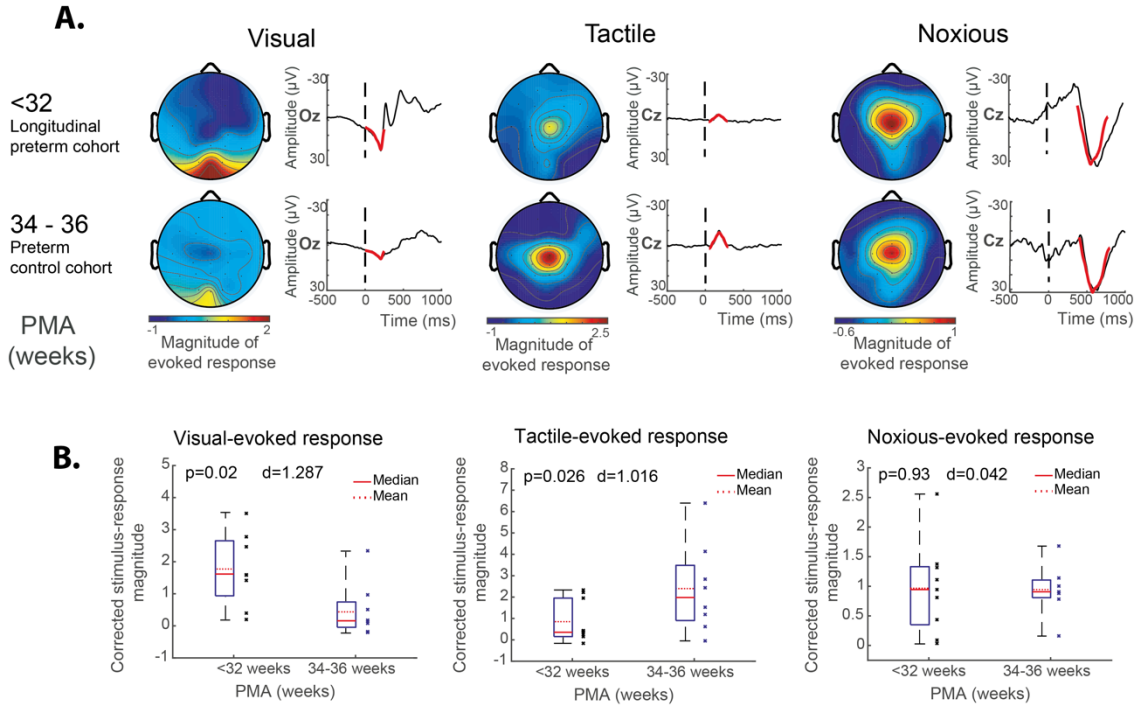


Figure 4.9. Comparison of the stimulus-evoked responses between preterm babies at <32 weeks GA and an older, independent preterm control group at 34–36 weeks GA (both at matched PMA of 34–37 weeks).
(A) Comparison of the spatial distribution of the stimulus-evoked response magnitudes between the two preterm groups with the corresponding average EEG activity and the template overlaid in red. (B) Comparison of the size of the stimulus-evoked response magnitudes between the two preterm groups at the spatial location of peak activity. Visual-evoked response is calculated at channel Oz, tactile-evoked response at Cz, and noxious-evoked response at Cz. The p-values of the comparison between both groups are shown, using a linear random intercepts regression model (corrected for baseline changes in pre-stimulus activity, PMA, and trial number confounds) *p<0.05. The d-values show Cohen's d effect size.

4.6.5 Reflex withdrawal in response to noxious stimulation.

Reflex withdrawal was observed in response to noxious stimulation. Visual comparison of the responses shows that the amplitude and duration changes with PMA, but no significant differences were found in RMS values. Comparisons between different groups in the longitudinal cohort showed no significant difference, and this was also the case when the longitudinal groups were compared with the preterm control cohort (see figure 4.10).

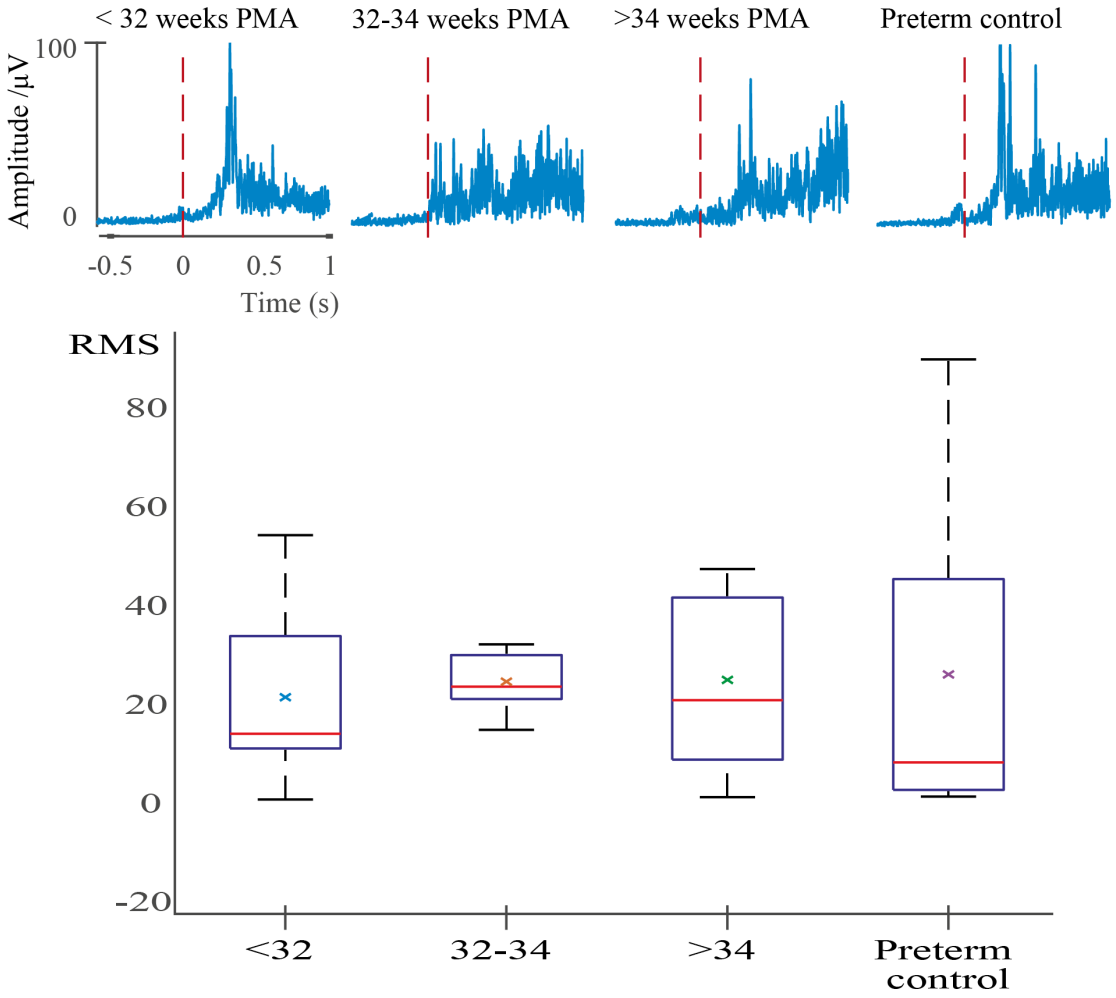


Figure 4.10 EMG activity in response to noxious stimulation.
On the top row, the average EMG activity for each group in the longitudinal cohort and the preterm control cohort are shown. Dotted lines represent the point of stimulationOn the bottom row, boxplot showing the calculated RMS values for each cohort of infants. Red lines represent the median range and crosses show the mean RMS for each group.

4.7 Discussion

A consequence of premature birth is the long-term exposure to the neonatal care environment which can adversely affect early neurodevelopment. In this study the aim was to track the maturation of specific components from stimulus-evoked brain activity in a longitudinal preterm cohort to elucidate when these components emerge and how they compare to age matched preterm control infants. Understanding how the preterm brain responds to the ex-utero

environment is key for optimising the supportive care and environment of infants in the neonatal care setting.

In this study, using a template approach of analysis, the maturational evolution of stimulus-evoked brain activity in response to visual, tactile and noxious stimulation is demonstrated in healthy preterm infants aged 28-37 PMA. Given that the visual and tactile pathways are amongst the earliest developing sensory systems, the localised topography of evoked activity to the occipital and mid central region observed in the youngest infants (<32 weeks PMA) was not surprising. In both cases the activity became more refined with PMA (shown in figures 4.3,4.5,4.7). In contrast, noxious evoked activity does not appear organised in the somatosensory area of the brain until after 34 weeks PMA, corroborating with previous studies suggesting that noxious activity in infants is not specific until later in gestation (111,209,211).

Interestingly, although the topography of visual and tactile evoked activity was already distinct in the primary sensory areas of the brain in the youngest infants, the developmental trajectory of the activity differed. Whilst visual evoked activity appears to increase with PMA, the intensity of tactile evoked activity decreased across the period studied. Noxious evoked activity matures differently and the increased magnitude of evoked activity with PMA, reflects the switch from non-specific neuronal bursts of activity (showing a poor fit to the template and low magnitudes) to the more refined response seen after 34 weeks PMA.

In accordance with previous longitudinal preterm studies, the increasing complexity of the visual evoked response is reproduced in this cohort. Infants

<30 weeks PMA showed a basic negative waveform preceded by a positive peak from 30 weeks GA onwards (187,189,190,240). The visual temporal template used in this study captured the emergence of the early positive peak (P200) which increases in magnitude with PMA. Its appearance between 30-36 weeks GA is thought to reflect the increasing number of stellate and other association neurons and their connections with the pyramidal cells which occurs around this time (190). The absence of this peak by term age has been associated with adverse outcomes such as the development of cerebral palsy (264). It is worth noting, that visual inspection of the individual trial averages showed delta brush activity after the initial visual evoked response in n=9 infants <34 weeks and n= 1 >34 weeks PMA. Although this is only a visual observation, this corroborates with the observations made by Colonnese et al (2010) (180).

In contrast to visual evoked activity, the evoked activity to tactile stimulation consisted of a negative waveform which was present in infants <32 weeks PMA and did not significantly change in morphology across the period studied. Instead, this peak is reliably observed in the youngest infants and reduces in amplitude with PMA. The topography of the response is consistent with the somatotopic representation of the foot in the somatosensory cortex (265), and the morphology, latency and developmental trend captured by the tactile template is similar to the N2 peak which emerges around 31- 34 weeks corrected GA (12,199,209,225,226,248,253,266). Stimulation of the hand has been shown to shift in topography from a contralateral central source to the midline (199), which has been suggested to represent bilateral integration of somatosensory information (267) and inter- hemispheric communication which occurs in the last trimester of gestation. The reduction in amplitude observed to hand stimulation is potentially

influenced by the increasing inter-hemispheric inhibition mediated by the corpus callosum (199,268). In this context, it is possible that the reduction in magnitude of evoked response seen in this study reflects this inhibition even though the shift in topography is not apparent due to the somatotopic location of the foot. Clinically, preterm infants show higher sensitivity to touch during handling for cares than term infants which could be explained by the fact that preterm infants have a very thin epidermal barrier which is more prone to damage and higher density of mechanoreceptors (99). Some studies suggest that preterm infants do not habituate to tactile stimulation and instead show sensitisation (64).

The appearance of noxious specific evoked activity after 34 weeks GA has been reported previously (111,209,211) using similar methods to those used in this thesis. Here we have again demonstrated that the magnitude of the response increases with PMA after 34 weeks GA. In younger infants <34 weeks PMA, the clinical lance either evoked delta brush activity or no response which explained the poor fit of the noxious template and the low magnitude weights. This switch in morphology has been attributed to a transition in the processing of noxious input related to the structural development of thalamocortical connections and the formation of callosal and association pathways (209). In contrast to somatosensory and visual input, which to some extent may be present in utero, the developing infant would not normally be exposed to noxious input in the womb, therefore the development of an early pain circuit is not necessary before birth. Pain is a complex experience with sensory and affective components involving multiple pain regions, and this switch may represent the first stage in the development of central pain processing (209).

The processing of noxious information is different to other sensory modalities. Unlike the somatosensory and visual cortex, there is no dedicated brain region for pain. Instead, noxious stimulation evokes a diffuse pattern of activity which coordinates or synchronises to create the sensory, emotional, motivational and cognitive experience of pain (106). The sensory- discriminative aspect of pain must involve the primary and secondary somatosensory areas of the brain (269) as without a functional framework of sensory receptors, afferent connections to the spinal cord and thalamocortical connections, which are in place by the second trimester of pregnancy, cortical processing of external input would not be possible (104). The discrimination between tactile and noxious stimulation is thought to occur around 35-37 weeks GA which coincides with the switch from non-specific delta brush activity to nociceptive specific brain activity in the infant brain, a refinement in reflex withdrawal (101,111) and discriminative behaviours (211) to noxious input, demonstrating the ensemble of key developmental milestones that lay down the foundations of nociceptive processing (106).

In this study, the latency of the trial-averaged activity for each stimulus was additionally manually measured to check for peak latency decreases which are commonly reported in evoked potential studies. Across the gestational ages in this data, no significant decreases in latency were observed for any stimulus modality. The positive component of the visual evoked response emerges during the period studied here but decreases in latency are most prominent from 37 weeks GA onwards with the initiation of myelination in the optic radiation (244). This would explain why latency decreases were not observed in our longitudinal data set. It has also been suggested that decreases in latency do not occur in a linear fashion but in a stepwise pattern reflecting synchronised myelination in

several parts of the visual pathway (244). In contrast, latency of cortical somatosensory responses has consistently been reported to decrease with gestational age (226,266) but this was not observed in our data. The discrepancy between these results could be due to failure to control for different sleep states, known to influence latency of tactile evoked brain activity (209). In six of the ten infants, no tactile evoked response was visible in the last recording session (infants >36 PMA) when sleep states are more established. It is also possible that the discrepancy is due to the age range of infants studied. In contrast with the initial P1 component which gradually decreases in latency with conceptional age, the latency of P2 and N2 dramatically reduces around 30 weeks conceptional age (cGA) but then only gradually continues to shorten (226). The infants in this study had a mean PMA of 30.4 weeks therefore a latency shift across the PMA studied may be more gradual and therefore not significant. It was also difficult to assess latency from noxious evoked brain activity. 4 out of 28 recorded heel lances showed no detectable response and in 10 out of 28 the response was a negative deflection of which half of them were clear delta brush activity. This variability in morphology and polarity of the peak made it difficult to establish developmental latency shifts. Furthermore, our template approach was not optimized for this purpose. However, the latency of nociceptive specific activity is shorter in term infants compared to preterms (209) and latency continues to reduce with age, as the adult equivalent of this response has a much shorter latency (270). Latency is associated with myelination, a process which continues through childhood in all sensory modalities.

Comparisons between the longitudinal cohort at >34 weeks PMA and the preterm control cohort born and studied between 34-36 weeks PMA in this study

showed interesting differences. The data suggests that the positive deflection captured by the visual template is significantly higher in magnitude in the longitudinal cohort compared to the preterm control, suggesting that the emergence of this component of the response is either accelerated or potentiated by early exposure to visual input. This observation has also been reported by several authors (16,240,271). If retinal maturation is the main contributor to the initial visual evoked response, then the effects observed here could be a result of over exposure to light directly affecting the retina. Preterm infants have thin eyelids so even though they are closed for most of the time, they are more vulnerable to over exposure to light. This is supported by observations that some visual abilities like ocular motility, object tracking, and fixation develop prenatally, but visual experience is crucial to acquire other visual abilities such as attention at a distance, ocular alignment, and convergence. Premature infants are more efficient at these skills than full-term infants of similar PMA (272–274). In preterm monkeys, visual experience appears to influence the maturation of the visual cortex by strengthening, modifying, and/or eliminating synapses that are already formed rather than regulating the rate of synaptogenesis (275). Interestingly, over the first two postnatal years, preterm infants show a delay in the latency of this positive peak when comparing low birthweight and very low birthweight preterm infants to healthy term controls. The pattern of maturation was similar in all groups, and the differences in the latencies generally became smaller so that by 18 months no significant latency differences could be seen between these groups (188). This suggests that although the emergence of the component in visual evoked activity is accelerated due to premature exposure to visual input, myelination of the optic nerve may be delayed.

On the other hand, the negative peak evoked by tactile stimulation was more reduced in the longitudinal cohort compared to the preterm control. In previous studies of posterior tibial stimulation, somatosensory evoked potentials matured at a similar rate independent of preterm birth in terms of morphology and latency (14,226). It is plausible that in-utero stimulation of the somatosensory system may be a factor influencing the different rates of maturation between the visual and tactile sensory system. It is also possible that some adaptation to living in the ex utero environment does occur in preterm infants, as it is not beneficial to survival to react in an exaggerated way every time they are being touched. Fabrizi et al 2011 showed that infants near term age responded similarly to a tactile and noxious stimulus that those born more preterm, suggesting that born near term age can also affect somatosensory processing as the differentiation between touch and nociceptive information is still not fully developed.

There was no significant difference between the noxious evoked magnitudes from the longitudinal preterm cohort and the preterm control cohort in this study. This may be counter-intuitive as infants from the longitudinal preterm cohort had more skin breaking procedures compared to the control group, and would have been handled more frequently by clinical staff for general cares and clinical assessment. Repetitive pain-related stress in preterm infant has been shown to have long-term consequences on somatosensory processing (i.e., quantitative sensory testing) (48,276), sensitivity to pain (143) and response to pain (239,277). In 2010, Slater et al compared the noxious evoked brain activity of preterm infants at term age with term infants. In this study, the evoked brain activity was significantly higher in magnitude in the ex-preterm cohort. However, the infants included in the ex-preterm cohort (n=7) included 3 infants

with grade 4 IVH (intraventricular haemorrhage) who would have required a higher number of painful procedures to support and monitor their care during their stay in the neonatal unit. It is possible that in that study the comparison reflects the high burden of painful procedures and pathology rather than normal maturation of noxious evoked brain activity which was the aim of this study. It is likely that the timing and duration of repetitive painful procedures also contributes to this result. Infants who are less sick in the first 24 hours of life have improved motor pathway development (278) and brain microstructure is not impaired by premature birth in the absence of brain injury or prolonged oxygen exposure (279,280). This emphasizes that exposure to systemic illness, brain injury and the environment and manner in which infants are cared for are the main determinants of adverse brain development (279). The infants in the longitudinal preterm cohort had no neurological conditions, when ventilated invasively it was for short periods, and the mean number of painful procedures decreased with PMA (see demographics table 4.1), therefore it is plausible that the infants in this cohort may not be adversely affected by long term exposure to painful procedures. To put this into perspective, the sickest of infants can have more than 12 procedures that are deemed painful per day (147), and this includes common procedures such as clinical heel lancing used in this study. In this cohort the mean number of painful procedures at the last recording session was 37.5, which is much lower than the average that would be expected for this population.

The effects on the maturation of neurosensory development trajectories observed in this study support the concept of the critical period of neurosensory development (192). In this context, preterm extrauterine experience interferes with endogenous stimulation (typically by sleep disturbance) and exogenous

stimulation that is earlier than biologically expected or overwhelming, including light, noise, touch or pain. An environment that is not GA-appropriate and stressful may add to the morbidities related to preterm birth.

Developmental care is to a large degree based on the understanding of preterm ex-utero neurosensory development. It consists of individualized strategies based on the infant's skills and vulnerabilities and is aimed at supporting the infant's regulation and development (124). One of its pillars is the reduction of the exposure to inappropriate stimuli, as well as pain and stress (281,282). Furthermore, the sensorial environment is adapted to provide physiological stimuli aimed at promoting behavioural and postural regulation and comfort. The environment is respectful of the endogenous neural stimulation and organization that occurs during REM sleep. Importantly, parents are supported in their role, and parent-child attachment is promoted, as attachment provides consistent physical nurturance and supportive social interaction (281,283–286). Although studies have shown contradictory results, there is evidence to support a positive impact of developmental care on short- and long-term outcomes (129,284,287–291).

The impact of preterm birth on development is not limited to the preterm period and consists of a combination of events over the course of many months which lead to cerebral white matter injury and subsequent dysmaturational events in the white matter and neuroaxonal structures (292). This encephalopathy of prematurity is likely to be caused by events which are critical to optimal development. Animal studies suggest that it may be possible to prevent, counteract, or ameliorate these dysmaturation processes which lead to

neurocognitive deficits (292). In the context of this study, moving towards a more individualised developmental care approach in NICU settings is a way of regulating the effects of experiential factors on development. An MRI study comparing the regional cortical volumes of pre-term and full-term infants at 8 years of age (293) showed significant reductions in brain volumes, especially over sensorimotor cortices, between the two groups. This finding was also associated with poorer visual and cognitive outcomes. Smyser et al 2010, also found differences in the resting state of sensory resting state pathways, with thalamocortical connections most affected by prematurity (276). These studies and others highlight the importance of optimal exposure of auditory and visual experience and reduction of pain and stress during the preterm period, and the importance of other factors in infancy relating to brain maturation including environmental enrichment, breastfeeding and social economic factors (122) which have all been linked with impaired brain maturation and neurocognitive function later in life

Although some of the observations reported in this study have been described in previous studies, the methods used to characterise the evoked responses to different types of stimulation are novel. The templates capture specific components from sensory evoked activity which change with gestation and would be difficult to compare across modalities without a common quantitative measure. The combination of visual inspection and data analysis techniques to quantify the emergence and trajectory of cortical evoked activity in response to visual, tactile and noxious stimulation provides invaluable insights into the physiological underpinnings of developmentally based pathology and a starting point from which to interpret and understand the aberrant pathways of brain

development that produce disease (38). Given that the neonatal population is a particularly difficult group in which to obtain clinical assessments of sensory and neurological function, evoked potentials can provide invaluable information, especially in sick neonates whose clinical examination may not reflect the outcome.

4.8 Strengths, limitations and future work

This study is the first to look at the longitudinal development of clinically-relevant noxious (i.e. heel lance), tactile and visual evoked potentials in the same cohort of infants during the period of prematurity. Moreover, the infants included in this longitudinal study had remarkably straightforward clinical journeys in NICU factoring out as much as possible the influence of injury rather than preterm birth. Evoked responses were characterized using temporal templates which made it possible to compare patterns of activity across sensory modalities and between the longitudinal and cross-sectional cohorts of infants. This work contributes to our understanding of normal ex-utero development, laying the groundwork for future hypothesis driven research studies to look at the impact of pathology or the effects of neuroprotective interventions. However, there are several important limitations of this work.

Vigilance states affect the characteristics of evoked potentials, but this was not investigated as part of this study. Whenever possible, visual stimulation was presented when infants appeared awake (determined by responsiveness to handling, EEG, ECG and respiration during the recording session) and tactile stimulation when the infant was asleep (determined by the same factors of

responsiveness, EEG, ECG and respiration). However, due to the restricted timing of the recording sessions, it was not feasible to wait until the infant changed state of alertness. In many cases the infant was in indeterminate sleep during periods of stimulation suggesting the infant was being aroused by the stimulus. However, in spite of this, the methods used in this thesis helped to overcome this limitation, and in an effort to account for variability caused by differences in sleep state, the pre-stimulus weight was subtracted from the weight of the response. Future work investigating the effects of sleep state may elucidate the influences of sleep on sensory development further.

Another important limitation is that the temporal templates were derived for the dominant activity present in term infants, and activity that will have been dominant during the preterm period was not analysed. For example, Colonnese et al 2010 (180) reported delta brush activity around 800 ms post stimulus in preterm infants which disappears by term age. As delta brush activity was not present in the full-term control group, the switch could only be visually described. This is a more general limitation of using temporal templates, as it cannot be assumed that a single peak reflects a singular process that is stable over time. However, the discussion of the results is based on comparisons with previous work where similar waveforms were observed.

It was also not within the scope of this study to assess the development of the infants included in this study after discharge from hospital. As development is an ongoing process, it would be important to report that these infants did not show any developmental disabilities later in life, especially if these results are to be used as a reference for future work including prognosis. To delineate the

differential maturation between longitudinal and cross-sectional data, it would be interesting in future studies to recruit control preterm infants at different gestational ages.

4.9 Conclusion

Using a novel template approach of analysis, it was possible to characterise the developmental changes in specific components of evoked brain activity and corroborate the results with previous findings. By using this approach, it was also possible to highlight the possible influence of ex-utero experience on sensory development which is a topic increasingly becoming more important as multiple lines of evidence suggest that the environment and way in which premature infants are cared for, plays a key role in brain development and long- term outcome.

Chapter 5 THE IMPACT OF NEONATAL HYPOXIC-ISCHEMIC ENCEPHALOPATHY ON SOMATOSENSORY PROCESSING

In Chapter 3 temporal templates were designed using a healthy full -term cohort of infants and in Chapter 4 they were projected onto longitudinal data collected in prematurely-born infants to track the trajectory of specific components in the waveform of responses to visual, tactile and noxious stimulation. In this chapter, the templates were projected onto the evoked responses to tactile and noxious stimulation in infants diagnosed with hypoxic-ischaemic encephalopathy (HIE) and an independent group of full-term healthy infants to investigate the impact of brain injury on somatosensory processing.

5.1 What is HIE

Neonatal encephalopathy (NE) is an umbrella term for the complex presentation of infants born with an altered level of consciousness, inability to initiate or maintain respiration and incidence of seizures. These infants may display multiorgan as well as neurological dysfunction. There is no specific aetiology for NE and there are multiple causes leading to its presentation, one of them being the prolonged deprivation of oxygen to the brain around the time of birth which leads to HIE. Due to the lack in specific aetiology of the different subtypes of NE, HIE and NE have been used interchangeably. There are no specific biomarkers

of HIE and it is difficult to prove the presence of cerebral hypoxic ischaemia with the exception of well characterised animal models and particular cases of neonatal stroke (294). To determine the likelihood that an acute hypoxic-ischaemia event has occurred around the time of delivery which could contribute to NE, The American College of Obstetricians and Gynecologists (ACOG) task force on NE and neurologic outcome made a statement to try and clarify these terms (295).

The incidence of HIE in England is reported to be between 2.4-2.8 per 1000 live births (296), and around 80% of the infants admitted to intensive care with HIE are born at term (>37 weeks gestational age, GA). Perinatal asphyxia leading to HIE remains one of the major causes of death and disability in term born infants and is the most common form of brain injury found in newborns around the time of birth.

Although there has been much progress regarding the pathophysiology of perinatal brain damage from animal models of brain injury, in the newborn infant it has been difficult to adapt the findings into safe neuroprotective treatments. Furthermore, although clinical correlates of HIE such as neurologic examination and biochemical markers are key determinants of the severity of encephalopathy initially, the challenge in neonatal care continues to be identifying infants who are most at risk for permanent injury, and those who are likely to benefit from treatment.

5.2 Animal studies of HIE

A wide range of animal species and models of hypoxic ischaemia have been used to study the underlying biochemical and cellular processes leading to brain injury, and its developmental consequences.

The rat model of perinatal asphyxia characterised extensively first by Rice and colleagues (297), and by Vannucci and colleagues in the 1980s (297,298), is the most established and reproducible model used to look at the effects of perinatal brain injury. Seven-day old rodents have been demonstrated to have the analogous brain maturity to model a third trimester fetus, and these studies have furthered our understanding of the mechanisms of brain injury, particularly within gray matter brain structures. The rat model is also suitable for long-term survival studies and to test for possible interventions. However due to its size, it is not possible to evaluate systemic effects of various treatments or to allow frequent biochemical measurements. Other small animal species have also successfully been used to model acute changes after a hypoxic insult, but there is limited information about each of the models in terms of changes in cerebral blood flow and metabolism.

While small animal studies have been essential for our understanding of the underlying biochemical mechanisms of perinatal HIE, these studies also lack the more complex neurological features of the condition. Gray/white matter proportion and substantial cortical folding (299) are better represented in larger animal species and are more comparable to humans.

Classical studies performed by Myers (1972) (300) investigated the effects of asphyxia in near term rhesus monkeys and categorised 2 patterns of brain damage that corresponded to the degree of hypoxia and/or ischaemia and the presence of acidemia. The pathological brain changes experienced by this cohort of monkeys closely resemble the changes observed in human newborns. These early primate studies also demonstrated that the immature fetal brain is more tolerant to asphyxial insult than the mature brain, that ischaemia is required for brain damage to occur, and that the nature and extent of injury can be predictive of neuropathologic changes (i.e. basal ganglia-thalamus and peri-rolandic cortex pattern, with commonly associated involvement of the hippocampus and the brainstem, following 'acute near total asphyxia'; or watershed pattern involving the white matter and cortex in the border zones of the anterior, middle and posterior cerebral arteries, following 'prolonged partial asphyxia').

Other large animal species, such as fetal- neonatal sheep models and newborn piglet models, have advanced our understanding of the effects of asphyxia on regional changes in cerebral blood flow and energy metabolism (302–306). The concept of 'secondary energy failure' (as described below) has been elaborated through animal models, in particular with newborn piglets. Furthermore, these animal experiments have allowed the investigation of neuroprotective strategies, including therapeutic hypothermia. The latter has, in turn, proven clinically effective and safe in human infants with HIE, and nowadays constitutes the standard of care for these patients (307).

In summary, no animal model is ideal for reflecting the complexity of human brain pathology, but these studies have elucidated important components in the

sequence and evolution of the phases of energy failure, cellular damage, and dysfunction after a hypoxic injury, as well as neuroprotection targets and interventions.

5.3 Pathophysiology of HIE

Hypoxic ischaemic brain damage is an evolving process. It begins during the insult and extends into the recovery period after resuscitation (reperfusion interval). Tissue injury takes the form of either selective neuronal necrosis or infarction (308), and is associated with two phases of pathologic events that culminate in brain injury.

The primary (initial) stage of energy failure is a predictable phenomenon characterised by a reduction in cerebral blood flow, depriving the brain of oxygen and nutrients, and leading to a shift in anaerobic glycolysis. High energy phosphorylated compounds such as adenosine triphosphate (ATP) and phosphocreatine are depleted, tissue acidosis is prominent, and there is an accumulation of lactic acid, calcium, and free radicals (309). This phase is also associated with acute intracellular derangements such as loss of cell membrane functions, defective osmoregulation, inhibition of protein synthesis, and the increased release of excitatory neurotransmitters such as glutamate.

Resolution of the primary energy failure within a specific time interval reverses the processes by increasing high energy phosphorylated metabolites and intracellular pH and promoting the recycling of neurotransmitters. This reversal and recovery is affected by factors such as maturation, preconditioning events, substrate availability, body temperature, and simultaneous disease processes.

Even though recovery may occur after the primary energy failure, a secondary interval of energy failure may follow at a time remote from the initial event. This secondary event is dependent on the extent of the primary event and is different in that the declines in phosphocreatine and ATP are not accompanied by brain acidosis. This secondary phase is also known as the delayed phase of injury, during which neurons and oligodendroglia continue to die for longer periods (310).

When infarction occurs, the immediate area surrounding the infarct (penumbra) consists of neurons undergoing either necrosis or apoptosis. This penumbral area appears most amenable to reversal of cellular injury, so the interval between the primary and secondary energy failure represents a latent phase that corresponds to a therapeutic window for intervention. If the insult is not interrupted, this cascade ultimately leads to acute or primary cell death.

In combination with and beyond the outlined phases of acute and secondary energy failure, a tertiary phase of damage may persist for months or years after the initial injury. These persistent brain changes may include myelin deficits, reduced plasticity, and altered cell number which could be caused by persistent inflammation and epigenetic changes causing blockade of oligodendrocyte maturation, impaired neurogenesis and axonal growth or altered synaptogenesis (311).

5.4 Concept of Neuroprotection: Therapeutic Hypothermia

The discovery of the observation that most injury caused by hypoxia-ischemia evolves with a delayed onset has been followed by the proof-of-principle that intervention after the hypoxic-ischemic insult (during the so-called therapeutic window) reduces brain injury and its consequences. Amongst the many experimentally neuroprotective interventions discovered, moderate hypothermia is the only one currently clinically available for infants with perinatal HIE.

The concept of neuroprotection by therapeutic hypothermia (TH), emerged from observations that people who nearly drowned in cold water had remarkably good outcomes. This was followed by testing of therapeutic hypothermia in animal studies (312–314). Models of asphyxia in piglets who were either maintained normothermic or treated with hypothermia at 30, 33.5 or 35ºC demonstrated a U-shaped temperature dependence for delayed cell death, and an inverted U-shaped temperature dependence for microglial activation (315). An overall neuroprotective effect was observed in piglets cooled to 33.5 and 35ºC, whereas cooling to 30ºC and normothermia were associated with significantly worse brain injury in areas such as the thalamus, putamen, and caudate nucleus (315). Moreover, cooling to 30ºC leads to abnormal metabolic homeostasis (316).

At least three randomised controlled trials comparing moderate hypothermia and conventional treatment for HIE in term infants are thought to be important milestones in the treatment of perinatal brain injury. In the CoolCap trial (309), infants were randomised to either treatment with a selective head cooling device, with a rectal temperature of 34.5ºC for 72 hours, or conventional treatment.

Although primary outcome of death or severe disability was not significantly different between the two groups, outcome was significantly better in the hypothermia group when the most severe cases of encephalopathy were excluded.

The National Institute of Child Health and Human Development (NICHD) trial of whole-body hypothermia for neonates with HIE (317) and the Total Body Hypothermia for Neonatal Encephalopathy trial (TOBY) (318) enrolled large numbers of infants. Meta-analysis of these and other randomised controlled trials has concluded that therapeutic hypothermia is beneficial in term infants with HIE (319). Cooling is associated with a reduction in death and disability at 18 to 24 months (risk ratio 0.75 [0.68, 0.83]). Longer-term follow up studies of some of the major trials show that hypothermia also results in improved neurocognitive outcomes in middle childhood (6,319–323).

There are no clinical trials of hypothermia for mild HIE. The available studies were designed for moderate or severe HIE, and incidentally some patients with mild HIE were included, but the statistical power was insufficient for this subgroup (324,325).

Despite the exclusion of infants with mild HIE from hypothermia trials, a "therapeutic creep" has been observed, so that TH has been extended to babies with mild NE in many NICUs (326–328). The reasons for this change include clinician's beliefs that therapeutic hypothermia may also be advantageous for mild HIE, medicolegal concerns or misdiagnosis (326). The cases of stage 1 HIE

who are offered therapeutic cooling are likely to represent the most severe forms within the spectrum of mild HIE.

Given the risk of sequelae in some children with mild HIE and the plausibility of the beneficial effect of therapeutic hypothermia, some authors find it reasonable to carry out clinical trials in this population (326). However, difficulties potentially faced when undertaking such studies are likely to include the need for a large sample size based on a lower probability of unfavourable outcome, and the requirement for carefully weighing risks and benefits. Possible adverse effects of cooling that are considered acceptable for high-risk infants (i.e. those with moderate or severe HIE) may be more of a concern for infants with mild HIE. These deleterious effects of cooling include: stress and need for sedatives (and the resulting risk of neurotoxicity); separation from parents; delay in oral feeding; impact on breastfeeding; and invasive procedures.

Two non-randomised cohort studies of hypothermia in mild HIE have shown improvement in MR spectroscopy biomarkers, reduction of white matter injury on MRI and neurodevelopmental outcomes almost free of sequelae at 2 years (329,330). There are currently at least two registered phase II clinical trials of neuroprotective treatments in HIE, "Optimising the Duration of Cooling in Mild Encephalopathy (COMET)" and "Mild Encephalopathy in the Newborn Treated With Darbepoetin (MEND)". Consensus is that, while awaiting results from randomised controlled trials evaluating the safety and efficacy of therapeutic hypothermia and other neuroprotective strategies in mild HIE, currently available evidence is insufficient to recommend routine therapeutic cooling for

infants with mild HIE. Furthermore, the focus should remain on not taking away this opportunity from newborns with the mildest forms of moderate HIE.

The precise mechanism of neuroprotection produced with hypothermia is still unclear but reducing the temperature of the brain appears to suppress many of the pathways leading to delayed cell death. Pathways involved in the synthesis of free radicals and nitric oxide are suppressed, cerebral energy phosphates are preserved, and cerebral acidosis and lactate are reduced. It also influences cell-signalling cascades, which are key factors in the initiation of neuronal injury in the primary phase. These changes may preserve cerebral energy metabolism, reduce cytotoxic cerebral oedema and intracranial pressure, and inhibit apoptosis. Therefore, the therapeutic effects of hypothermia impact both the primary injury and the latent phase of hypoxic ischaemic brain injury (331).

The therapeutic response to hypothermia is dependent on the interval between the time of insult and the initiation of treatment, the severity of the insult, and the duration and depth of hypothermia. Most studies indicate that the maximum benefit is gained if treatment is started within 6 hours of birth and continued for 72 hours (332). Although beneficial effects have been observed when moderate hypothermia was maintained for 12- 24 hours, it has been suggested that 72 hours may be required if the interval before inducing hypothermia is prolonged or milder degrees of hypothermia are employed (320). The therapeutic effect of cooling diminishes linearly with the time of start of cooling (304,333), which means that the intervention has to start as soon as possible after birth. This is of particular importance in transfer cases, when neonates have to be passively cooled until they arrive in a neonatal unit equipped to offer cooling (334). Both

human and experimental data suggest that cooling for longer than 72 hours or to lower than 33.5ºC is not associated with better outcomes and may even be harmful (335,336). These results support the current regimen of cooling at 33.5ºC for 72 hours.

Given that therapeutic hypothermia is now standard of care, other potential treatments are being investigated in combination with hypothermia. Leading examples include the use of erythropoietin, melatonin, stem cells, Xenon, growth factors and blockade of Connexin hemichannels (335). These will not be discussed any further in this thesis.

5.5 Clinical Management of infants with HIE

5.5.1 Clinical Neurologic Evaluation

Any newborn suspected to have central or peripheral nervous system dysfunction is assessed using a comprehensive neurologic examination. The suspicion of dysfunction may be due to history (i.e perinatal asphyxia) or a physical finding detected during the routine neonatal assessment (i.e weak extremities). The first neurological examination is performed at birth, with observations of the newborn's alertness, activity and tone. These factors may be affected by gestational age, maternal anaesthesia or narcotic administration, other maternal medications, prenatal exposure to drugs, placental insufficiency or neonatal illness. The Apgar score is a clinical indicator of the infants' physical condition at birth. A score from 0 to 10 is given at 1, 5 and 10 minutes after birth. Low Apgar scores can be due to other causes apart from asphyxia, such as

prematurity, infection, or maternal anaesthesia. Prolonged depression of the Apgar score (less than 5 at 10 minutes) is indicative of poor outcome.

Infants showing signs of a perinatal hypoxic-ischaemic insult display an altered level of consciousness, reactivity, tone and often have seizures. These signs can be graded using clinical scales of which the three-tier staging system developed by Sarnat & Sarnat (1976) (337) is the most widely used, although various groups have introduced modifications to the original scale, and its application has changed with the introduction of TH. The original Sarnat staging combined clinical and electrographic findings to give an indication of the severity of the insult. More recently, the stages do not incorporate EEG findings or the presence of seizures (301). Infants are reassessed daily as HIE symptoms evolve over the first few days of life (337) and improve over time (323,331).

Clinical Feature	Stage 1	Stage 2	Stage 3
Level of consciousness	Hyperalert	Lethargic or obtunded	Stuporous
Spontaneous movement	Frequent symmetrical	Decreased	Absent
Autonomic system	Generalized sympathetic	Generalized parasympathetic	Both systems depressed
Pupils	Mydriasis	Miosis	Variable; poor light reflex
Heart rate	Tachycardia	Bradycardia	Variable (loss of heart rate variability)
Gastrointestinal motility	Normal or decreased	Increased; passing meconium	Variable
Primitive reflexes			
Suck	Weak	Weak or absent	Absent
Moro	Strong	Weak; incomplete	Absent
Tonic neck	Slight	Strong	Absent
Olfactory response	Strong	Weak	Absent
Myotendon stretch reflex	Brisk	Brisk	Absent
Neuromuscular control			
Muscle tone	Normal	Mild hypotonia	Flaccid
Posture	Normal or mild distal flexion	Strong distal flexion	Intermittent decerebration, fisting, thumb adduction
Central tone	Normal	Decreased	Flaccid

Figure 5.1 Sarnat scale for neonatal encephalopathy.
Taken from Sarnat et al (2020) (301).

5.5.2 Biochemical markers of HIE

Umbilical cord blood pH, base deficit, and lactate are often interpreted clinically as surrogate markers for tissue hypoxia-ischemia. Other more sophisticated serum and cerebrospinal fluid (CSF) biomarkers investigated in neonatal HIE include inflammatory cytokines, neuronal biomarkers, and metabolomic profiling (338–343). The rationale for biomarkers is that they could provide real-time measurement of injury, allowing to identify those neonates who might benefit from neuroprotective treatment, as well as to monitor the progression of injury, and the response to neuroprotection. Furthermore, biomarkers could

inform regarding the timing, patterns and pathways of injury, and research into biomarkers could lead to the recognition of novel neuroprotection targets. Despite this promise, there are no currently available biomarkers widely recommended for clinical use.

5.5.3 Phases and Methods of Therapeutic Hypothermia

Phases of hypothermia are: (1) induction of cooling to the desired core temperature of 33.5ºC; (2) maintenance of the target core temperature for the desired duration of 72 hours; and (3) rewarming in a controlled fashion at a slow rate of ≤0.5 °C per hour (172)

Servo-controlled cooling devices are commonly used nowadays. They consist of a base connected to a blanket or a suit which circulates water. The system uses feedback from core and skin temperature probes on the patient. Its algorithm regulates the temperature of the circulating water to achieve the target core temperature. These servo-controlled hypothermia devices offer better temperature stability than manual systems, with minimal nursing input to ensure that core temperature remains within the target, although it is essential to check that the temperature probes remain in situ.

5.5.4 Supportive care of the infant with HIE undergoing Therapeutic Hypothermia

The implementation of therapeutic hypothermia requires significant understanding of how temperature affects all organ systems and interventions used in neonatal intensive care (344). Education and training in resuscitation, including avoidance of hyperthermia, early identification of eligible infants, and

initiation of early hypothermia followed by safe transport to the referral centre, are crucial steps of the neuroprotection chain. Adequate primary clinical care, seizure therapy, neuromonitoring, neuroimaging and specialised follow-up are all part of neurocritical care of the infant with HIE (345).

5.6 Pain in HIE

Evaluating pain in infants who are incapable of self-report is challenging (see Chapter 1: Section 1.5, and this problem becomes more complex in infants diagnosed with a brain injury such as HIE.

During their first week of life, infants with HIE experience a higher burden of essential clinical procedures that are deemed painful compared to healthy term infants. In addition, many of the procedures take place over the first 3 days of life when the infant is undergoing hypothermia treatment. Exposure to painful procedures so early in life can add to the already high risk of neurological impairment caused by the brain injury. And it is likely that the combination of drugs, the encephalopathy and hypothermia contribute to the neonate's inability to express pain.

Mechanical, chemical and thermal stimuli can all elicit the sensation of pain, and it has been suggested that all nociceptive neurons are cold sensitive, though some require very low temperatures for activation to occur (346). Both cold and pain are transmitted through the spinal cord via the anterolateral system to various parts of the brain that contribute to different aspects of the experience of pain.
While cold is known to attenuate certain modalities of pain (i.e. application of a cold compress to alleviate pain after an injury or for a headache), noxious

mechanical stimulation is increased in cold conditions. It is possible that nociceptors may be sensitised by cold and become hypersensitive. The physiology contributing to these effects is unclear. Infants undergoing hypothermia treatment are cooled to a core temperature of 33-34 °C, not low enough to cause pain, but may cause discomfort especially when induced for a long period of time.

Painful experiences can be measured using physiological, biochemical and behavioural measures. However, in order for a noxious stimulus to be perceived as painful it needs to be transmitted to the cortex. It is therefore possible that in the absence of language measuring changes in noxious-evoked brain activity may be a more direct way of quantifying nociception rather than relying on motor responses that are not directly related to the sensory experience. In spite of this, in newborn neonatal units the observation of changes in facial expression using validated scales is the most common method for assessing pain. While it is still an important factor in determining pain in infants with HIE, signs of pain may be subtle or absent due to the encephalopathy itself or medications and or treatment options. For example, Stevens and colleagues have shown that infants with a higher risk of neurological impairment showed less facial expression in response to noxious stimuli than infants with low risk, and no increase in heart rate or decrease in oxygen saturation (135,347). This raises the possibility that these infants experience more pain, which may not be recognized using conventional pain assessment tools. In contrast Oberlander et al (2002) (348) found no differences in pain expression in low weight preterm infants with parenchymal brain injury and healthy matched controls, suggesting that even when brain activity is likely to be substantially modulated by pathology, this may

not be reflected in downstream observations of facial expression changes. It is clear that behavioural and autonomic changes to noxious stimuli are potentially difficult to interpret if these approaches are used as the only pain assessment tools. This is particularly important given that several studies suggest that even in the absence of behavioural changes, nociceptive activity can still be recorded in the cortex (234).

Even though it is not clear whether sedation-analgesia, aimed at mitigating the response to pain or stress during TH, improves neuroprotection (349), this practice is based on the observation that stress and/or pain seem to counteract the neuroprotective benefits of TH in preclinical models (350). In a retrospective study of neonates with HIE, newborns treated with opioids had less brain damage when assessed using neuroimaging techniques, and better long-term neurological outcome (351). In a multicentre European randomised control trial of TH, a greater therapeutic effectiveness of hypothermia was observed compared to the results reported in previous clinical trials (number needed to treat of 4 versus 6-9). This observation was attributed to the fact that in this study, all the neonates treated with TH were also treated with morphine or an equivalent dose of fentanyl (352). However, in a secondary analysis of the NICHD TH randomised controlled trial, sedation-analgesia exposure showed no association with outcome (353). Unresolved issues regarding sedation-analgesia in infants with HIE undergoing cooling include the suitability, type and dose of the drug used, as well as the clinical scales or neurophysiological tools used to assess pain or stress.

5.7 Diagnostic investigation and Neuroimaging in HIE

At the time of birth, clinical evaluation and cerebral function monitoring (CFM) are the tools of choice to assess the severity of HIE. Electroencephalography (EEG) remains the gold standard for assessing electrophysiological changes after the initial diagnosis of HIE but is not easily accessible and requires trained clinical staff to perform and report the findings. MRI is the gold standard neuroimaging technique to define the pattern and the extent of neonatal brain hypoxic-ischemic injury and offers a reliable guide to prognosis. Brain ultrasound provides a valuable and inexpensive bedside alternative. Near-infrared spectroscopy (NIRS) is a tool to monitor regional cerebral oximetry. HIE is associated with an increase in regional cerebral oxygen saturation, which is correlated with poor outcome (354). This is due to reduced oxygen utilization and impaired autoregulation of cerebral perfusion.

The role of brain ultrasound and MRI in infants diagnosed with HIE will be briefly described in this section. CFM will be succinctly described in the electrophysiology section, which is mainly dedicated to EEG and evoked potentials, as these are the techniques used in this thesis.

5.7.1 Cranial ultrasonography (CUS)

Cranial ultrasonography (CUS) is a safe, reliable and readily available bedside neuroimaging technique for the infant before the fontanels close. Though MRI provides high-resolution depiction of all areas of the brain and has become the gold standard neuroimaging modality at all ages, CUS continues to be the first line and most widely used imaging technique to study the neonatal brain.

CUS is highly sensitive for screening of brain injury, such as intracranial haemorrhage, hydrocephalus, and cystic lesions within the parenchyma (337). It can also be used to exclude brain malformations. However, CUS is less reliable than MRI to depict neonatal hypoxic-ischemic injury, and CUS changes following HIE take hours or days to appear. All in all, CUS is complimentary to MRI in the assessment of the infant with HIE.

5.7.2 Magnetic Resonance Imaging (MRI)

Conventional MRI, with T1- and T2-weighted images, has been shown to have good diagnostic ability to detect brain hypoxic-ischemic injury at the end of the first week of life. The advancement of MR diffusion-weighted imaging (DWI) and magnetic resonance spectroscopy (MRS) means lesions can be recognised within the first days of life.

MRI patterns of injury vary depending on the duration and severity of the hypoxic-ischaemic insult. Acute asphyxia often results in injury of the most metabolically active tissues, including the basal ganglia, thalami, putamen, hippocampi, brainstem, and the corticospinal tract (307). Injury to the deep grey nuclei can lead to dyskinetic cerebral palsy. Common comorbidities include

cognitive impairment, communication and feeding problems, epilepsy and visual deficit (355,356). Subacute ('chronic') asphyxia is most often associated with watershed injury involving the cortex and the white matter (307). This does not usually result in motor impairment, while common long-term disabilities include cognitive deficit, behavioural disorders, epilepsy and post-neonatal microcephaly (355,356).

With the advent of hypothermia treatment for infants with HIE, several authors have investigated the effects of hypothermia on MRI findings. In brief, hypothermia seems to reduce the likelihood of injury in the basal ganglia-thalami, white matter and corticospinal tract (357–359). However, the predictive value of MRI for subsequent neurological impairment is not affected by therapeutic hypothermia (358–360).

5.7.3 Electrophysiology in HIE

5.7.3.1 Cerebral Function Monitoring in NICU

Most neonatal units now use cerebral function monitoring (CFM) or amplitude integrated EEG (aEEG) to monitor brain activity throughout hypothermia treatment. It is also useful to support the presence of encephalopathy and the decision to start hypothermia treatment (320). The technique was initially developed to monitor the depth of anaesthesia during surgery, and after cardiac arrest in adults, but has subsequently been adapted for the use in asphyxiated neonates.

CFM/aEEG uses up to 4 electrodes placed in specific locations (usually in central [C4 and C3] and parietal [P3 and P4] electrode positions) and is compact enough

to be used at the bedside. aEEG provides a basic electrocortical recording and shows a compressed and rectified trace of brain activity. Although it lacks the more detailed information multichannel EEG recordings offer, background amplitude can be defined (361) and traces can be categorised into background patterns (362), similar to those produced by conventional EEG. aEEG is useful to assess sleep-wake cycling and allows seizure detection. In the pre-hypothermia era, it was shown that aEEG background amplitude within 6 hours of birth was highly predictive of long-term outcome (363). Early aEEG has been demonstrated to be less predictive of outcome in infants treated with hypothermia compared to normothermia. Infants with good outcome normalise background patterns of activity within 24 hours when kept normothermic (364), and by 48 hours when treated with hypothermia (365).

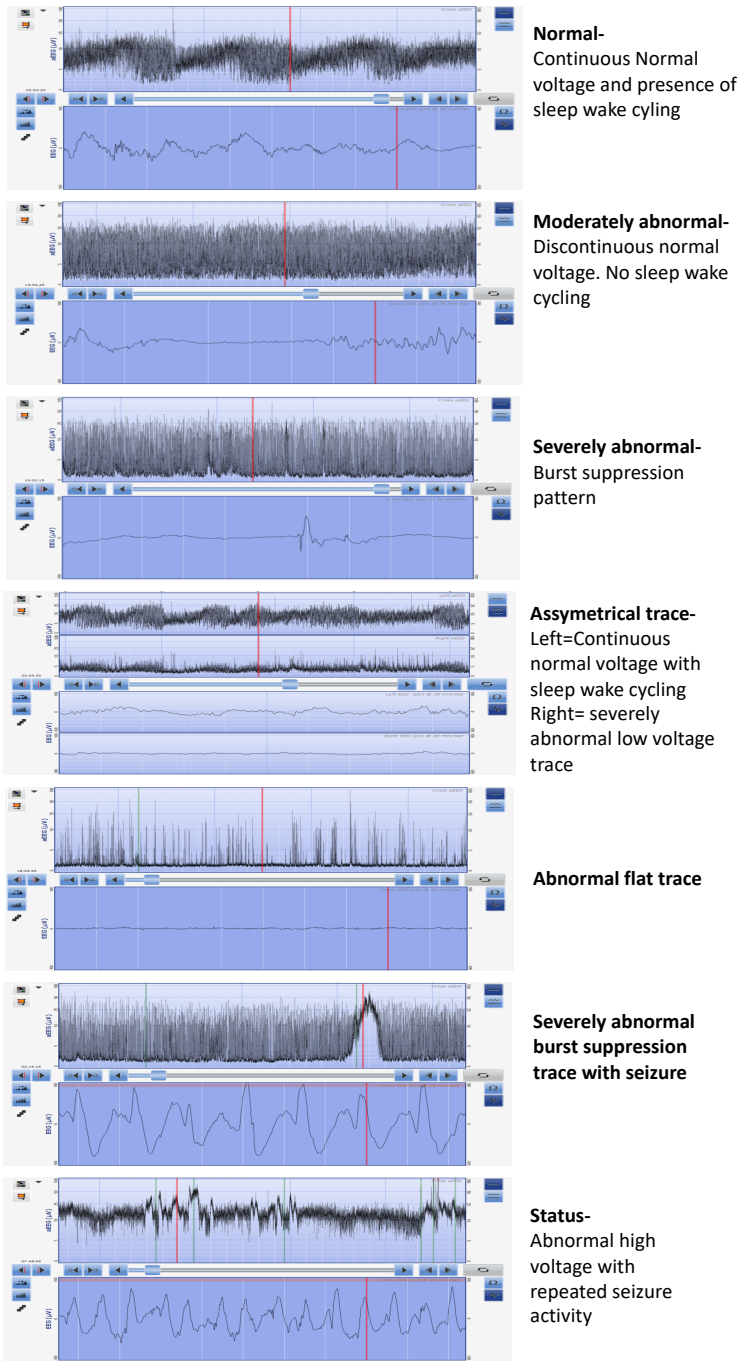


Figure 5.2 Examples of cerebral function monitoring (CFM) traces.

Examples taken with permission from the Neonatal Unit at the John Radcliffe Hospital, Oxford, OUH NHS Trust.

5.7.3.2 The value of EEG in HIE

Pre hypothermia

The role of EEG in HIE has been well documented since Monod et al in 1972. These early studies (337,366–368) provided evidence for a prognostically significant tier staging system combining clinical and EEG findings in neonates with HIE. The authors agreed that EEG was a valuable prognostic tool but it was most useful in extreme cases, i.e a normal EEG was highly predictive of a normal outcome whereas a severely abnormal background EEG was highly predictive of severe developmental sequelae or death. In newborns whose EEG showed moderate abnormalities, outcome was a lot harder to predict. EEG background abnormalities included: very discontinuous trace with inter-burst intervals exceeding 10-20 seconds, burst suppression pattern, or a very low amplitude inactive EEG. Good prognostic features include improvement of EEG background amplitude, decrease in discontinuity, or appearance of sleep-wake cycles within 12–24 h after birth. Due to the evolution of changes after a hypoxic ischaemic insult, it is recommended that EEG recordings be performed in the second and sixth day from birth with a follow up every 3-4 days for 2 weeks in neonates whose EEG remained abnormal (337,368). More recent studies have focused on early EEG's performed within 3 days of birth, which show a strong correlation with outcome even for minor developmental sequelae (369,370).

The duration of abnormalities is an important factor in predicting outcome, as early recovery is associated with better prognosis. EEG background evolves and usually improves over the first 3 days of life; therefore, the prognostic utility of EEG is dependent on the time when the recording was performed (368,369,371,372). Murray et al (2009) (369) found that EEG recordings recovered

by 48 hours after birth, thereby reducing the predictive value of a normal EEG after 48 hours. Although fast EEG recovery indicates a better prognosis, this does not always lead to normal outcome. Persistence of moderate abnormalities for 2 weeks or mild abnormalities for 3 weeks is indicative of poor outcome (368). In addition, an initial EEG showing a very abnormal background can improve with recovery of the acute brain damage, so an initial EEG near the time of insult is more indicative of outcome than an EEG performed a few weeks later giving a falsely optimistic prognosis (172). For these reasons most authors suggest performing continuous video EEG monitoring or serial EEG's to accurately assess the severity of insult (372,373).

Neonatal seizures cause acute local energy depletion, alterations in cerebral blood flow and nutrient supply, and induce neuronal disintegration (374,375). More significant energy depletion occurs with seizure propagation and rapid or accumulation of seizures which induces subsequent apoptosis. This suggests that a high seizure burden increases the likelihood of neurological impairment. Status epilepticus, defined as seizure burden more than 20- 30 minutes per hour, has consistently been associated with poor short and long-term outcomes (172). However, the presence of seizures has been reported to be less predictive of outcome than abnormalities in the background EEG despite their importance (345,376–378) . The onset of seizures, often within 24 hours of birth, signals the secondary injury phase and may aggravate the initial brain damage (374,375). Seizure onset may vary according to severity of the insult (379). Animal models of hypoxia have shown that recurrent neonatal seizures may be linked to the formation of excessive excitatory circuits in the hippocampus and lead to epilepsy (380,381). Severity and pattern of MRI injury are risk factors for neonatal

seizures and there is a relationship with the development of epilepsy (382). Prolonged and recurrent seizures initiate a pathophysiological cascade contributing to persistent inflammation, a component of brain white matter damage which gives rise to CP (383).

It is also important to note that infants with HIE commonly have poor correlation between clinical signs & symptoms and EEG seizure activity (384,385), making clinical seizures a poor indicator of seizure onset. This highlights the important role of EEG in the diagnosis of seizures in the neonate. Electrographic-only, or electrographic seizure activity with no clinical correlate, has been associated with severe sequelae in neonates with HIE (386,387). Recently, the 2017 International League Against Epilepsy (ILAE) Classification scheme was modified by the Neonatal Seizures Task Force to better define seizure types relevant to this age group (207).

Post hypothermia

Since hypothermia protocols have been adopted in neonatal units, a few authors have re-evaluated the role of EEG in HIE. Nash et al (2011) (388) performed continuous video EEG monitoring in 41 neonates during hypothermia and rewarming. The study agreed with previous literature and showed that EEG background features are still valuable when hypothermia is used. The results only differed in 2 main aspects. Firstly, extremely discontinuous patterns even after rewarming did not predict a poor outcome as seen in the pre-cooling era. Secondly, although previous studies suggest a gradual normalisation of EEG patterns during the first 3 days of life, in some of the neonates the background worsened over the course of treatment. This study only looked at short-term

outcomes by correlating the EEG findings with MRI around 5 days after birth. Similar studies by Mariani et al 2008 and Hamelin et al 2011 (389,390), also concluded that EEG remains a useful tool for the evaluation of outcomes in infants even when hypothermia is employed but continued to emphasise the importance of early EEGs and long-term monitoring or serial EEGs in neonates at high risk of neurological sequelae.

The neuroprotective effects of TH on brain metabolism, cell death, and overall reduction in neuronal excitability and seizure propagation (391), would explain why seizure burden also appears to be significantly reduced in infants undergoing TH, especially in infants with moderate HIE (392). Wusthoff et al., 2011 found 65% of participants had electrographic seizures during or immediately after treatment with hypothermia (393). A study by Elshorbagy et al., found that 31% of infants had seizures during TH, and electrographic seizure activity without a clinical change was reported in 42 % of them (394). These reports are similar to those from the pre-hypothermia era (395). A recent study (396) looking at seizure burden and outcome in HIE concluded that abnormal outcomes were associated with a high seizure burden irrespective of hypothermia treatment even though the total seizure burden was significantly lower in neonates with TH. In this study, cerebral palsy (CP) and epilepsy were associated with either high total seizure burden or high maximum seizure burden (defined as seizures for more than 13 minutes per hour).

5.7.3.3 The role of Evoked Potentials in HIE

Pre hypothermia

Visual, auditory and somatosensory pathways pass through brain areas that are selectively vulnerable to asphyxia. For this reason, evoked potentials were extensively investigated in the 1970's in the context of HIE. However, these early studies are based on small samples, retrospective data, or evaluated only short term follow up (less than 18months), therefore these studies lacked robust evidence and were methodologically limited (397).

In a recent review looking at the value of different prognostic tests and outcome in neonates with HIE, amplitude EEG, conventional EEG and visual evoked potentials (VEPs) were found to be the most promising neurophysiologic techniques (397). Some studies have looked at the value of using a combination of tests, and VEPs and somatosensory evoked potentials (SEPs) are often the most robust combination (218). Brainstem auditory evoked potentials (BAEPs) in combination with any of the other modalities was somewhat useful, but as a single test did not seem to provide more information, even though it seemed to be the most consistently preserved.

Several studies have investigated the normal maturation of visual evoked potentials in term and preterm infants (19,190). Also see Chapter 4. Later studies investigating the effects of perinatal disorders and asphyxia on VEPs (24,25,398,399) found that although a normal VEP in the first week of life was not consistently a good indicator of prognosis, an abnormal VEP was highly predictive of an abnormal outcome with a high degree of specificity. These studies compared their results with normal values (190), and used characteristics

such as abnormal waveforms with missing components, increased latency of the response (compared to healthy controls), or absence of a response, to grade the severity of abnormality (218).

In adults with hypoxia, bilaterally absent SEPs have been found to be the most valuable early predictor of poor neurological outcome, but the predictive value in neonates is less well established. Studies by several authors (21,23) found them most reliable at predicting motor impairment, and in a study by Suppiej et al (400), the bilateral absence of SEP's correctly identified neonates at risk of cerebral palsy, the most severe form of abnormal motor outcome of HIE. This study also compared findings with VEP's performed in the same group of neonates. The study concluded that VEPs did not significantly add to the neurological prognosis but did identify severe outcomes and future visual impairments if abnormal in the first week of life.

Post hypothermia

The effects of hypothermia on evoked potentials have been mainly investigated in adults and children undergoing heart surgery. Moderate hypothermia (19-25ºC) significantly increases the latency of all sensory modalities, and with lower temperatures (severe hypothermia below 20ºC) sensory responses are no longer present (401). Long latency responses are affected by hypothermia sooner than short latency responses (402). A study looking at moderate hypothermia at 33.5ºC in adults, showed that although VEPs increased in latency and decreased in amplitude under cooling conditions, the difference was not significant, and concluded that cooling to 33.5ºC is on the borderline for significant alterations in brain electrical activity (403). While hypothermia-induced changes in SEP latency

are well defined, amplitude behaves unpredictably (404), with reports of no change, decreased amplitude, and even increases in amplitude (405).

Although pre-cooling literature suggests that the use of VEP by themselves and combined with other tests performs well as an early predictor of abnormal outcome, there is no literature on the role of VEPs with the advent of cooling. Similarly, there is no literature on the role of auditory evoked potentials in neonates who have been cooled. Previous literature on brainstem AEPs suggests that although these may not have been as useful in the pre-cooling era, they may be less distorted by cooling and may be more predictive nowadays.

The sensory modality that has been investigated the most is SEP's. The predictive value of SEP's in neonates treated with hypothermia is debatable. The studies performed on neonates use small sample sizes and remain inconclusive. Some authors suggest that SEP's performed during hypothermia have a low prognostic value (406), while others show that it remains highly predictive of outcome (407). Two studies investigated SEP´s after rewarming (408,409), and in contrast with previous literature, the absence of a response did not necessarily indicate a poorer prognosis. Evoked potentials are not routinely performed in neonatal units but may provide important information regarding the severity of brain injury and guide to prognosis, especially in cases of moderate HIE.

5.8 Study aims

- To investigate the impact of HIE on the evoked brain activity in response to tactile and noxious stimulation during and after hypothermia treatment, compared to healthy term infants.

5.9 Methods

5.9.1 Hospital setting

The intensive care unit in Oxford is a referral centre which provides hypothermia treatment to approximately 40 infants per year. Here is a summary of the procedures followed in the neonatal unit at the John Radcliffe Hospital in Oxford (Oxford University Hospitals NHS Trust).

Trained medical staff assess infants at risk of HIE soon after birth based on the presence of the following clinical surrogates of perinatal hypoxic-ischemic insult: Apgar score of ≤5 at 10 minutes after birth; continued need for resuscitation, including endotracheal or mask ventilation, at 10 minutes after birth; pH <7.00 or base deficit ≥16 mmol/L in umbilical cord or any blood sample [arterial, venous or capillary] within 60 minutes of birth (320). Within the first 6 hours of life, the presence or absence of encephalopathy and its severity is established using clinical neurologic examination (337). It is also routine practice to perform amplitude-integrated electroencephalogram (aEEG) in infants being assessed for clinical encephalopathy. Medical staff perform a two-channel aEEG (needle-electrode placement C3-P3, C4-P4), which records continuously via a combined aEEG/ EEG digital device (CFM Olympic Brainz Monitor; Natus). This recording is started soon after birth, and in infants not deemed encephalopathic only short recordings of ≥1 hour are obtained. For infants treated with hypothermia, aEEG

is recorded continuously until rewarming is completed. Patterns and voltages of aEEG backgrounds are documented at <6h (precooling), 12, 18, 24, 36, 48, 60 and 72h (361,362). The background pattern changes from abnormal to normal and development of sleep-wake cycling is noted. Clinical and electrographic seizures and their treatment are also logged.

In our neonatal intensive care unit, treatment with hypothermia constitutes standard management for newborns with HIE. This group of infants undergo whole-body hypothermia, whereby their core temperature is cooled to 33.5°C for 72 hours. They are nursed on a cooling blanket in which fluid circulates; the temperature of the fluid is regulated automatically (Criticool; MTRE Advanced technologies). After 72 hours, rewarming occurs in intervals of 0.5°C per hour till a rectal temperature of 36.5°C is reached. Local guidance is followed in terms of integral management of HIE, including antibiotic treatment, ventilatory and circulatory care, management of seizures, blood products, fluids, electrolytes and nutrition. In this unit, all infants treated with hypothermia undergo sedation via morphine infusion.

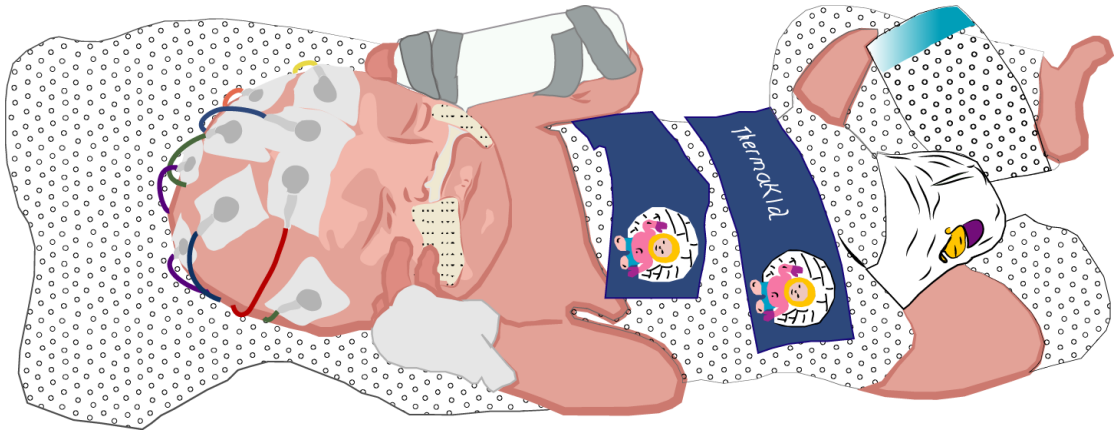


Figure 5.3 Infant with hypothermia blanket.

5.9.2 Participating infants

Two independent cohorts of infants were considered for this study. Full demographic information is detailed in table 5.1.

Control Group: Fourteen healthy full-term infants were included in this cohort. Infants had no documented neurological, cardiac or respiratory conditions. Three infants were on antibiotic treatment for suspected infection, but none were admitted to the neonatal unit.

HIE group: Twenty infants admitted for intensive care and therapeutic hypothermia treatment were included in this cohort. All infants had a diagnosis of HIE but no other neurological conditions, brain malformations, genetic or metabolic disorders. Local procedures as described in Section 5.9.1 were followed for all infants. All infants were given morphine infusion for the duration of hypothermia treatment as per local protocol, which was discontinued around the time normothermia was reached. Four infants were treated with anticonvulsant medication. During hypothermia treatment three infants had been given single bolus doses of phenobarbitone and one infant was given 2 bolus doses of phenobarbitone and phenytoin prior to the recording taking place. After hypothermia treatment two infants were on maintenance doses of phenobarbitone. The recommended loading dose of 20 to 40 mg/kg followed by a maintenance dose of 3 to 5 mg/kg/day in 1 to 2 divided doses was used in the study patients.

Table 5.1 Infant demographics

Mean and (range) is displayed for continuous variables. Discrete variables are expressed as number or range.

	Control group	HIE group	
Number of participants	14	20	
Female	11	6	
GA at birth (weeks)	39.3 (37.3 - 42.3)	39.5 (35.9 - 42)	
Antenatal problems			
Prolonged rupture of membranes	0	3	
Diabetes/Gestational diabetes	0	2	
Maternal infection/ other	4	3	
Reduced fetal movements	0	1	
Meconium aspiration	0	1	
Type of delivery			
Emergency caesarean	2	8	
Ventouse / forceps	6	5	
Normal Vaginal Delivery	3	5	
Apgar at 1 min	9.2 (7- 10)	1.9 (0 – 7)	
Conditions during admission			
• Neurological conditions	0	20	
• Cardiac conditions	0	1	
• Respiratory support			
▪ Invasive mechanical ventilation <24 hours	0	7	
▪ Invasive mechanical ventilation > 24 hours	0	5*	
• Treated suspected infection	3	17	
Test occasion	1	1	2
PMA at study (weeks)	39 (37- 42.1)	39.9 (36.3-42)	40.0 (36.6- 42.7)
PNA at study (days)	2 (0 - 4)	2.3 (1- 3)	6 (3- 8)
HIE grade (mild:moderate:severe)	n/a	10:07:03	08:06:03
Cooling time at study (hrs)	n/a	51.7 (18-71)	
Time since cooling stopped at study (hrs)	n/a		42 (6- 120)
Respiratory Support			
▪ Invasive mechanical ventilation (SIPPV/ HFOV)	0	4	3
▪ Respiratory support (HFT/LFT)	0	5	1
Medications			
◊ Analgesia (Morphine)	0	20	0
◊ Muscle relaxants	0	1	0
◊ Antibiotics	3	19	11
◊ Anti-convulsant	0	4	2
Prior pain (n=10 infants)	n/a	6.36 (0- 24)	9.44 (0- 25)

PMA= post menstrual age PNA=post-natal age
SIPPV= Synchronised intermittent positive pressure ventilation
HFOV= High frequency oscillatory ventilation
HFT= High flow nasal cannulae LFT= Low flow oxygen therapy
Prior Pain = number of skin breaking procedures and endotracheal tube/oral suction

5.9.3 Experimental design

The control group was studied on a single occasion in the post-natal ward during their first week after birth, whereas the HIE group was studied on two occasions: once during hypothermia treatment, and once as long as possible after reaching normothermia.

All infants remained in the unit for the duration of the hypothermia treatment (first test occasion). In n=2 cases the infants were transferred to another neonatal unit soon after rewarming, therefore the second test occasion could not be performed, and in one case the parents did not consent to taking part in the second part of the study. An illustration of the experimental design is depicted in figure 5.4.

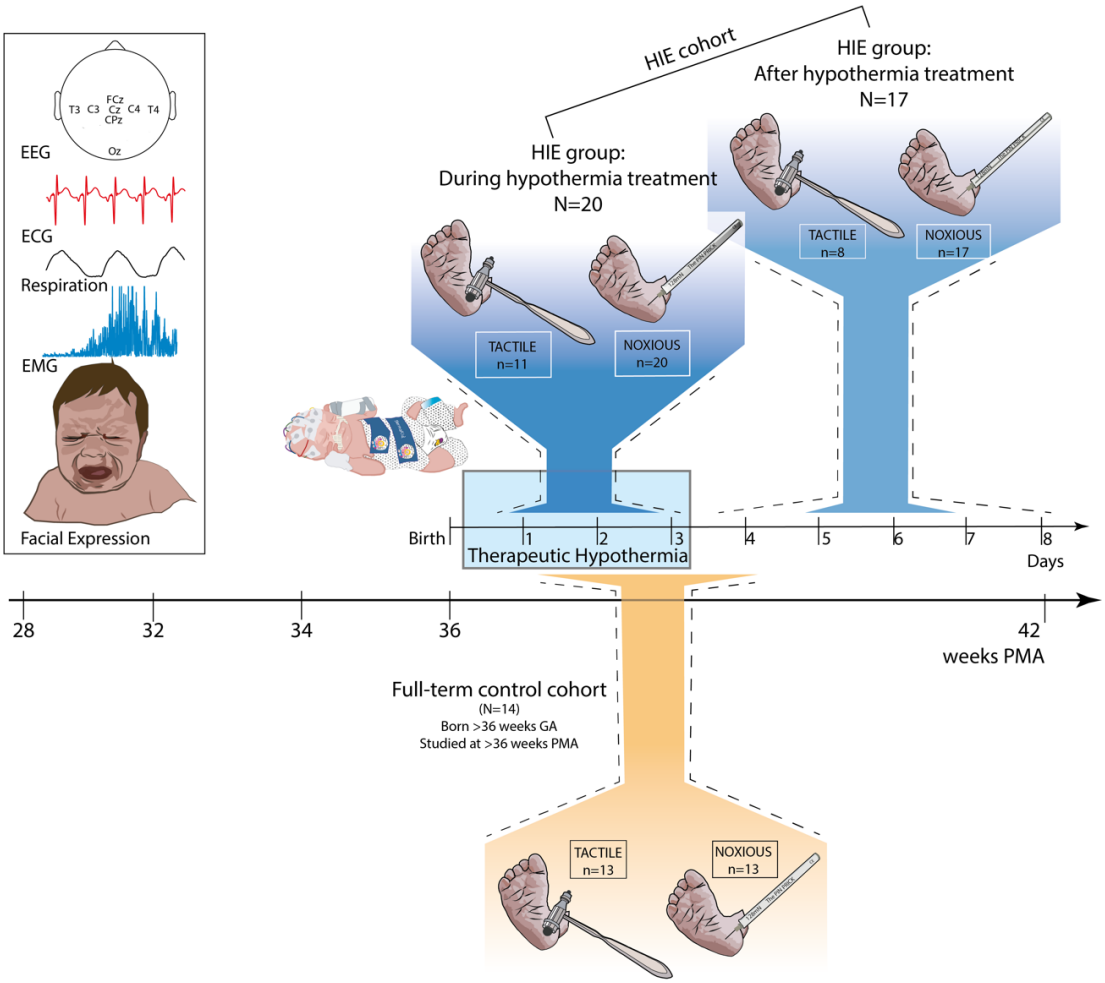


Figure 5.4 Experimental design.
On the left- Data acquisition (EEG, ECG, EMG and facial expression). On the right- experimental procedure. N denotes number of infants in the cohort and n denotes the number of infants included in the analysis for each stimulus.

5.9.4 Data acquisition

EEG activity and reflex withdrawal were recorded using the equipment described in Chapter 2: Section 2.5. Electrocardiogram (ECG) and respiration was recorded to aid in artefact detection and to monitor sleep state. Behavioural and physiological measures were only recorded in a subset of n=10 infants in the HIE Group.

5.9.5 Stimulation techniques

Non-noxious tactile stimulation and experimental noxious stimulation were applied to both groups of infants. Each stimulus was applied in a train of 10 trials for the control group, and 20-30 trials in the HIE group, with an inter-stimulus time of at least 10 seconds. The order the stimuli were applied, and the choice of foot being stimulated for each test occasion were randomised.

Tactile stimulation was applied to n=13/14 infants in the control group. A total of 117 trials were included in the analysis. In the HIE group, tactile stimulation was applied to 11 infants in study session 1 (203 trials included in analysis) and a reduced number (n=8) infants in study session 2 (177 trials). Full details of the modified tendon hammer used for tactile stimulation are detailed in Chapter 2: Section 2.6.1.2.

Experimental noxious stimulation was applied to the heel of the infant using the 128mN PinPrick (MRC Systems) device detailed in Chapter 2: Section 2.6.2.2. Fourteen infants from the control group were stimulated using this device. One infant was excluded from the analysis as most of the trials were contaminated by movement/ muscle artefact (total of 116 trials included in the analysis). In the HIE group noxious stimulation was applied to all infants (n=20) in study occasion 1 (335 trials included in analysis), and n=17 in study session 2 (257 trials included).

5.10 Analysis

5.10.1 EEG Analysis

In Chapter 2: General Methods, the responses recorded after tactile and noxious stimulation in a group of 15 infants was used to create temporal templates which could be used to calculate a magnitude for the evoked brain activity. Here we projected these templates to a new independent group of healthy term infants and infants with a diagnosis of HIE in order to quantify the magnitude of tactile and noxious activity.

The templates were projected onto the trial averaged activity using the same methods outlined in chapters 3 and 4 with two important differences. Firstly, the reduced number of electrodes used to record the EEG of infants in the full-term healthy control group (Chapter 2: Section 2.5.1) meant that all analysis had to be carried out with a limited number of electrodes for consistency. This also meant that an average reference was calculated using those same 8 recording electrodes. Secondly, although there were no differences in the woody filtering of the tactile temporal template (maximum shift of +/- 100 ms), the woody filtering was altered to +/- 100ms for noxious, in contrast to the +/- 50 ms shift allowed in Chapter 4. How well the template characterised the evoked activity was established by visual inspection of the control group trial averages which then determined the amount of Woody filtering of the temporal template.

In order to define a threshold for identifying whether an evoked response was present, the distribution of the weights obtained from projecting the template on to the background preceding the point of stimulus were calculated for the control

group and the HIE group (during and after cooling calculated separately). If the weight of the evoked potential post stimulus was greater than 80% of the distribution of the background weights, then a response was deemed present.

Peak latencies were measured manually by GSM when a response was visible. If the waveform had a bifid shape, then the middle of the two peaks was taken as the peak latency.

5.10.2 EMG analysis

EMG activity recorded from the leg being stimulated was processed using the methods described in Chapter 2: Section 2.7.2. Data was analysed after noxious stimulation only.

5.10.3 Behavioural and physiological analysis

Behaviour and physiology were assessed using the Premature Infant Pain Profile- Revised (PIPP-R) (see Chapter 2). Behaviour was assessed by reviewing facial videos to time the duration of facial expression changes over 30 seconds after the stimulus. Heart rate and oxygen saturation were also assessed for changes in the 30 seconds immediately following the stimulus compared to baseline. Total PIPP-R scores, behaviour and physiological changes were obtained after the first and last pinprick stimulus per recording session.

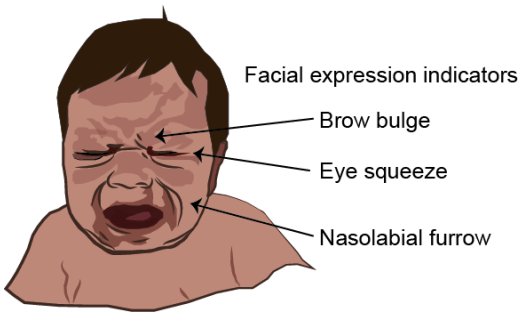


Figure 5.5 Illustration of infant showing the three indicators of facial expression used in the PIPP-R.

5.11 Statistical analysis

Differences in the mean weights between the two groups (control vs HIE) were compared using one-way ANOVA (corrected for trial number), and differences within the HIE group were compared using a repeated measures ANOVA. Spearman's rank order correlation was run to determine the relationship between HIE severity and the magnitude of tactile and noxious evoked activity during and after hypothermia treatment. Changes in physiological, behavioural and total PIPP-R scores were compared using repeated measures ANOVA.

5.12 Results

5.12.1 Tactile- evoked responses are reduced in infants with HIE compared to healthy term infants.

To evaluate the impact of HIE on tactile processing, the magnitude of tactile-evoked activity from healthy term infants was compared with the responses of the HIE group (during and after hypothermia treatment). The healthy term control group showed tactile evoked responses characterised by a dominant early negative peak (N1), captured by the template, followed by a positive – negative- positive waveform (figure 5.6 A). This morphology can also be seen in the response from the HIE group during TH albeit with a reduced amplitude. The initial negative (N1) waveform evoked in the HIE group after hypothermia is very low in amplitude and the following deflections are absent. When the magnitude of tactile evoked activity was compared between groups (Figure 5.6 B), infants with HIE showed significantly lower weights in comparison to healthy control infants, whether they were studied during (one-way ANOVA $p=0.045$) or after hypothermia treatment ($p=0.001$). However, no difference in magnitude was seen between the two studies in the HIE group ($p=0.128$).

Overall, an observable tactile-evoked response was evoked in 92 % (12/13) of infants in the healthy term control group, whereas in the HIE group there was no response in n=5/11 (45%) during cooling and 4/8 (50%) after cooling (figure 5.6 C).

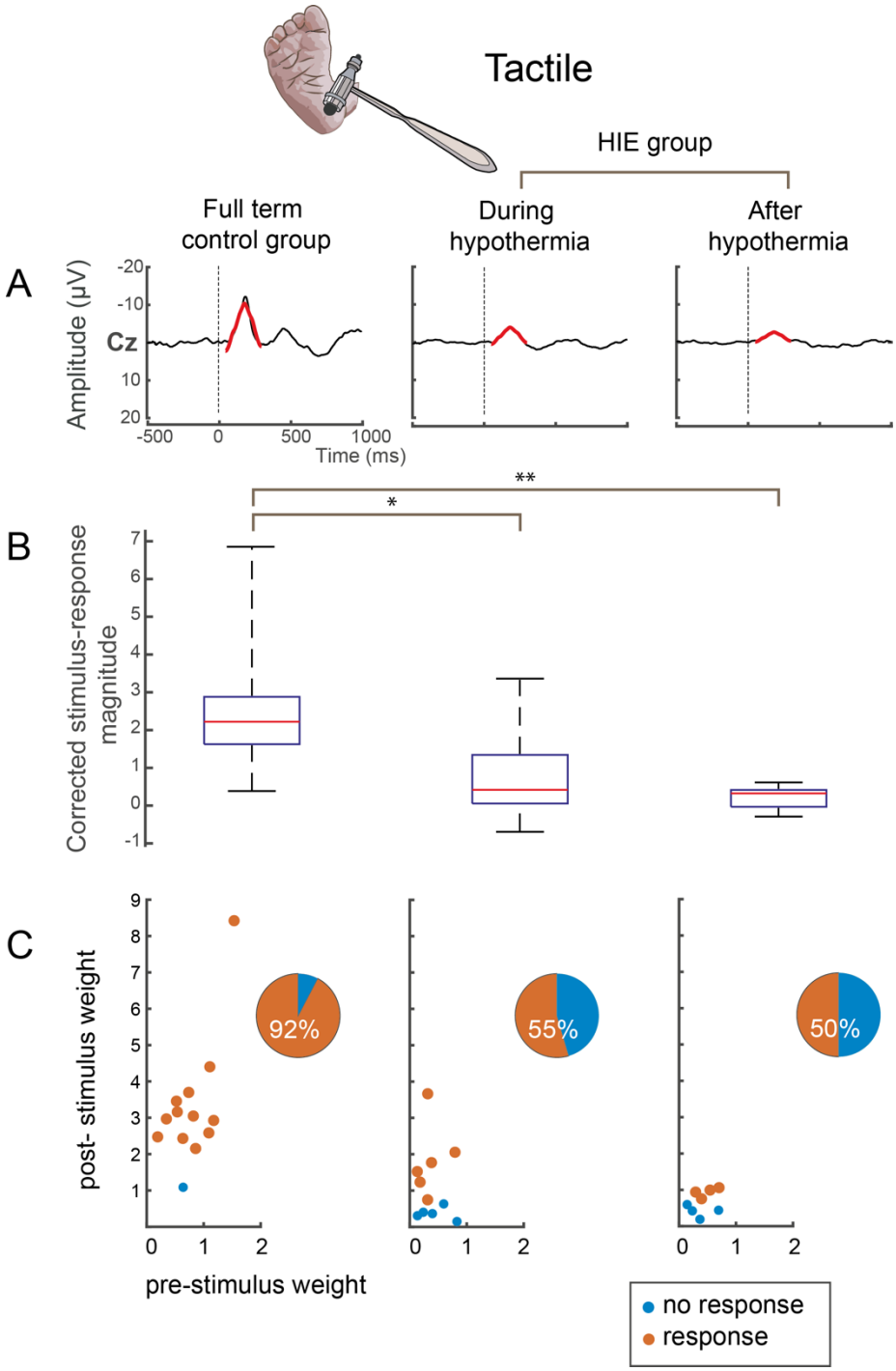


Figure 5.6 Tactile-evoked brain activity.
A) The group trial-averaged EEG activity in response to tactile stimulation with the template overlaid in red B) Comparison of the magnitudes of evoked response from the midline central electrode (Cz) between the full term healthy control group, and the HIE group recordings during and after hypothermia treatment. The significance bars represent the comparison between the means, ANOVA (corrected for trial number confound) *p<0.05. C) Scatter plots showing the pre stimulus and post stimulus weights for each infant per group. The red dots represent the presence of an evoked response and blue dots represent the absence of a response. Whether a response was present was determined by a threshold (see section 5.11.1). The corresponding pie charts show the percentage of responses for each group.

5.12.2 Compared to healthy controls, the magnitude of noxious evoked activity is significantly reduced in infants with HIE.

Infants diagnosed with HIE showed a significant reduction in noxious evoked brain activity compared to healthy controls. Noxious experimental stimulation evoked a well- defined positive waveform which is also seen in infants with HIE with much lower amplitudes (figure 5.7 A). When the magnitudes of the healthy term infants were compared to the HIE group, there was a significant reduction in the mean weights calculated during TH (one- way ANOVA $p=0.0191$) and after TH ($p=0.0196$). However, there were no significant differences in the mean weights between the recording sessions in infants with HIE (repeated measures ANOVA $p=0.741$) (figure 5.7 B).

In the healthy control group, n=11/13 (85%) of infants had an observable noxious-evoked response compared to the pre stimulus period. The number of responses decreased in the HIE group. During TH the number of responses reduced to n= 9/20 (45%) and n=6/16 (38%) after hypothermia treatment (figure 5.7 C).

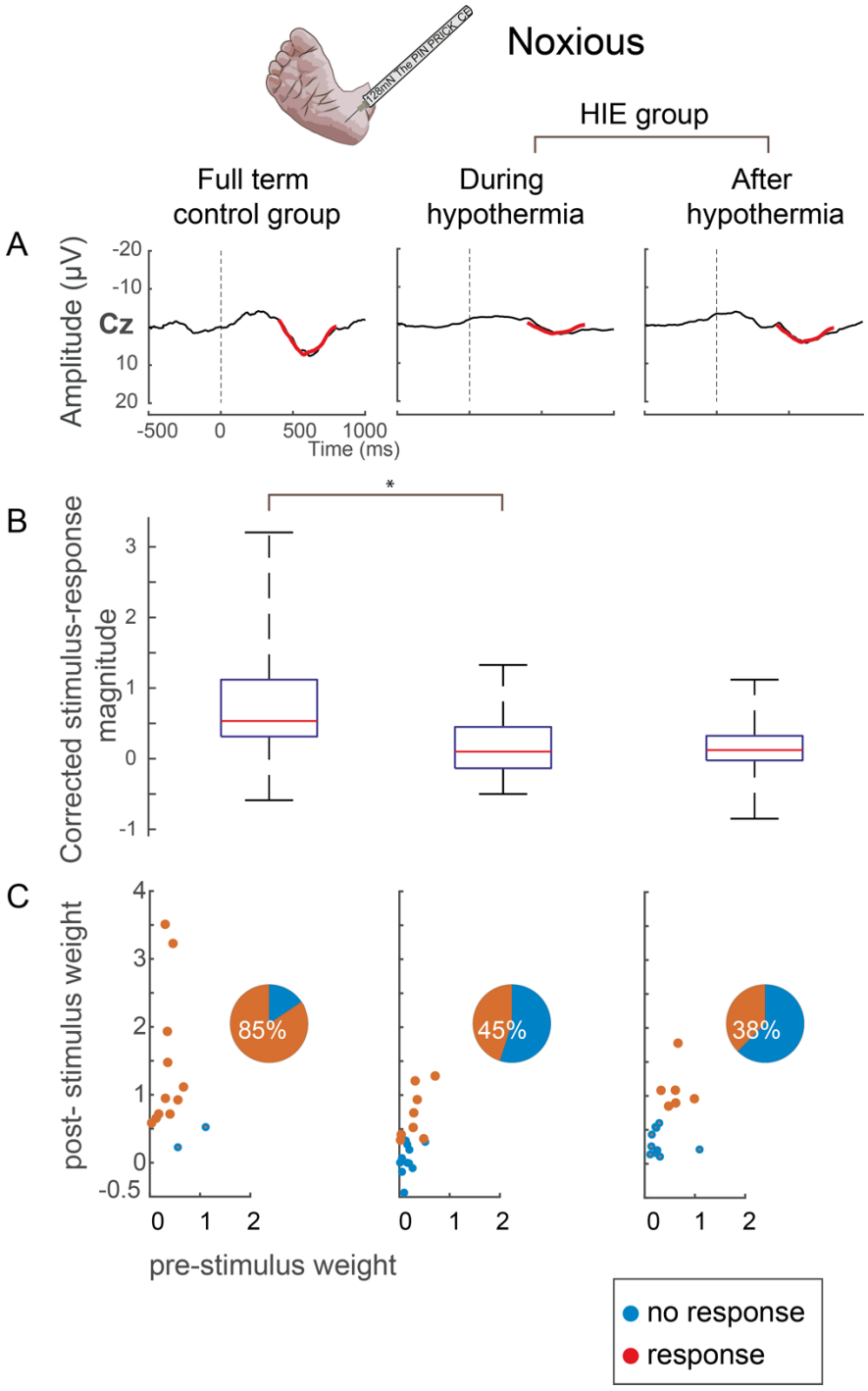


Figure 5.7 Noxious-evoked brain activity.
A) The group trial-averaged EEG activity in response to noxious stimulation with the template overlaid in red B) Comparison of the magnitudes of evoked response from the midline central electrode (Cz) between the full term healthy control group, and the HIE group recordings during and after hypothermia treatment. The significance bars represent the comparison between the means, ANOVA (corrected for trial number confound) *$p<0.05$. C) Scatter plots showing the pre stimulus and post stimulus weights for each infant per group. The red dots represent the presence of an evoked response and blue dots represent the absence of a response. Whether a response was present was determined by a threshold (see section 5.11). The corresponding pie charts show the percentage of responses for each group.

5.12.3 Peak latencies are delayed in infants with HIE compared to healthy term infants: visual inspection of latency.

To establish if latencies to the specific components of the tactile and noxious evoked activity measured by the temporal template differed between the groups, peak latencies were manually measured from the individual trial- averaged activity for each stimulus. Full term healthy infants showed peak latencies of 169 ± 48 ms (mean ± standard deviation) and 615 ± 105 ms for tactile and noxious respectively. Peak latencies increased in the HIE group during TH to 210 ± 29 ms for tactile and 670 ± 104 ms for noxious, but only 6 infants had a measurable response after tactile stimulation and 8 infants after noxious stimulation. When the HIE group was studied after TH latencies decreased to 168 ± 67 ms for tactile and 594 ± 88 ms for noxious, however, a response could only be measured in 4 infants after tactile stimulation and in 6 infants after noxious stimulation. This is summarised in Table 5.2.

Table 5.2 Peak latency values for tactile and noxious evoked brain activity.

Mean ± standard deviation is given for peak latency. Discrete values are expressed as a single number (ms= milliseconds)

	Healthy full-term control group	HIE: during hypothermia	HIE: after hypothermia
Tactile			
Number of infants	13	11	8
Absent response	1	5	4
Peak latency / ms	169 ± 48	210 ± 29	168 ± 67
Noxious			
Number of infants	13	20	16
Absent response	2	12	10
Peak latency / ms	615 ± 105	670 ± 104	594 ± 88

5.12.4 HIE severity was not associated with the magnitude of tactile or noxious evoked brain activity.

In this study, half of the infants (n=10) treated with hypothermia treatment had baseline clinical features consistent with mild encephalopathy, n=7 infants were graded as having moderate encephalopathy and three with severe HIE. Of the three infants who did not have a second recording session, n=2 were diagnosed as mild and n=1 was moderate HIE. No significant relationship was found between HIE severity and magnitude of tactile evoked activity during cooling (Spearman's rho p=0.609) or after cooling (p=0.77). Similarly, no association was found between HIE severity and noxious evoked activity recorded during (p=0.758) and after hypothermia treatment (p=0.566).

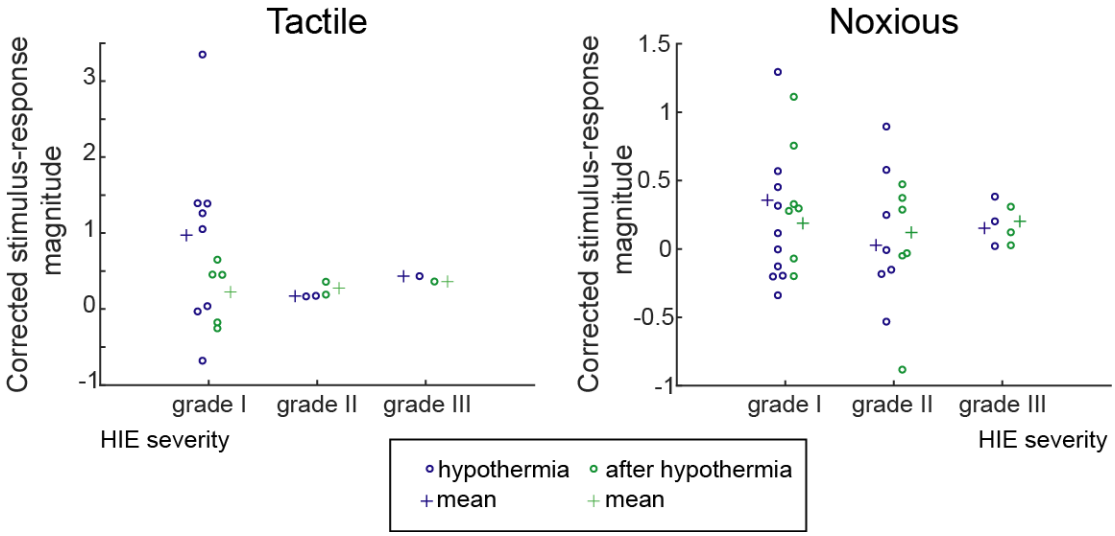


Figure 5.8 Relationship between severity of HIE and the magnitude of evoked response to tactile and noxious stimulation.
HIE severity (according to the clinical notes) was compared with the magnitude of evoked response during hypothermia (blue dots) and after hypothermia (green dots). Crosses show the mean.

Of the three infants with severe HIE, only one infant was stimulated using both types of stimulation and only a tactile evoked response was observed in the first test occasion but not the second after TH. The other 2 grade III cases showed

noxious evoked responses during TH but not after. Four infants with grade II HIE were stimulated with noxious stimulation only, two showed responses at both test occasions, one had a response during but did not have a second recording, and one showed no responses. Of the two grade II infants who were stimulated with both stimuli, one showed absent responses and the other infant showed responses to both stimuli but only during TH. Infants in the mild grade I HIE group were also varied. Noxious stimulation only was performed in two infants, one showed no responses and the other only showed a response during TH. Eight infants were stimulated with both types of stimuli. 5 showed clear evoked responses during TH, which were also present in two of them after TH (n=2 infants did not have a second recording). Noxious evoked activity was observed in four infants during TH, one of them also showed a response after treatment and 2 others had responses after TH even though they had shown no response during TH.

5.12.5 Reflex withdrawal to noxious stimulation is reduced in infants with HIE compared to healthy infants.

Reflex withdrawal to noxious stimulation was reduced in infants with HIE compared to healthy infants (figure 5.9 A). However, although there was a significant difference between healthy infants and the HIE group during hypothermia (corrected ANOVA p=0.008), no significant difference was found between the HIE group after hypothermia and the control group(p=0.116) or the HIE group after hypothermia (p=0.1) (figure 5.9 B).

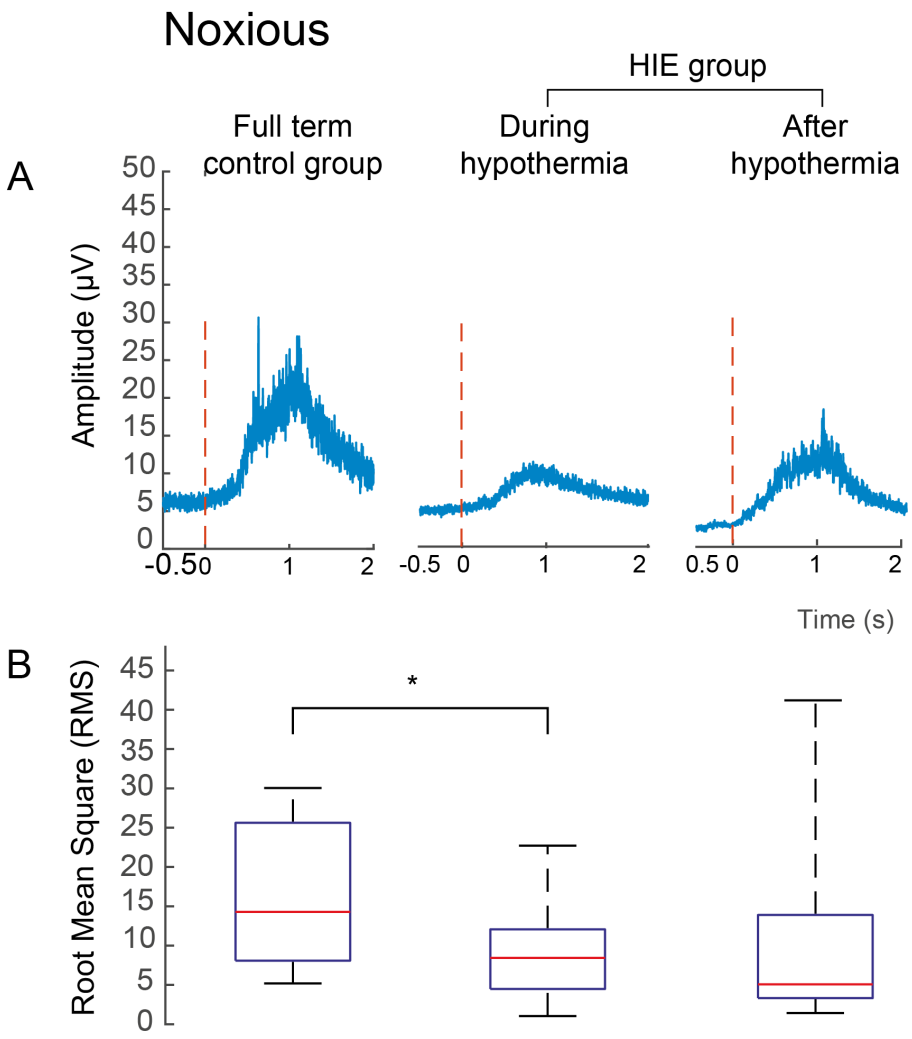


Figure 5.9 EMG response to noxious stimulation.

A) The group averaged EMG activity in response to noxious stimulation for the three groups. B) Comparison of the root mean square (RMS) for each group. The significance bars represent the comparison between the means, ANOVA (corrected for trial number confound) *p<0.05.

5.12.6 Infants with HIE do not show significant behavioural or physiological changes in response to experimental noxious stimulation.

In seven infants PIPP-R scores were calculated after the first and last experimental noxious stimuli was applied in a train of 20-30 trials. The mean total PIPP-R score was 1.8 ± 0.8 (mean ± standard error of the mean) after the first stimulation during hypothermia treatment, and 2.3 ± 0.67 after the last stimulation. The mean PIPP score increases slightly to 3.1 ± 1.05 after the first

stimulation and 2.8 ± 0.95 after the last stimulation after hypothermia treatment. No significant difference was observed between the first and last stimulation either during or after hypothermia treatment and between the two test occasions. The three facial expression components of the PIPP score were combined to give a total facial expression score. No significant changes were observed between the first and last stimulation in either test occasion or between the two testing sessions. Physiological changes in heart rate and oxygen saturation also did not show any significant changes. These findings are summarised in figure 5.10.

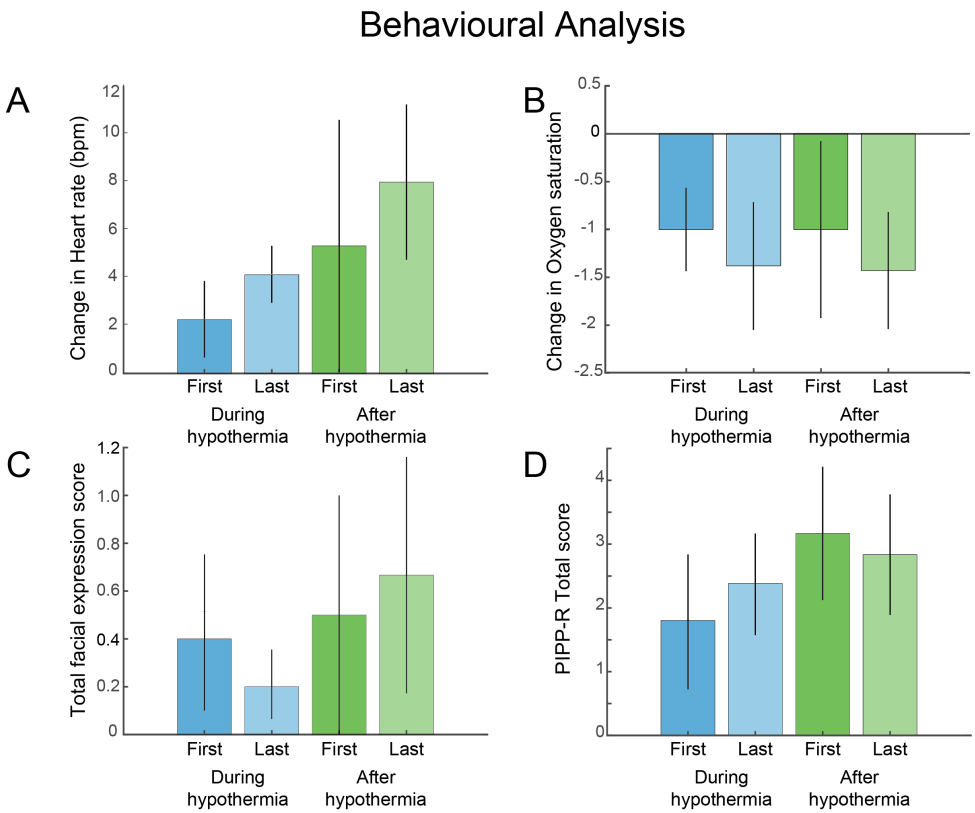


Figure 5.10 Behavioural and physiological analysis.

The bar charts show the A) heart rate change in beats per minute B) the change in oxygen saturation, C) the total combined score for facial expression changes, and D) the total PIPP-R score, for the first and last pinprick stimulation in each recording session (during and after TH) for n=7 infants from the HIE cohort. Error bars show the standard error of the mean.

5.13 Discussion

In this study the aim was to investigate the impact of neonatal HIE on somatosensory processing of tactile and noxious stimulation, using the same template approach of analysis from Chapter 3. Early outcome detection in newborns with HIE is essential for guiding individual treatment decisions. Somatosensory evoked potentials were valuable adjuncts to EEG pre-cooling era and this appears to also be the case nowadays. However, the combination of neurological changes involved in recovery from the initial insult, the possible secondary injury after the event, hypothermia treatment, and medications make it a challenging cohort to investigate. This is the first study to show how tactile and noxious evoked activity is impacted by neonatal HIE, therefore the discussion here is mostly based on comparisons with published studies where somatosensory evoked potentials are evoked using electrical stimulation.

In general, infants with HIE in this study show a reduced magnitude of evoked activity in response to tactile and noxious stimulation compared to healthy term infants. This was the case regardless of when the recording session took place, during or after hypothermia treatment. In the HIE group, there was no significant differences in the magnitude of evoked response but tactile -evoked activity was greater in magnitude during hypothermia treatment, whereas noxious stimulation appeared to be more reduced during hypothermia treatment compared to after.

Studies on adults treated with hypothermia for heart surgery or intra-operative monitoring (403,410,411) have shown that SEP's are resistant to the therapeutic levels of neonatal hypothermia (33 -34 ºC) for HIE which is a key advantage of

this technique compared to other electrophysiological investigations (412). Therefore, the reduction in evoked activity cannot be attributed to the hypothermia treatment. It is more likely that the general reduction in magnitude observed in the infants with HIE compared to healthy term infants is due to the encephalopathy itself, and the effects of analgesic, sedative and anticonvulsant medications. Cortical SEPs are more sensitive to hypoxia than the EEG. In adults, changes in SEP due to hypoxia correlate with reductions in brain high-energy phosphate concentrations and therefore may indicate CNS ischemia (410).

In this study, all infants in the HIE group were treated with a continuous infusion of morphine hydrochloride between 5-20 μg/kg/h. It is common practice to give morphine sedation during TH to alleviate the discomfort from cooling and to enhance neuroprotection (413). However, its use is being debated given that sedation can interfere with neurologic assessment and affect the interpretation of diagnostic tools such as EEG and aEEG. Sedation has also been shown to prolong mechanical ventilation, cause haemodynamic compromise (335), and may prolong hospital stay without any additional neuroprotective benefit to the infant (349). Róka et al compared serum morphine concentrations between neonates with HIE treated with hypothermia and infants kept at normothermia (414). All of the infants were treated with a continuous infusion of morphine-hydrochloride, with the rate adjusted according to clinical status. The authors found serum morphine concentrations at 24 to 72 hours after birth of 292 ng/mL (137–767 ng/mL) in the hypothermia-treated infants and 206 ng/mL (88–327 ng/mL) in the infants on normothermia ($p=0.014$), despite similar morphine infusion rates and cumulative doses. Importantly, serum morphine concentrations reached a steady state after 24 hours in the normothermic infants

but continued to increase throughout the assessment period in the hypothermia group. Frymoyer et al compared the pharmacokinetics of morphine during therapeutic hypothermia for HIE (415) to the data during normothermia (416), and concluded that with cooling, morphine clearance was decreased by 46.7% (15.6% per ºC). They performed Monte Carlo simulations to evaluate the dosing strategy that achieved optimal morphine concentrations and recommended a loading dose of 50 μg/kg followed by a maintenance continuous infusion dose of 5 μg/kg or intermittent dosing of 40–50 μg/kg every 6 hours. Favié et al also showed that morphine clearance was reduced under hypothermia by 20.7% (or 6.89% per ºC), and metabolite clearance was reduced by 14.7% (or 4.91% per ºC) (417).

Four infants in this study were also treated with anticonvulsant medication, 2 of whom continued to be treated with maintenance doses at the time of the second test occasion. Phenobarbital is metabolized in the liver by CYP2C9, and inactive metabolites are renally excreted together with 25% of unchanged phenobarbital (418). None of the published studies on phenobarbital pharmacokinetics in infants with HIE undergoing cooling have shown a substantial effect of therapeutic hypothermia on phenobarbital clearance (419–425), though some report higher plasma concentrations and longer half-lives than expected in normothermia.

Morphine infusion is known to cause changes on the EEG background such as the increase in periods of electrical quiescence, increase in the amount of interictal epileptiform activity and can affect reactivity to external stimulation (426,427). Morphine is also known to affect the latency of somatosensory evoked potentials

while amplitude changes appear to be more variable. Pathak et al. 1984 (428) reported posterior tibial nerve SSEP latency to increase by approximately 10–15% and amplitude to decrease by 20% after induction of anaesthesia with 0.25 mg/kg morphine in adults. Amplitude continued to decrease to approximately 10% of control during subsequent morphine infusion.

In this study, peak latencies were delayed in the HIE group during hypothermia compared with the control group but returned to normal values after hypothermia treatment reflecting the reversible effects of morphine on somatosensory evoked potentials. Nevertheless, although peak latencies were longer in the HIE group during hypothermia treatment, no significant differences were found in the magnitude of evoked activity in response to tactile and noxious activity during and after hypothermia treatment. Although serum morphine concentrations were not determined in this study, it is likely that these increased during the hypothermia period and remained high at the time of the second test occasion (414). This would explain why no significant difference was observed between the two test occasions. It could also explain why there is a possible increase in the magnitude of noxious evoked brain activity after TH as morphine levels are decreasing.

A recent review (4) looking at predictors of outcomes in HIE following hypothermia treatment, found 3 articles investigating somatosensory evoked potentials. Only one (407) performed SEP during hypothermia treatment and this was found to be predictive of outcome at a median age of 21 months. The others were performed around day 7 (408,409) and showed lower sensitivity and specificity in comparison. Cainelli et al (408) also performed visual evoked

potentials and found them to be more predictive than SEP in those infants with normal MRI. As there was no significant differences between the evoked responses to tactile and noxious stimulation between the recording sessions, this study did not provide any evidence on the appropriate timing of SEP recording.

In clinical practice, routine somatosensory evoked potentials are rarely performed on newborns even though they are complimentary to EEG and are better suited for identifying motor conditions such as cerebral palsy (CP) (400) especially in moderate cases of HIE. It is possible that altering stimulation techniques in this population may enhance our ability to perform and interpret these responses in NICU. However, in this study, the limited numbers of infants did not allow for associations between HIE severity and the magnitude of evoked response, and developmental outcome was not collected, both essential for establishing if the methods used here are prognostically and clinically useful.

An interesting finding in this study was the number of infants with baseline clinical features consistent with mild HIE and treated with hypothermia (n=10/20), reflecting the 'therapeutic creep' of infants with mild NE being treated with TH. Though mild HIE has traditionally been considered to have an excellent prognosis, with virtually no risk of long-term disability, more recent studies show that some children may have long-term difficulties. This may explain the clinical decision to start hypothermia treatment. A recent systematic review showed an abnormal outcome in 86 of the 341 infants with mild HIE (25%) included in the studies reviewed (324). Studies with follow-up to at least school age revealed that IQ was slightly lower in infants with mild HIE when compared to controls (429,430). However, there are few studies specifically designed to

ascertain the outcome of mild HIE, and mild forms are likely underrepresented in HIE cohort studies. In a recent multicentre, prospective study of infants with mild HIE not treated with hypothermia, disability occurred in 7 of 43 children with follow-up data available (16%) (431).

The magnitude of the reflex withdrawal in response to noxious stimulation was reduced in infants with HIE compared to the controls. Consistent with the EEG activity, reflex activity appeared to increase after hypothermia treatment, but this was not a significant finding. Reflex withdrawal has been shown to be significantly correlated with noxious evoked brain activity, and both increase in magnitude with increasing intensity of stimulus (216). No significant correlations were found in this study.

Experimental noxious stimulation with a force of 128 mN has been used previously to investigate nociception in preterm infants near term age and full-term infants, but it has never been used in infants with a neonatal encephalopathy such as HIE. For this reason, PIPP scores were calculated in order to evaluate its suitability to use in this cohort of infants. The PIPP-R score has been well validated for acute pain, can be used in infants of varying gestational ages (135,149,432), and performs well in infants who are ventilated (433). Signs of distress in this population of infants include tachycardia, facial grimacing and irritability. An elevated heart rate is suggestive of either distress or persistent seizures. In healthy term infants, behavioural and physiological changes (using PIPP-R grading) in response to experimental noxious stimulation, did not show significant changes in total PIPP score or combined facial expression score. In this study, the infants showed no significant changes in the

total PIPP score and each of the three behavioural and physiological components of the score. Furthermore, scores from the infants with HIE were lower than those reported in Hartley et al (216).

5.14 Strengths, limitations, and future work

This is the first study to quantify the responses to tactile and noxious evoked brain activity in infants with HIE during and after hypothermia treatment. Given that the current view is that a combination of multimodal brain monitoring gives additional prognostic information, this study could potentially be very important. The acquisition methods in this study are more practical for cot-side testing and the novel analysis methods could provide us with insights on other aspects of clinical importance such as the effects of analgesia as well as the integrity of the somatosensory pathway.

The main limitations of this study, which can be addressed in future work, were the limited numbers of infants in the HIE group which did not allow for associations between the magnitude of evoked response and clinical HIE grading, and the lack of short and long term follow up information regarding development. Future work should include associations between stimulus-evoked brain activity and short-term outcomes: combined outcome of death or moderate-severe injury on MRI, and long-term neurodevelopmental outcomes at 18-24 months. It may also be useful in future studies to look at the serum levels of morphine around the time of study to establish a better understanding of the accumulative effects of morphine on brain activity.

5.15 Conclusion

Using the analytical approach developed in the first results chapter of this thesis, it was possible to show that in infants with HIE tactile and noxious evoked brain activity is significantly reduced compared to healthy term infants. Stimulus evoked brain activity did not differ between the two study sessions performed around day 2 and 5 in infants with HIE likely showing the effects of sedation during hypothermia treatment. Nonetheless, this study showed that it is possible to investigate nociception using experimental noxious stimulation without distressing the infant and therefore could be used in future studies to assess the effects of sedation.

Chapter 6 DISCUSSION

The aim of this research work was to develop a method of quantifying the evoked brain responses to sensory stimulation in preterm and term infants in order to investigate development and the impact of preterm birth, and to investigate the impact of brain injury on early neurosensory development. In this chapter a summary of the key results and conclusions of this thesis is given followed by a discussion on the strengths and limitations and future directions for this work.

6.1 Summary of results

In Chapter 3, I demonstrate that in full term infants the brain can discriminate between different sensory stimuli in a spatially distinct, stimulus specific manner. By characterising the responses to visual, tactile, and noxious stimulation, using a consistent approach across modality I was able to develop a framework of temporal templates that could be used to better understand the development of sensory processing during the period of prematurity, and the impact of prematurity and pathology on the evoked patterns of activity in term-equivalent or near term infants. The use of templates to analyse evoked brain activity has several advantages over conventional methods of analysis. Importantly, using temporal templates of activity allows for objective quantification of the response and is a way of comparing modalities using a single analytical approach. There are limitations to this method, such as the need to use Woody filtering to account for variability in individual latencies. For this thesis it was of particular interest to develop a method that could be applied to

measure the evoked activity to different sensory stimuli across a wide range of gestational ages. Templates allowed for that flexibility while managing to appreciate significant effects and changes in the evoked activity, as was shown in Chapters 4 and 5.

When the templates were applied onto the longitudinal preterm cohort investigated in Chapter 4, distinct developmental trajectories in the spatial distribution and magnitude of stimulus-evoked activity with post menstrual age (PMA) was observed. From <32 weeks PMA, visual and tactile stimulation evoked topographically organised activity over the occipital and mid central regions of the brain respectively, whereas noxious stimulation did not show a localised response in the mid central area until >34 weeks PMA. Interestingly, although responses to visual and tactile stimuli were already distinct in terms of topography in the youngest infants, the direction of their developmental trajectories differed. Whilst the magnitude of visual- evoked activity increased with PMA, tactile evoked brain activity decreased in magnitude with PMA. The magnitude of noxious evoked brain activity increased with PMA, and this was observed as a switch in morphology whereby infants <34 weeks either showed no observable response or evoked delta brush activity and in infants >34 weeks a positive deflection around 400-700 ms after the noxious stimulus was observed. This is the first study to show the longitudinal development of these responses across the preterm period between 28- 37 weeks PMA. As many authors suggest, the presence, emergence and disappearance of components from evoked brain responses are potentially more indicative of maturation during the preterm period than variations in latency. This was demonstrated in our data.

Maturational factors and experience play a key role in the specialisation of neural circuits. Therefore, to assess if early life experience alters normal development, the same temporal templates were projected to an independent age-matched preterm control cohort of infants. Comparisons between the last grouping of the longitudinal infant recordings (born <32 weeks GA and studied >34 weeks PMA) and the preterm control group (born and studied >34 weeks PMA) showed that specific components of visual and tactile evoked brain activity may be altered by ex-utero exposure to the environment. Our results corroborate previous studies showing that the emergence of the component measured by the visual temporal template is accelerated in preterm infants. Tactile evoked activity was also significantly lower in magnitude in the longitudinal cohort compared to the preterm control suggesting a more adaptive response to the environment in the infants who had been exposed to it the longest. The fact that there was no significant difference in the magnitude of noxious evoked activity between the two group reflects how healthy and stable the infants were in the longitudinal cohort. Prior pain experience was relatively low in this cohort 37.5 (25 - 65 up to the last test occasion) compared to the sickest preterm infants who require up to 50 painful procedures a day. These results support the idea of individualised developmental care in the neonatal setting.

In Chapter 5, the same analytical approaches were used to measure somatosensory tactile and noxious evoked responses in infants diagnosed with HIE at birth compared to healthy term infants. As would have been expected, the magnitude of evoked response was significantly reduced in infants with HIE, in response to either stimulus compared to healthy term infants. Furthermore, infants with HIE were less likely to produce a response that was detectable above

the background EEG activity. There were no significant differences between the two recording sessions (during and after hypothermia treatment) in the HIE group, however, it is possible that the magnitude of tactile evoked activity appeared to reduce even more after hypothermia treatment, whereas noxious evoked activity appeared to increase. The trend observed in the noxious evoked activity was similar to the reflex withdrawal, increasing confidence in the validity of these results, and although no significant difference was seen between the two recording sessions, it did appear that there was a trend towards an increase in the magnitude of the responses in the second recording session. PIPP-R scores showed that noxious stimulation with a force of 128mN used in this study does not evoke behavioural signs of distress and therefore can feasibly be used to study nociception in this population of infants.

6.2 Strengths and Limitations of this research

A strength of this research comes from the careful recruitment of infants, how we stimulated the infants and the novel analysis approach used to compare evoked activity across gestational age and types of stimulation. The challenges and limitations involved in the process of acquiring this data are discussed further here. Research investigations in neonates tend to include small sample sizes and, in this thesis, this was a limitation. This was likely due to the inherent challenges involved in recruiting infants to research studies. In particular, this thesis involved the recruitment of very preterm infants (Chapter 4) and infants born in poor condition (Chapter 5), which proved challenging cohorts of interest. Additionally, infants who met the eligibility criteria for all chapters were infrequent. Healthy full- term infants do not stay in hospital for longer than 24 hours unless they have been admitted to the neonatal unit for reasons that would

have made them not eligible to take part. The longitudinal preterm and control preterm cohorts also had strict eligibility criteria as it was of great importance that the infants in these cohorts had no neurologic conditions and did not require invasive mechanical ventilation for long periods so they could represent healthy premature individuals with minimal complications. More flexibility was allowed to recruit infants in Chapter 5. This was supposed to allow for infants who were diagnosed with mild HIE and were not treated with hypothermia treatment. However, although around half of the infants recruited to take part in the study were diagnosed with mild HIE and treated with hypothermia, no infants with mild HIE without hypothermia treatment were identified.

Another limitation with the data presented in Chapter 4 was the requirement of a clinically essential heel lance. The frequency with which infants require blood tests varies during their treatment in the neonatal unit. The infants in the longitudinal preterm cohort tended to require bloods almost daily during the first couple of weeks after birth, but this reduced to once a week and was rarely needed after 35-36 weeks GA. Recording test occasions needed to be arranged at a time when a clinical blood test was required, and this was sometimes challenging as infants in the preterm cohort rarely required clinical heel lancing. This combination of factors reduced the frequency of recording occasions and affected how long we could study the infants for.

The application of experimental noxious stimulation in Chapter 3 and 5 overcame the issue with timing and requirement of clinical blood tests. It would not have been possible to record clinical heel lances from infants undergoing hypothermia treatment as central lines are in situ, so lancing is not clinically

required. Healthy full-term infants do not usually require clinical blood tests until day 5 (when the newborn screening or 'heel-prick test´ is performed) therefore the application of the experimental noxious (Pinprick) stimulus increased the number of infants who could take part in the study. The reason for not using the Pinprick in the preterm cohorts was that although it is a low intensity stimulus, the feasibility and appropriateness of its use in preterm infants has not been investigated.

Perhaps the most significant limitation to this work was that an identifiable auditory evoked response was not recorded in the healthy full term control group (Chapter 3). The equipment used to deliver the tone using headphones was new to the group, therefore parameters that were deemed safe to use in premature infants were chosen based on previous literature. However, the literature is very varied, with different studies using different delivery methods, tone frequency, duration and intensity. It is likely that the reason a cortical auditory evoked response was not recorded was due to the parameters not being optimal. A tone delivered with a low intensity may not have been complex enough to produce an evoked response, or perhaps if the intensity of the sound had been higher, then a tone would have evoked a response. The duration of the tone being so short likely also contributed. In any case, new parameters need to be chosen in any further studies involving the recording of auditory evoked potentials.

6.3 Future work

The results of this thesis set the groundwork for many different avenues of research in this field. It would be interesting to recruit more infants longitudinally to create robust 'normal values' so that further comparisons can

be made. Introducing independent preterm control cohorts at earlier stages, may have also highlighted different aspects of the maturation of evoked response and provide evidence for potential changes that could promote appropriate sensory development in the neonatal setting. In addition, preterm infants with different degrees of IVH or other types of preterm brain injury could be investigated to assess the value of recording evoked potentials to noxious and non-noxious stimulation for prediction of neurodevelopmental outcome. For future work on nociception, it would also be beneficial to investigate the feasibility of using low intensity experimental noxious stimulation in preterm infants.

In this thesis it was not possible to compare the visual evoked responses of infants with HIE during and after hypothermia treatment due to lack of a healthy term control group. It was also not possible to separate the degrees of HIE severity due to small numbers of infants. However, it would be interesting to investigate if the altered processing of somatosensory information during the early days after an insult to the brain also affects visual sensory processing and how. Also, given the challenges of performing somatosensory evoked potentials in infants, it is of clinical significance to investigate if using methods such as the tendon hammer or the Pinprick (used in this thesis), may provide significant information regarding somatosensory processing which can be adopted readily during routine EEG recordings.

Though it was out of the scope of this thesis, in future it will be possible to correlate the neonatal neurosensory data to neurodevelopmental outcomes. Developmental follow up is an ongoing process and though the infants in

Chapters 3 and 4 were all deemed healthy during their stay in the neonatal unit this may have changed. Similarly, evaluating outcome is ultimately the best way of assessing if early detection methods are sensitive enough to predict prognosis.

Lastly, the possible confounds of sleep state were not assessed in this thesis but given the evidence that at least visual and tactile evoked activity is influenced by sleep state, this would be another interesting avenue of research using the template approach of analysis for comparisons.

6.4 Concluding remarks

Temporal templates developed in this thesis provide a novel approach of analysis of stimulus-evoked brain activity in infants and give insight regarding the development of neurosensory systems early in life. This approach has provided a framework for developing our understanding of critical periods of neurodevelopment, how these are modulated by exposure to the neonatal care unit environment, and the impact of HIE and its treatment on somatosensory processing. Understanding early neurosensory processing both in the healthy term infant and the preterm infant is essential for improving developmental care practices. Evoked potentials are valuable adjuncts to the recording of electroencephalography (EEG), and novel acquisition and analysis methods like the ones used in this thesis could potentially change how we assess neurological development of sensory function in infants in the future.

Chapter 7 REFERENCES

1 Walani SR. Global burden of preterm birth. International Journal of Gynecology & Obstetrics . 2020 Jul 1;150(1):31–3. Available from: https://doi.org/10.1002/ijgo.13195

2. Volpe JJ. Brain injury in premature infants: a complex amalgam of destructive and developmental disturbances. The Lancet Neurology . 2009;8(1):110–24. Available from: http://www.sciencedirect.com/science/article/pii/S1474442208702941

3. de Vries LS, Jongmans MJ. Long-term outcome after neonatal hypoxic-ischaemic encephalopathy. Archives of Disease in Childhood - Fetal and Neonatal Edition . 2010 May 1;95(3):F220 LP-F224. Available from: http://fn.bmj.com/content/95/3/F220.abstract

4. Ouwehand S, Smidt LCA, Dudink J, Benders MJNL, de Vries LS, Groenendaal F, et al. Predictors of Outcomes in Hypoxic-Ischemic Encephalopathy following Hypothermia: A Meta-Analysis. Neonatology . 2020;117(4):411–27. Available from: https://www.karger.com/DOI/10.1159/000505519

5. van Handel M, Swaab H, de Vries LS, Jongmans MJ. Long-term cognitive and behavioral consequences of neonatal encephalopathy following perinatal asphyxia: a review. European journal of pediatrics . 2007/04/11. 2007 Jul;166(7):645–54. Available from: https://pubmed.ncbi.nlm.nih.gov/17426984

6. Azzopardi D. Predictive value of the amplitude integrated EEG in infants with hypoxic ischaemic encephalopathy: data from a randomised trial of therapeutic hypothermia. Archives of Disease in Childhood - Fetal and Neonatal Edition . 2014 Jan 1;99(1):F80 LP-F82. Available from: http://fn.bmj.com/content/99/1/F80.abstract

7. Moore T, Hennessy EM, Myles J, Johnson SJ, Draper ES, Costeloe KL, et al. Neurological and developmental outcome in extremely preterm children born in England in 1995 and 2006: the EPICure studies. BMJ . 2012;345. Available from: https://www.bmj.com/content/345/bmj.e7961

8. Cheong JLY, Spittle AJ, Burnett AC, Anderson PJ, Doyle LW. Have outcomes following extremely preterm birth improved over time? Seminars in Fetal and Neonatal Medicine . 2020;25(3):101114. Available from: http://www.sciencedirect.com/science/article/pii/S1744165X20300391

9. Blencowe H, Lee ACC, Cousens S, Bahalim A, Narwal R, Zhong N, et al. Preterm birth–associated neurodevelopmental impairment estimates at regional and global levels for 2010. Pediatric Research . 2013;74(1):17–34. Available from: https://doi.org/10.1038/pr.2013.204

10. Gibson NA, Brezinova V, Levene MI. Somatosensory evoked potentials in the term newborn. Electroencephalography and Clinical Neurophysiology/Evoked Potentials Section . 1992;84(1):26–31. Available from: https://www.sciencedirect.com/science/article/pii/016855979290065J

11. Hrbek A. Visual Evoked Responses in the Newborn Infant. International Ophthalmology Clinics . 1969;9(4). Available from: https://journals.lww.com/internat-ophthalmology/Fulltext/1969/00940/Visual_Evoked_Responses_in_the_Newborn_Infant.13.aspx

12. Hrbek A, Karlberg P, Olsson T. Development of visual and somatosensory evoked responses in pre-term newborn infants. Electroencephalography and Clinical Neurophysiology . 1973;34(3):225–32. Available from: http://www.sciencedirect.com/science/article/pii/0013469473902496

13. Klimach VJ, Cooke RW. Maturation of the neonatal somatosensory evoked response in preterm infants. Developmental medicine and child neurology. 1988 Apr;30(2):208–14.

14. White CP, Cooke RWI. Maturation of the Cortical Evoked Response To Posterior-Nerve Stimulation in the Preterm Neonate. Developmental Medicine & Child Neurology . 1989 Oct 1;31(5):657–64. Available from: https://doi.org/10.1111/j.1469-8749.1989.tb04052.x

15. Willis J, Duncan C, Bell R. Short-latency somatosensory evoked potentials in perinatal asphyxia. Pediatric Neurology . 1987;3(4):203–7. Available from: http://www.sciencedirect.com/science/article/pii/0887899487900178

16. Whyte HE, Pearce JM, Taylor MJ. Changes in the VEP in preterm neonates with arousal states, as assessed by EEG monitoring. Electroencephalography and Clinical Neurophysiology/Evoked Potentials Section . 1987;68(3):223–5. Available from: http://www.sciencedirect.com/science/article/pii/016855978790030X

17. Hrbek A, Hrbková M, Lenard H-G. Somato-sensory, auditory and visual evoked responses in newborn infants during sleep and wakefulness. Electroencephalography and Clinical Neurophysiology . 1969;26(6):597–603. Available from: http://www.sciencedirect.com/science/article/pii/0013469469900066

18. Pihko E, Lauronen L, Wikström H, Taulu S, Nurminen J, Kivitie-Kallio S, et al. Somatosensory evoked potentials and magnetic fields elicited by tactile stimulation of the hand during active and quiet sleep in newborns. Clinical Neurophysiology . 2004;115(2):448–55. Available from: http://www.sciencedirect.com/science/article/pii/S1388245703003493

19. Ellingson RJ. Electroencephalograms of normal, full-term newborns immediately after birth with observations on arousal and visual evoked responses. Electroencephalography and Clinical Neurophysiology .

1958;10(1):31–50. Available from:
http://www.sciencedirect.com/science/article/pii/0013469458901019

20. Majnemer A, Rosenblatt B. Evoked potentials as predictors of outcome in neonatal intensive care unit survivors: Review of the literature. Pediatric Neurology . 1996;14(3):189–95. Available from:
http://www.sciencedirect.com/science/article/pii/0887899496000495

21. Majnemer A, Rosenblatt B, Riley PS. Prognostic significance of multimodality evoked response testing in high-risk newborns. Pediatric Neurology . 1990;6(6):367–74. Available from:
http://www.sciencedirect.com/science/article/pii/088789949090002I

22. Pike AA, Marlow N. The role of cortical evoked responses in predicting neuromotor outcome in very preterm infants. Early Human Development . 2000;57(2):123–35. Available from:
http://www.sciencedirect.com/science/article/pii/S0378378299000614

23. Taylor MJ, Murphy WJ, Whyte HE. Prognostic reliability of somatosensory and visual evoked potentials of asphyxiated term infants. Developmental Medicine & Child Neurology . 1992 Jun 1;34(6):507–15. Available from: https://doi.org/10.1111/j.1469-8749.1992.tb11471.x

24. Muttitt SC, Taylor MJ, Kobayashi JS, MacMillan L, Whyte HE. Serial visual evoked potentials and outcome in term birth asphyxia. Pediatric Neurology . 1991;7(2):86–90. Available from:
http://www.sciencedirect.com/science/article/pii/0887899491900023

25. Whyte HE, Taylor MJ, Menzies R, Chin KC, MacMillan LJ. Prognostic utility of visual evoked potentials in term asphyxiated neonates. Pediatric Neurology . 1986;2(4):220–3. Available from:
http://www.sciencedirect.com/science/article/pii/0887899486900512

26. Moultrie F, Slater R, Hartley C. Improving the treatment of infant pain. Current Opinion in Supportive and Palliative Care . 2017;11(2). Available from:
https://journals.lww.com/co-

supportiveandpalliativecare/Fulltext/2017/06000/Improving_the_treatment_of_infant_pain.9.aspx

27. Hartley C, Duff EP, Green G, Mellado GS, Worley A, Rogers R, et al. Nociceptive brain activity as a measure of analgesic efficacy in infants. Science Translational Medicine . 2017 May 3;9(388):eaah6122. Available from: http://stm.sciencemag.org/content/9/388/eaah6122.abstract

28. Vasung L, Abaci Turk E, Ferradal SL, Sutin J, Stout JN, Ahtam B, et al. Exploring early human brain development with structural and physiological neuroimaging. NeuroImage . 2019;187:226–54. Available from: http://www.sciencedirect.com/science/article/pii/S1053811918306566

29. Stiles J, Jernigan TL. The basics of brain development. Neuropsychology review. 2010 Dec;20(4):327–48.

30. Ladher R, Schoenwolf GC. Making a Neural Tube: Neural Induction and Neurulation BT - Developmental Neurobiology. In: Rao MS, Jacobson† M, editors. Boston, MA: Springer US; 2005. p. 1–20. Available from: https://doi.org/10.1007/0-387-28117-7_1

31. Herschkowitz N. Brain development in the fetus, neonate and infant. Biology of the neonate. 1988;54(1):1–19.

32. Wood JG, Martin S, Price DJ. Evidence that the earliest generated cells of the murine cerebral cortex form a transient population in the subplate and marginal zone. Developmental Brain Research . 1992;66(1):137–40. Available from: http://www.sciencedirect.com/science/article/pii/016538069290150U

33. White T, Su S, Schmidt M, Kao C-Y, Sapiro G. The development of gyrification in childhood and adolescence. Brain and Cognition . 2010;72(1):36–45. Available from: http://www.sciencedirect.com/science/article/pii/S0278262609002012

34. Olson EC, Walsh CA. Smooth, rough and upside-down neocortical development. Current Opinion in Genetics & Development . 2002;12(3):320–7.

Available from:
http://www.sciencedirect.com/science/article/pii/S0959437X02003052

35. Kostovic I, Rakic P. Developmental history of the transient subplate zone in the visual and somatosensory cortex of the macaque monkey and human brain. Journal of Comparative Neurology . 1990 Jul 15;297(3):441–70. Available from: https://doi.org/10.1002/cne.902970309

36. Allendoerfer KL, Shatz CJ. The Subplate, A Transient Neocortical Structure: Its Role in the Development of Connections between Thalamus and Cortex. Annual Review of Neuroscience . 1994 Mar 1;17(1):185–218. Available from: https://doi.org/10.1146/annurev.ne.17.030194.001153

37. Kostovic I, Rakic P. Development of prestriate visual projections in the monkey and human fetal cerebrum revealed by transient cholinesterase staining. The Journal of Neuroscience . 1984 Jan 1;4(1):25 LP – 42. Available from: http://www.jneurosci.org/content/4/1/25.abstract

38. Tau GZ, Peterson BS. Normal Development of Brain Circuits. Neuropsychopharmacology . 2010 Jan;35(1):147–68. Available from: https://doi.org/10.1038/npp.2009.115

39. Duque A, Krsnik Z, Kostović I, Rakic P. Secondary expansion of the transient subplate zone in the developing cerebrum of human and nonhuman primates. Proceedings of the National Academy of Sciences . 2016 Aug 30;113(35):9892 LP – 9897. Available from: http://www.pnas.org/content/113/35/9892.abstract

40. Ghosh A, Antonini A, McConnell SK, Shatz CJ. Requirement for subplate neurons in the formation of thalamocortical connections. Nature . 1990;347(6289):179–81. Available from: https://doi.org/10.1038/347179a0

41. Ghosh A, Shatz CJ. A role for subplate neurons in the patterning of connections from thalamus to neocortex. Development (Cambridge, England). 1993 Mar;117(3):1031–47.

42. Kostović I, Jovanov-Milošević N. The development of cerebral connections during the first 20–45 weeks' gestation. Seminars in Fetal and Neonatal Medicine . 2006;11(6):415–22. Available from: http://www.sciencedirect.com/science/article/pii/S1744165X06000680

43. Kostović I, Judaš M, Radoš M, Hrabač P. Laminar Organization of the Human Fetal Cerebrum Revealed by Histochemical Markers and Magnetic Resonance Imaging. Cerebral Cortex . 2002 May 1;12(5):536–44. Available from: https://doi.org/10.1093/cercor/12.5.536

44. Huisman TA, Martin E, Kubik-Huch R, Marincek B. Fetal magnetic resonance imaging of the brain: technical considerations and normal brain development. European Radiology . 2002;12(8):1941–51. Available from: https://doi.org/10.1007/s00330-001-1209-x

45. Vasung L, Lepage C, Radoš M, Pletikos M, Goldman JS, Richiardi J, et al. Quantitative and Qualitative Analysis of Transient Fetal Compartments during Prenatal Human Brain Development. Frontiers in neuroanatomy. 2016;10:11.

46. Ghosh A, Shatz CJ. Involvement of subplate neurons in the formation of ocular dominance columns. Science . 1992 Mar 13;255(5050):1441 LP – 1443. Available from: http://science.sciencemag.org/content/255/5050/1441.abstract

47. Ball G, Pazderova L, Chew A, Tusor N, Merchant N, Arichi T, et al. Thalamocortical Connectivity Predicts Cognition in Children Born Preterm. Cerebral Cortex . 2015 Nov 1;25(11):4310–8. Available from: https://doi.org/10.1093/cercor/bhu331

48. McQuillen PS, Ferriero DM. Perinatal Subplate Neuron Injury: Implications for Cortical Development and Plasticity. Brain Pathology . 2005 Jul 1;15(3):250–60. Available from: https://doi.org/10.1111/j.1750-3639.2005.tb00528.x

49. Bystron I, Blakemore C, Rakic P. Development of the human cerebral cortex: Boulder Committee revisited. Nature Reviews Neuroscience . 2008;9(2):110–22. Available from: https://doi.org/10.1038/nrn2252

50. Garel C, Chantrel E, Brisse H, Elmaleh M, Luton D, Oury J-F, et al. Fetal Cerebral Cortex: Normal Gestational Landmarks Identified Using Prenatal MR Imaging. American Journal of Neuroradiology . 2001 Jan 1;22(1):184 LP – 189. Available from: http://www.ajnr.org/content/22/1/184.abstract

51. Hüppi PS, Warfield S, Kikinis R, Barnes PD, Zientara GP, Jolesz FA, et al. Quantitative magnetic resonance imaging of brain development in premature and mature newborns. Annals of Neurology . 1998 Feb 1;43(2):224–35. Available from: https://doi.org/10.1002/ana.410430213

52. Goldman PS, Galkin TW. Prenatal removal of frontal association cortex in the fetal rhesus monkey: Anatomical and functional consequences in postnatal life. Brain Research . 1978;152(3):451–85. Available from: http://www.sciencedirect.com/science/article/pii/0006899378911034

53. Karzbrun E, Tshuva RY, Reiner O. An On-Chip Method for Long-Term Growth and Real-Time Imaging of Brain Organoids. Current Protocols in Cell Biology . 2018 Dec 1;81(1):e62. Available from: https://doi.org/10.1002/cpcb.62

54. Karzbrun E, Kshirsagar A, Cohen SR, Hanna JH, Reiner O. Human Brain Organoids on a Chip Reveal the Physics of Folding. Nature physics . 2018/02/19. 2018 May;14(5):515–22. Available from: https://pubmed.ncbi.nlm.nih.gov/29760764

55. Lancaster MA. Crinkle-Cut Brain Organoids. Cell Stem Cell . 2018;22(5):616–8. Available from: http://www.sciencedirect.com/science/article/pii/S1934590918301735

56. Huttenlocher PR, Dabholkar AS. Regional differences in synaptogenesis in human cerebral cortex. Journal of Comparative Neurology . 1997 Oct 20;387(2):167–78. Available from: https://doi.org/10.1002/(SICI)1096-9861(19971020)387:2%3C167::AID-CNE1%3E3.0.CO

57. Bourgeois JP, Goldman-Rakic PS, Rakic P. Synaptogenesis in the prefrontal cortex of rhesus monkeys. Cerebral cortex (New York, NY : 1991). 1994;4(1):78–96.

58. Rakic P, Bourgeois JP, Goldman-Rakic PS. Synaptic development of the cerebral cortex: implications for learning, memory, and mental illness. Progress in brain research. 1994;102:227–43.

59. Rakic P, Bourgeois JP, Eckenhoff MF, Zecevic N, Goldman-Rakic PS. Concurrent overproduction of synapses in diverse regions of the primate cerebral cortex. Science (New York, NY). 1986 Apr;232(4747):232–5.

60. Penn AA, Shatz CJ. Brain Waves and Brain Wiring: The Role of Endogenous and Sensory-Driven Neural Activity in Development. Pediatric Research . 1999;45(4):447–58. Available from: https://doi.org/10.1203/00006450-199904010-00001

61. Graven SN. Early neurosensory visual development of the fetus and newborn. Clinics in Perinatology . 2004;31(2):199–216. Available from: http://www.sciencedirect.com/science/article/pii/S0095510804000326

62. Graven SN, Browne J v. Sensory Development in the Fetus, Neonate, and Infant: Introduction and Overview. Newborn and Infant Nursing Reviews . 2008;8(4):169–72. Available from: http://www.sciencedirect.com/science/article/pii/S1527336908001311

63. Graven SN, Browne J v. Auditory Development in the Fetus and Infant. Newborn and Infant Nursing Reviews . 2008;8(4):187–93. Available from: https://www.sciencedirect.com/science/article/pii/S1527336908001347

64. André V, Durier V, Beuchée A, Roué J-M, Lemasson A, Hausberger M, et al. Higher tactile sensitivity in preterm infants at term-equivalent age: A pilot study. PLOS ONE . 2020 Mar 5;15(3):e0229270. Available from: https://doi.org/10.1371/journal.pone.0229270

65. Graven SN, Browne J v. Sleep and Brain Development: The Critical Role of Sleep in Fetal and Early Neonatal Brain Development. Newborn and Infant Nursing Reviews . 2008;8(4):173–9. Available from: http://www.sciencedirect.com/science/article/pii/S1527336908001323

66. Calciolari G, Montirosso R. The sleep protection in the preterm infants. The Journal of Maternal-Fetal & Neonatal Medicine . 2011 Oct 1;24(sup1):12–4. Available from: https://doi.org/10.3109/14767058.2011.607563

67. Parmelee Jr. AH, Wenner WH, Akiyama Y, Schultz M, Stern E. Sleep States in Premature Infants. Developmental Medicine & Child Neurology . 1967 Feb 1;9(1):70–7. Available from: https://doi.org/10.1111/j.1469-8749.1967.tb02212.x

68. Kurth S, Olini N, Huber R, LeBourgeois M. Sleep and Early Cortical Development. Current sleep medicine reports . 2015/02/03. 2015 Mar;1(1):64–73. Available from: https://pubmed.ncbi.nlm.nih.gov/26807347

69. Scher MS, Johnson MW, Holditch-Davis D. Cyclicity of neonatal sleep behaviors at 25 to 30 weeks' postconceptional age. Pediatric research. 2005 Jun;57(6):879–82.

70. Scher MS, Steppe DA, Banks DL. Postnatal adaptation of brain function in full-term neonates as assessed by EEG sleep analyses. Sleep. 1995 Sep;18(7):531–5.

71. Scher MS, Steppe DA, Dahl RE, Asthana S, Guthrie RD. Comparison of EEG Sleep Measures in Healthy Full-Term and Preterm Infants at Matched Conceptional Ages. Sleep . 1992 Sep 1;15(5):442–8. Available from: https://doi.org/10.1093/sleep/15.5.442

72. de Asis-Cruz J, Kapse K, Basu SK, Said M, Scheinost D, Murnick J, et al. Functional brain connectivity in ex utero premature infants compared to in utero fetuses. NeuroImage . 2020;219:117043. Available from: https://www.sciencedirect.com/science/article/pii/S1053811920305292

73. Mirmiran M, Maas YGH, Ariagno RL. Development of fetal and neonatal sleep and circadian rhythms. Sleep Medicine Reviews . 2003;7(4):321–34. Available from: http://www.sciencedirect.com/science/article/pii/S1087079202902431

74. Zhang LI, Poo M. Electrical activity and development of neural circuits. Nature Neuroscience . 2001;4(11):1207–14. Available from: https://doi.org/10.1038/nn753

75. Batalle D, Hughes EJ, Zhang H, Tournier J-D, Tusor N, Aljabar P, et al. Early development of structural networks and the impact of prematurity on brain connectivity. NeuroImage . 2017;149:379–92. Available from: http://www.sciencedirect.com/science/article/pii/S1053811917300885

76. Jha SK, Jones BE, Coleman T, Steinmetz N, Law C-T, Griffin G, et al. Sleep-Dependent Plasticity Requires Cortical Activity. The Journal of Neuroscience . 2005 Oct 5;25(40):9266 LP – 9274. Available from: http://www.jneurosci.org/content/25/40/9266.abstract

77. Kayser MS, Yue Z, Sehgal A. A Critical Period of Sleep for Development of Courtship Circuitry and Behavior in Drosophila Science . 2014 Apr 18;344(6181):269 LP – 274. Available from: http://science.sciencemag.org/content/344/6181/269.abstract

78. Holditch-Davis D, Scher M, Schwartz T, Hudson-Barr D. Sleeping and waking state development in preterm infants. Early human development. 2004 Oct;80(1):43–64.

79. Scher MS, Richardson GA, Salerno DG, Day NL, Guthrie RD. Sleep Architecture and Continuity Measures of Neonates with Chronic Lung Disease. Sleep . 1992 May 1;15(3):195–201. Available from: https://doi.org/10.1093/sleep/15.3.195

80. Bennet L, Walker DW, Horne RSC. Waking up too early – the consequences of preterm birth on sleep development. The Journal of Physiology

. 2018 Dec 1;596(23):5687–708. Available from: https://doi.org/10.1113/JP274950

81. Bertelle V, Mabin D, Adrien J, Sizun J. Sleep of preterm neonates under developmental care or regular environmental conditions. Early human development. 2005 Jul;81(7):595–600.

82. van den Hoogen A, Teunis CJ, Shellhaas RA, Pillen S, Benders M, Dudink J. How to improve sleep in a neonatal intensive care unit: A systematic review. Early human development. 2017 Oct;113:78–86.

83. Ludington-Hoe SM, Johnson MW, Morgan K, Lewis T, Gutman J, Wilson PD, et al. Neurophysiologic assessment of neonatal sleep organization: preliminary results of a randomized, controlled trial of skin contact with preterm infants. Pediatrics. 2006 May;117(5):e909-23.

84. Stangenes KM, Fevang SK, Grundt J, Donkor HM, Markestad T, Hysing M, et al. Children born extremely preterm had different sleeping habits at 11 years of age and more childhood sleep problems than term-born children. Acta paediatrica (Oslo, Norway : 1992). 2017 Dec;106(12):1966–72.

85. Graven SN, Browne J v. Visual Development in the Human Fetus, Infant, and Young Child. Newborn and Infant Nursing Reviews . 2008;8(4):194–201. Available from: http://www.sciencedirect.com/science/article/pii/S1527336908001359

86. Shatz CJ. Emergence of order in visual system development. Journal of Physiology-Paris . 1996;90(3):141–50. Available from: https://www.sciencedirect.com/science/article/pii/S0928425797814131

87. Wiesel TN, Hubel DH. Ordered arrangement of orientation columns in monkeys lacking visual experience. Journal of Comparative Neurology . 1974 Dec 1;158(3):307–18. Available from: https://doi.org/10.1002/cne.901580306

88. Hubel DH, Wiesel TN. Ferrier lecture. Functional architecture of macaque monkey visual cortex. Proceedings of the Royal Society of London Series B, Biological sciences. 1977 Jul;198(1130):1–59.

89. Hubel DH, Wiesel TN. Receptive fields, binocular interaction and functional architecture in the cat's visual cortex. The Journal of Physiology . 1962 Jan 1;160(1):106–54. Available from: https://doi.org/10.1113/jphysiol.1962.sp006837

90. Hubel DH, Wiesel TN. The period of susceptibility to the physiological effects of unilateral eye closure in kittens. The Journal of Physiology . 1970 Feb 1;206(2):419–36. Available from: https://doi.org/10.1113/jphysiol.1970.sp009022

91. Polley DB, Seidl AH, Wang Y, Sanchez JT. Comprehensive Developmental Neuroscience: Neural Circuit Development and Function in the Heathy and Diseased Brain: Chapter 2. Functional Circuit Development in the Auditory System. Elsevier Inc. Chapters; 2013.

92. Moore JK, Linthicum FH. The human auditory system: A timeline of development. International Journal of Audiology . 2007 Jan 1;46(9):460–78. Available from: https://doi.org/10.1080/14992020701383019

93. Pujol R, Lavigne-Rebillard M. Development of Neurosensory Structures in the Human Cochlea. Acta Oto-Laryngologica . 1992 Jan 1;112(2):259–64. Available from: https://doi.org/10.1080/00016489.1992.11665415

94. Hall III PhD JW. Development of the Ear and Hearing. Journal of Perinatology . 2000;20(1):S12–20. Available from: https://doi.org/10.1038/sj.jp.7200439

95. Morris BH, Philbin MK, Bose C. Physiological Effects of Sound on the Newborn. Journal of Perinatology . 2000;20(1):S55–60. Available from: https://doi.org/10.1038/sj.jp.7200451

96. Lahav A, Skoe E. An acoustic gap between the NICU and womb: a potential risk for compromised neuroplasticity of the auditory system in preterm infants. Frontiers in neuroscience . 2014 Dec 5;8:381. Available from: https://pubmed.ncbi.nlm.nih.gov/25538543

97. Steeds CE. The anatomy and physiology of pain. Surgery - Oxford International Edition . 2016 Feb 1;34(2):55–9. Available from: https://doi.org/10.1016/j.mpsur.2015.11.005

98. Fitzgerald M, Shaw A, MacIntosh N. Postnatal development of the cutaneous flexor reflex:comparative study of preterm infants and newborn rat pups. Developmental Medicine & Child Neurology . 1988 Aug 1;30(4):520–6. Available from: https://doi.org/10.1111/j.1469-8749.1988.tb04779.x

99. Hatfield LA. Neonatal pain: What's age got to do with it? Surgical neurology international . 2014 Nov 13;5(Suppl 13):S479–89. Available from: https://pubmed.ncbi.nlm.nih.gov/25506507

100. Fitzgerald M. What do we really know about newborn infant pain? Experimental Physiology . 2015 Dec 1;100(12):1451–7. Available from: https://doi.org/10.1113/EP085134

101. Cornelissen L, Fabrizi L, Patten D, Worley A, Meek J, Boyd S, et al. Postnatal Temporal, Spatial and Modality Tuning of Nociceptive Cutaneous Flexion Reflexes in Human Infants. PLOS ONE . 2013 Oct 4;8(10):e76470. Available from: https://doi.org/10.1371/journal.pone.0076470

102. Abraira VEE, Ginty DDD. The sensory neurons of touch. Neuron . 2013 Aug 21;79(4):618–39. Available from: https://doi.org/10.1016/j.neuron.2013.07.051

103. Lowery CL, Hardman MP, Manning N, Clancy B, Whit Hall R, Anand KJS. Neurodevelopmental Changes of Fetal Pain. Seminars in Perinatology . 2007;31(5):275–82. Available from: https://www.sciencedirect.com/science/article/pii/S0146000507000687

104. Kostović I, Judaš M. The development of the subplate and thalamocortical connections in the human foetal brain. Acta Paediatrica . 2010 Aug 1;99(8):1119–27. Available from: https://doi.org/10.1111/j.1651-2227.2010.01811.x

105. Fitzgerald M. The development of nociceptive circuits. Nature Reviews Neuroscience . 2005;6(7):507–20. Available from: https://doi.org/10.1038/nrn1701

106. Verriotis M, Chang P, Fitzgerald M, Fabrizi L. The development of the nociceptive brain. Neuroscience . 2016;338:207–19. Available from: http://www.sciencedirect.com/science/article/pii/S0306452216303311

107. Goksan S, Hartley C, Emery F, Cockrill N, Poorun R, Moultrie F, et al. fMRI reveals neural activity overlap between adult and infant pain. eLife . 2015 Apr 21;4:e06356. Available from: https://pubmed.ncbi.nlm.nih.gov/25895592

108. Duff EP, Moultrie F, van der Vaart M, Goksan S, Abos A, Fitzgibbon SP, et al. Inferring pain experience in infants using quantitative whole-brain functional MRI signatures: a cross-sectional, observational study. The Lancet Digital Health . 2020;2(9):e458–67. Available from: http://www.sciencedirect.com/science/article/pii/S2589750020301680

109. Hathway GJ, Koch S, Low L, Fitzgerald M. The changing balance of brainstem-spinal cord modulation of pain processing over the first weeks of rat postnatal life. The Journal of physiology . 2009/04/29. 2009 Jun 15;587(Pt 12):2927–35. Available from: https://doi.org/10.1113/jphysiol.2008.168013

110. Goksan S, Baxter L, Moultrie F, Duff E, Hathway G, Hartley C, et al. The influence of the descending pain modulatory system on infant pain-related brain activity. eLife. 2018 Sep;7.

111. Hartley C, Moultrie F, Gursul D, Hoskin A, Adams E, Rogers R, et al. Changing Balance of Spinal Cord Excitability and Nociceptive Brain Activity in Early Human Development. Current Biology . 2016;26(15):1998–2002. Available from: http://www.sciencedirect.com/science/article/pii/S0960982216305516

112. Jones L, Fabrizi L, Laudiano-Dray M, Whitehead K, Meek J, Verriotis M, et al. Nociceptive Cortical Activity Is Dissociated from Nociceptive Behavior in Newborn Human Infants under Stress. Current Biology . 2017;27(24):3846-3851.e3. Available from: https://www.sciencedirect.com/science/article/pii/S0960982217314008

113. Gursul D, Goksan S, Hartley C, Mellado GS, Moultrie F, Hoskin A, et al. Stroking modulates noxious-evoked brain activity in human infants. Current Biology . 2018;28(24):R1380–1. Available from: http://www.sciencedirect.com/science/article/pii/S0960982218314805

114. Bröring T, Oostrom KJ, Lafeber HN, Jansma EP, Oosterlaan J. Sensory modulation in preterm children: Theoretical perspective and systematic review. PLOS ONE . 2017 Feb 9;12(2):e0170828. Available from: https://doi.org/10.1371/journal.pone.0170828

115. Gerhardt KJ, Abrams RM. Fetal hearing: characterization of the stimulus and response. Seminars in perinatology. 1996 Feb;20(1):11–20.

116. Querleu D, Renard X, Versyp F, Paris-Delrue L, Crèpin G. Fetal hearing. European journal of obstetrics, gynecology, and reproductive biology. 1988 Jul;28(3):191–212.

117. Hall RW, Anand KJS. Physiology of Pain and Stress in the Newborn. NeoReviews . 2005 Feb 1;6(2):e61 LP-e68. Available from: http://neoreviews.aappublications.org/content/6/2/e61.abstract

118. Philpott-Robinson K, Lane SJ, Korostenski L, Lane AE. The impact of the Neonatal Intensive Care Unit on sensory and developmental outcomes in infants born preterm: A scoping review. British Journal of Occupational Therapy . 2017 Jun 29;80(8):459–69. Available from: https://doi.org/10.1177/0308022617709761

119. Niutanen U, Harra T, Lano A, Metsäranta M. Systematic review of sensory processing in preterm children reveals abnormal sensory modulation, somatosensory processing and sensory-based motor processing. Acta

Paediatrica . 2020 Jan 1;109(1):45–55. Available from: https://doi.org/10.1111/apa.14953

120. Altimier L, Phillips R. The Neonatal Integrative Developmental Care Model: Advanced Clinical Applications of the Seven Core Measures for Neuroprotective Family-centered Developmental Care. Newborn and Infant Nursing Reviews . 2016;16(4):230–44. Available from: http://www.sciencedirect.com/science/article/pii/S1527336916301118

121. Pickler RH, McGrath JM, Reyna BA, McCain N, Lewis M, Cone S, et al. A model of neurodevelopmental risk and protection for preterm infants. The Journal of perinatal & neonatal nursing . 2010;24(4):356–65. Available from: https://pubmed.ncbi.nlm.nih.gov/21045616

122. Leijser LM, Siddiqi A, Miller SP. Imaging Evidence of the Effect of Socio-Economic Status on Brain Structure and Development. Seminars in Pediatric Neurology . 2018;27:26–34. Available from: https://www.sciencedirect.com/science/article/pii/S1071909118300123

123. Grunau RVE, Whitfield MF, Petrie JH, Fryer LE. Early pain experience, child and family factors, as precursors of somatization: a prospective study of extremely premature and fullterm children. Pain. 1994 Mar;56(3):353–9.

124. Schneider J, Borghini A, Morisod Harari M, Faure N, Tenthorey C, le Berre A, et al. Joint observation in NICU (JOIN): study protocol of a clinical randomised controlled trial examining an early intervention during preterm care. BMJ Open . 2019 Mar 1;9(3):e026484. Available from: http://bmjopen.bmj.com/content/9/3/e026484.abstract

125. Pinelli J, Symington A. Non-nutritive sucking for promoting physiologic stability and nutrition in preterm infants. The Cochrane database of systematic reviews. 2005 Oct;(4):CD001071.

126. Foster JP, Psaila K, Patterson T. Non-nutritive sucking for increasing physiologic stability and nutrition in preterm infants. The Cochrane database of systematic reviews. 2016 Oct;10(10):CD001071.

127. Bond C. Positive Touch and massage in the neonatal unit: a British approach. Seminars in neonatology : SN. 2002 Dec;7(6):477–86.

128. Feldman R, Weller A, Sirota L, Eidelman AI. Skin-to-Skin contact (Kangaroo care) promotes self-regulation in premature infants: sleep-wake cyclicity, arousal modulation, and sustained exploration. Developmental psychology. 2002 Mar;38(2):194–207.

129. Symington A, Pinelli J. Developmental care for promoting development and preventing morbidity in preterm infants. The Cochrane database of systematic reviews. 2006 Apr;(2):CD001814.

130. Ream MA, Lehwald L. Neurologic Consequences of Preterm Birth. Current Neurology and Neuroscience Reports . 2018;18(8):48. Available from: https://doi.org/10.1007/s11910-018-0862-2

131. Raja SN, Carr DB, Cohen M, Finnerup NB, Flor H, Gibson S, et al. The revised International Association for the Study of Pain definition of pain: concepts, challenges, and compromises. Pain. 2020;161(9):1976–82.

132. Slater R, Cantarella A, Gallella S, Worley A, Boyd S, Meek J, et al. Cortical Pain Responses in Human Infants. Journal of Neuroscience . 2006;26(14):3662–6. Available from: https://www.jneurosci.org/content/26/14/3662

133. Bartocci M, Bergqvist LL, Lagercrantz H, Anand KJS. Pain activates cortical areas in the preterm newborn brain. Pain. 2006 May;122(1–2):109–17.

134. Eccleston C, Fisher E, Howard RF, Slater R, Forgeron P, Palermo TM, et al. Delivering transformative action in paediatric pain: a Lancet Child & Adolescent Health Commission. The Lancet Child & Adolescent Health . 2021;5(1):47–87. Available from: https://www.sciencedirect.com/science/article/pii/S2352464220302777

135. Stevens BJ, Gibbins S, Yamada J, Dionne K, Lee G, Johnston C, et al. The premature infant pain profile-revised (PIPP-R): initial validation and feasibility. The Clinical journal of pain. 2014 Mar;30(3):238–43.

136. Mitchell AJ, Green A, Jeffs DA, Roberson PK. Physiologic effects of retinopathy of prematurity screening examinations. Advances in neonatal care : official journal of the National Association of Neonatal Nurses . 2011 Aug;11(4):291–7. Available from: https://pubmed.ncbi.nlm.nih.gov/22123352

137. Ranger M, Grunau RE. Early repetitive pain in preterm infants in relation to the developing brain. Pain Management . 2013 Dec 19;4(1):57–67. Available from: https://doi.org/10.2217/pmt.13.61

138. Ranger M, Zwicker JG, Chau CMY, Park MTM, Chakravarthy MM, Poskitt K, et al. Neonatal Pain and Infection Relate to Smaller Cerebellum in Very Preterm Children at School Age. The Journal of pediatrics. 2015 Aug;167(2):292-8.e1.

139. Ranger M, Chau CMY, Garg A, Woodward TS, Beg MF, Bjornson B, et al. Neonatal pain-related stress predicts cortical thickness at age 7 years in children born very preterm. PloS one. 2013;8(10):e76702.

140. Brummelte S, Grunau RE, Chau V, Poskitt KJ, Brant R, Vinall J, et al. Procedural pain and brain development in premature newborns. Annals of neurology. 2012 Mar;71(3):385–96.

141. Vinall J, Miller SP, Bjornson BH, Fitzpatrick KP v, Poskitt KJ, Brant R, et al. Invasive Procedures in Preterm Children: Brain and Cognitive Development at School Age. Pediatrics . 2014 Mar 1;133(3):412 LP – 421. Available from: http://pediatrics.aappublications.org/content/133/3/412.abstract

142. Grunau RE, Whitfield MF, Petrie-Thomas J, Synnes AR, Cepeda IL, Keidar A, et al. Neonatal pain, parenting stress and interaction, in relation to cognitive and motor development at 8 and 18months in preterm infants. Pain . 2009;143(1):138–46. Available from: http://www.sciencedirect.com/science/article/pii/S0304395909001316

143. Doesburg SM, Chau CM, Cheung TPL, Moiseev A, Ribary U, Herdman AT, et al. Neonatal pain-related stress, functional cortical activity and visual-perceptual abilities in school-age children born at extremely low gestational age. PAIN® . 2013;154(10):1946–52. Available from: http://www.sciencedirect.com/science/article/pii/S0304395913001644

144. Hermann C, Hohmeister J, Demirakça S, Zohsel K, Flor H. Long-term alteration of pain sensitivity in school-aged children with early pain experiences. Pain. 2006 Dec;125(3):278–85.

145. Carbajal R, Rousset A, Danan C, Coquery S, Nolent P, Ducrocq S, et al. Epidemiology and Treatment of Painful Procedures in Neonates in Intensive Care Units. JAMA . 2008 Jul 2;300(1):60–70. Available from: https://doi.org/10.1001/jama.300.1.60

146. Courtois E, Droutman S, Magny J-F, Merchaoui Z, Durrmeyer X, Roussel C, et al. Epidemiology and neonatal pain management of heelsticks in intensive care units: EPIPPAIN 2, a prospective observational study. International journal of nursing studies. 2016 Jul;59:79–88.

147. Cruz MD, Fernandes AM, Oliveira CR. Epidemiology of painful procedures performed in neonates: A systematic review of observational studies. European journal of pain (London, England). 2016 Apr;20(4):489–98.

148. Ball G, Srinivasan L, Aljabar P, Counsell SJ, Durighel G, Hajnal J v, et al. Development of cortical microstructure in the preterm human brain. Proceedings of the National Academy of Sciences . 2013 Jun 4;110(23):9541 LP – 9546. Available from: http://www.pnas.org/content/110/23/9541.abstract

149. Stevens B, McGrath P, Gibbins S, Beyene J, Breau L, Camfield C, et al. Procedural pain in newborns at risk for neurologic impairment. Pain. 2003 Sep;105(1–2):27–35.

150. Duhn LJ, Medves JM. A Systematic integrative review of infant pain assessment tools. Advances in Neonatal Care . 2004;4(3). Available from:

https://journals.lww.com/advancesinneonatalcare/Fulltext/2004/06000/A_SYSTEMATIC_INTEGRATIVE_REVIEW_OF_INFANT_PAIN.8.aspx

151. Holsti L, Grunau RE. Initial validation of the Behavioral Indicators of Infant Pain (BIIP). PAIN . 2007;132(3):264–72. Available from: http://www.sciencedirect.com/science/article/pii/S0304395907000656

152. Lawrence J, Alcock D, McGrath P, Kay J, MacMurray SB, Dulberg C. The development of a tool to assess neonatal pain. Neonatal network : NN. 1993 Sep;12(6):59–66.

153. Desai SA, Nanavati RN, Jasani BB, Kabra N. Comparison of Neonatal Pain, Agitation, and Sedation Scale with Premature Infant Pain Profile for the Assessment of Acute Prolonged Pain in Neonates on Assisted Ventilation: A Prospective Observational Study. Indian journal of palliative care . 2017;23(3):287–92. Available from: https://pubmed.ncbi.nlm.nih.gov/28827932

154. Popowicz H, Kwiecień-Jaguś K, Olszewska J, Mędrzycka-Dąbrowska WA. Pain Scales in Neonates Receiving Mechanical Ventilation in Neonatal Intensive Care Units - Systematic Review. Journal of pain research . 2020 Jul 24;13:1883–97. Available from: https://pubmed.ncbi.nlm.nih.gov/32801846

155. Mörelius E, Hellström-Westas L, Carlén C, Norman E, Nelson N. Is a nappy change stressful to neonates? Early human development. 2006 Oct;82(10):669–76.

156. Comaru T, Miura E. Postural support improves distress and pain during diaper change in preterm infants. Journal of perinatology : official journal of the California Perinatal Association. 2009 Jul;29(7):504–7.

157. Verriotis M, Fabrizi L, Lee A, Cooper RJ, Fitzgerald M, Meek J. Mapping Cortical Responses to Somatosensory Stimuli in Human Infants with Simultaneous Near-Infrared Spectroscopy and Event-Related Potential Recording. eNeuro . 2016 May 13;3(2):ENEURO.0026-16.2016. Available from: https://pubmed.ncbi.nlm.nih.gov/27200413

158. Fabrizi L, Verriotis M, Williams G, Lee A, Meek J, Olhede S, et al. Encoding of mechanical nociception differs in the adult and infant brain. Scientific Reports . 2016;6(1):28642. Available from: https://doi.org/10.1038/srep28642

159. Whitehead K, Jones L, Laudiano Dray P, Meek J, Fabrizi L. Full 10-20 EEG application in hospitalised neonates is not associated with an increase in stress hormone levels. Clinical neurophysiology practice . 2017 Dec 19;3:20–1. Available from: https://pubmed.ncbi.nlm.nih.gov/30215001

160. Karbowski K. Sixty Years of Clinical Electroencephalography. European Neurology . 1990;30(3):170–5. Available from: https://www.karger.com/DOI/10.1159/000117338

161. André M, Lamblin M-D, d'Allest AM, Curzi-Dascalova L, Moussalli-Salefranque F, Nguyen The Tich S, et al. Electroencephalography in premature and full-term infants. Developmental features and glossary. Neurophysiologie Clinique/Clinical Neurophysiology . 2010;40(2):59–124. Available from: http://www.sciencedirect.com/science/article/pii/S0987705310000092

162. Smith JR. The Electroencephalogram During Normal Infancy and Childhood: III. Preliminary Observations on the Pattern Sequence During Sleep. The Pedagogical Seminary and Journal of Genetic Psychology . 1938 Dec 1;53(2):471–82. Available from: https://doi.org/10.1080/08856559.1938.10533822

163. Smith JR. The Electroencephalogram During Normal Infancy and Childhood: I. Rhythmic Activities Present in the Neonate and Their Subsequent Development. The Pedagogical Seminary and Journal of Genetic Psychology . 1938 Dec 1;53(2):431–53. Available from: https://doi.org/10.1080/08856559.1938.10533820

164. Tharp BR. Electrophysiological Brain Maturation in Premature Infants: An Historical Perspective. Journal of Clinical Neurophysiology . 1990;7(3). Available from:

https://journals.lww.com/clinicalneurophys/Fulltext/1990/07000/Electrophysiological_Brain_Maturation_in_Premature.2.aspx

165. Gibbs FA, Gibbs EL. Atlas of electroencephalography. Atlas of electroencephalography. Oxford, England: F. A. Gibbs, Boston City Hospital; 1941. 221.

166. Lindsley DB. Heart and brain potentials of human fetuses in utero. The American Journal of Psychology. 1942;55(3):412–6.

167. Hughes JG, Brown UA. Electroencephalography of the newborn: I. Studies on Normal, Full Term, Sleeping Infants. American Journal of Diseases of Children . 1948 Nov 1;76(5):503–12. Available from: https://doi.org/10.1001/archpedi.1948.02030030516003

168. Hahn JS, Tharp BR. Neonatal and paediatric encephalography. In: Electrodiagnosis in Clinical Neurology. Elsevier London, UK; 2005.

169. Drefus-Brisac C. The electroencephalogram of the premature infant. World neurology. 1962 Jan;3:5–15.

170. Lamblin MD, André M, Challamel MJ, Curzi-Dascalova L, d'Allest AM, de Giovanni E, et al. Électroencéphalographie du nouveau-né prématuré et à terme. Aspects maturatifs et glossaire. Neurophysiologie Clinique/Clinical Neurophysiology . 1999;29(2):123–219. Available from: https://www.sciencedirect.com/science/article/pii/S0987705399800513

171. Holmes GL. Morphological and physiological maturation of the brain in the neonate and young child. Journal of clinical neurophysiology : official publication of the American Electroencephalographic Society . 1986 Jul;3(3):209—238. Available from: https://doi.org/10.1097/00004691-198607000-00002

172. Boylan GB. Neurophysiology in the neonatal period. In: Pressler RM, Binnie CD, Cooper R, Robinson R, editors. Neonatal and paediatric clinical neurophysiology. Edinburgh: Churchill Livingstone/Elsevier; 2007. p. 169–220.

173. Biagioni E, Bartalena L, Boldrini A, Cioni G, Giancola S, Ipata AE. Background EEG activity in preterm infants: correlation of outcome with selected maturational features. Electroencephalography and Clinical Neurophysiology . 1994;91(3):154–62. Available from: https://www.sciencedirect.com/science/article/pii/0013469494900655

174. Hayakawa F, Okumura A, Kato T, Kuno K, Watanabe K. Dysmature EEG pattern in EEGs of preterm infants with cognitive impairment: maturation arrest caused by prolonged mild CNS depression. Brain and Development . 1997;19(2):122–5. Available from: https://www.sciencedirect.com/science/article/pii/S0387760496004913

175. Vecchierini M-F, André M, d'Allest AM. Normal EEG of premature infants born between 24 and 30 weeks gestational age: Terminology, definitions and maturation aspects. Neurophysiologie Clinique/Clinical Neurophysiology . 2007;37(5):311–23. Available from: https://www.sciencedirect.com/science/article/pii/S098770530700158X

176. Arichi T, Whitehead K, Barone G, Pressler R, Padormo F, Edwards AD, et al. Localization of spontaneous bursting neuronal activity in the preterm human brain with simultaneous EEG-fMRI. Kastner S, editor. eLife . 2017;6:e27814. Available from: https://doi.org/10.7554/eLife.27814

177. Allievi AG, Arichi T, Tusor N, Kimpton J, Arulkumaran S, Counsell SJ, et al. Maturation of Sensori-Motor Functional Responses in the Preterm Brain. Cerebral Cortex . 2016 Jan 1;26(1):402–13. Available from: https://doi.org/10.1093/cercor/bhv203

178. Whitehead K, Pressler R, Fabrizi L. Characteristics and clinical significance of delta brushes in the EEG of premature infants. Clinical Neurophysiology Practice . 2017;2:12–8. Available from: http://www.sciencedirect.com/science/article/pii/S2467981X16300191

179. Chipaux M, Colonnese MT, Mauguen A, Fellous L, Mokhtari M, Lezcano O, et al. Auditory Stimuli Mimicking Ambient Sounds Drive Temporal

"Delta-Brushes" in Premature Infants. PLOS ONE . 2013 Nov 11;8(11):e79028. Available from: https://doi.org/10.1371/journal.pone.0079028

180. Colonnese MT, Kaminska A, Minlebaev M, Milh M, Bloem B, Lescure S, et al. A conserved switch in sensory processing prepares developing neocortex for vision. Neuron. 2010 Aug;67(3):480–98.

181. Kaminska A, Delattre V, Laschet J, Dubois J, Labidurie M, Duval A, et al. Cortical Auditory-Evoked Responses in Preterm Neonates: Revisited by Spectral and Temporal Analyses. Cerebral Cortex . 2018 Oct 1;28(10):3429–44. Available from: https://doi.org/10.1093/cercor/bhx206

182. Milh M, Kaminska A, Huon C, Lapillonne A, Ben-Ari Y, Khazipov R. Rapid Cortical Oscillations and Early Motor Activity in Premature Human Neonate. Cerebral Cortex . 2007 Jul 1;17(7):1582–94. Available from: https://doi.org/10.1093/cercor/bhl069

183. Khazipov R, Luhmann HJ. Early patterns of electrical activity in the developing cerebral cortex of humans and rodents. Trends in Neurosciences . 2006;29(7):414–8. Available from: https://www.sciencedirect.com/science/article/pii/S0166223606000956

184. Ellingson RJ. Variability of visual evoked responses in the human newborn. Electroencephalography and Clinical Neurophysiology . 1970;29(1):10–9. Available from: http://www.sciencedirect.com/science/article/pii/0013469470900751

185. Hrbek A, Mareš P. Cortical evoked responses to visual stimulation in full-term and premature newborns. Electroencephalography and Clinical Neurophysiology . 1964;16(6):575–81. Available from: http://www.sciencedirect.com/science/article/pii/0013469464900483

186. Ciganek L. The EEG response (evoked potential) to light stimulus in man. Electroencephalography and clinical neurophysiology. 1961 Apr;13:165–72.

187. Chin KC, Taylor MJ, Menzies R, Whyte H. Development of visual evoked potentials in neonates. A study using light emitting diode goggles. Archives of Disease in Childhood . 1985 Dec 1;60(12):1166 LP – 1168. Available from: http://adc.bmj.com/content/60/12/1166.abstract

188. Feng J-J, Wang W-P, Guo S-J, Liu Z-W, Xu X. Flash Visual Evoked Potentials in Preterm Infants. Ophthalmology . 2013;120(3):489–94. Available from: http://www.sciencedirect.com/science/article/pii/S0161642012008093

189. Placzek M, Mushin J, Dubowitz LMS. Maturation of the visual evoked response and its correlation with visual acuity in preterm infants. Developmental Medicine & Child Neurology . 1985 Aug 1;27(4):448–54. Available from: https://doi.org/10.1111/j.1469-8749.1985.tb04567.x

190. Taylor MJ, Menzies R, MacMillan LJ, Whyte HE. VEP's in normal full-term and premature neonates: longitudinal versus cross-sectional data. Electroencephalography and Clinical Neurophysiology/Evoked Potentials Section . 1987;68(1):20–7. Available from: http://www.sciencedirect.com/science/article/pii/0168559787900669

191. Benavente I, Tamargo P, Tajada N, Yuste V, Oliván MJ. Flash Visually Evoked Potentials in the Newborn and their Maturation During the First Six Months of Life. Documenta Ophthalmologica . 2005;110(2):255–63. Available from: https://doi.org/10.1007/s10633-005-0818-0

192. Hensch TK. Critical period regulation. Annual Review of Neuroscience . 2004 Jun 24;27(1):549–79. Available from: https://doi.org/10.1146/annurev.neuro.27.070203.144327

193. Kræmer M, Abrahamsson M, Sjöström A. The neonatal development of the light flash visual evoked potential. Documenta Ophthalmologica . 1999;99(1):21–39. Available from: https://doi.org/10.1023/A:1002414803226

194. Suppiej A. General characteristics of evoked potentials. In: Pressler R, Binnie CD, Cooper R, Robinson R, editors. Neonatal and paediatric clinical neurophysiology. Edinburgh: Churchill Livingstone Elsevier; 2007. p. 111–50.

195. Cavalcanti HG, da Silva Nunes AD, da Cunha BKS, de Freitas Alvarenga K, Balen SA, Pereira A. Early exposure to environment sounds and the development of cortical auditory evoked potentials of preterm infants during the first 3 months of life. BMC Research Notes . 2020;13(1):303. Available from: https://doi.org/10.1186/s13104-020-05129-8

196. Romero ACL, Frizzo ACF, Chagas EFB, Isaac M de L. Cortical auditory evoked potential in babies and children listeners. Brazilian Journal of Otorhinolaryngology . 2020;86(4):395–404. Available from: https://www.sciencedirect.com/science/article/pii/S1808869418304798

197. Wunderlich JL, Cone-Wesson BK, Shepherd R. Maturation of the cortical auditory evoked potential in infants and young children. Hearing research. 2006 Feb;212(1–2):185–202.

198. Donadio A, Whitehead K, Gonzalez F, Wilhelm E, Formica D, Meek J, et al. A novel sensor design for accurate measurement of facial somatosensation in pre-term infants. PLOS ONE . 2018 Nov 16;13(11):e0207145. Available from: https://doi.org/10.1371/journal.pone.0207145

199. Whitehead K, Papadelis C, Laudiano-Dray MP, Meek J, Fabrizi L. The Emergence of Hierarchical Somatosensory Processing in Late Prematurity. Cerebral Cortex . 2019 May 1;29(5):2245–60. Available from: https://doi.org/10.1093/cercor/bhz030

200. Spreng M, Ichioka M. Slow cortical potentials following pain stimulation in man. Pflugers Archiv fur die gesamte Physiologie des Menschen und der Tiere. 1964 Apr;279:121–32.

201. Bromm B, Scharein E. Principal component analysis of pain-related cerebral potentials to mechanical and electrical stimulation in man. Electroencephalography and Clinical Neurophysiology . 1982;53(1):94–103. Available from: https://www.sciencedirect.com/science/article/pii/0013469482901092

202. Naka D, Kakigi R. Simple and Novel Method for Measuring Conduction Velocity of Aδ Fibers in Humans. Journal of Clinical Neurophysiology . 1998;15(2). Available from: https://journals.lww.com/clinicalneurophys/Fulltext/1998/03000/Simple_and_Novel_Method_for_Measuring_Conduction.8.aspx

203. Chao C-C, Hsieh S-C, Tseng M-T, Chang Y-C, Hsieh S-T. Patterns of contact heat evoked potentials (CHEP) in neuropathy with skin denervation: Correlation of CHEP amplitude with intraepidermal nerve fiber density. Clinical Neurophysiology . 2008;119(3):653–61. Available from: https://www.sciencedirect.com/science/article/pii/S1388245707006992

204. Chen ACN, Niddam DM, Arendt-Nielsen L. Contact heat evoked potentials as a valid means to study nociceptive pathways in human subjects. Neuroscience Letters . 2001;316(2):79–82. Available from: https://www.sciencedirect.com/science/article/pii/S0304394001023746

205. Chen ACN, Arendt-Nielsen L, Plaghki L. Laser-evoked potentials in human pain: I. Use and possible misuse. Pain Forum . 1998;7(4):174–84. Available from: https://www.sciencedirect.com/science/article/pii/S1082317498700017

206. Fabrizi L, Williams G, Lee A, Meek J, Slater R, Olhede S, et al. Cortical activity evoked by an acute painful tissue-damaging stimulus in healthy adult volunteers. Journal of Neurophysiology . 2013 Feb 20;109(9):2393–403. Available from: https://doi.org/10.1152/jn.00990.2012

207. Pressler, R.M., Cilio, M.R., Mizrahi, E.M., Moshé, S.L., Nunes, M.L., Plouin, P., Vanhatalo, S., Yozawitz, E., de Vries, L.S., Puthenveettil Vinayan, K., Triki, C.C., Wilmshurst, J.M., Yamamoto, H. and Zuberi, S.M. (2021), The ILAE classification of seizures and the epilepsies: Modification for seizures in the neonate. Position paper by the ILAE Task Force on Neonatal Seizures. Epilepsia, 62: 615-628. https://doi.org/10.1111/epi.16815

208. Slater R, Worley A, Fabrizi L, Roberts S, Meek J, Boyd S, et al. Evoked potentials generated by noxious stimulation in the human infant brain. European journal of pain (London, England). 2010 Mar;14(3):321–6.

209. Fabrizi L, Slater R, Worley A, Meek J, Boyd S, Olhede S, et al. A Shift in Sensory Processing that Enables the Developing Human Brain to Discriminate Touch from Pain. Current Biology . 2011;21(18):1552–8. Available from: http://www.sciencedirect.com/science/article/pii/S0960982211008852

210. Jones L, Laudiano-Dray MP, Whitehead K, Verriotis M, Meek J, Fitzgerald M, et al. EEG, behavioural and physiological recordings following a painful procedure in human neonates. Scientific Data . 2018;5(1):180248. Available from: https://doi.org/10.1038/sdata.2018.248

211. Green G, Hartley C, Hoskin A, Duff E, Shriver A, Wilkinson D, et al. Behavioural discrimination of noxious stimuli in infants is dependent on brain maturation. Pain . 2019 Feb;160(2):493–500. Available from: https://pubmed.ncbi.nlm.nih.gov/30422872

212. Worley A, Fabrizi L, Boyd S, Slater R. Multi-modal pain measurements in infants. Journal of neuroscience methods . 2012/01/24. 2012 Apr 15;205(2):252–7. Available from: https://pubmed.ncbi.nlm.nih.gov/22285660

213. Verriotis M, Jones L, Whitehead K, Laudiano-Dray M, Panayotidis I, Patel H, et al. The distribution of pain activity across the human neonatal brain is sex dependent. NeuroImage . 2018;178:69–77. Available from: https://www.sciencedirect.com/science/article/pii/S1053811918304373

214. Magerl W, Krumova EK, Baron R, Tölle T, Treede R-D, Maier C. Reference data for quantitative sensory testing (QST): refined stratification for age and a novel method for statistical comparison of group data. Pain. 2010 Dec;151(3):598–605.

215. Suzuki K, Baad-Hansen L, Pigg M, Svensson P. Assessment of Mechanical Pain Thresholds in the Orofacial Region: A Comparison Between

Pinprick Stimulators and Electronic Von Frey Device. Journal of oral & facial pain and headache. 2016;30(4):338–45.

216. Hartley C, Goksan S, Poorun R, Brotherhood K, Mellado GS, Moultrie F, et al. The relationship between nociceptive brain activity, spinal reflex withdrawal and behaviour in newborn infants. Scientific Reports . 2015;5(1):12519. Available from: https://doi.org/10.1038/srep12519

217. Hartley C, Moultrie F, Hoskin A, Green G, Monk V, Bell JL, et al. Analgesic efficacy and safety of morphine in the Procedural Pain in Premature Infants (Poppi) study: randomised placebo-controlled trial. The Lancet . 2018;392(10164):2595–605. Available from: https://www.sciencedirect.com/science/article/pii/S0140673618318130

218. Scalais E, François-Adant A, Nuttin C, Bachy A, Guérit JM. Multimodality evoked potentials as a prognostic tool in term asphyxiated newborns. Electroencephalography and Clinical Neurophysiology/Evoked Potentials Section . 1998;108(2):199–207. Available from: http://www.sciencedirect.com/science/article/pii/S0168559797000762

219. Vanhatalo S, Kaila K. Development of neonatal EEG activity: From phenomenology to physiology. Seminars in Fetal and Neonatal Medicine . 2006;11(6):471–8. Available from: http://www.sciencedirect.com/science/article/pii/S1744165X0600076X

220. Vanhatalo S, Lauronen L. Neonatal SEP – Back to bedside with basic science. Seminars in Fetal and Neonatal Medicine . 2006;11(6):464–70. Available from: http://www.sciencedirect.com/science/article/pii/S1744165X06000758

221. Vanhatalo S, Voipio J, Kaila K. Full-Band EEG (FbEEG): A New Standard for Clinical Electroencephalography. Clinical EEG and Neuroscience . 2005 Oct 1;36(4):311–7. Available from: https://doi.org/10.1177/155005940503600411

222. Grieve PG, Emerson RG, Fifer WP, Isler JR, Stark RI. Spatial correlation of the infant and adult electroencephalogram. Clinical Neurophysiology .

2003;114(9):1594–608. Available from:
https://www.sciencedirect.com/science/article/pii/S1388245703001226

223. Grieve PG, Emerson RG, Isler JR, Stark RI. Quantitative analysis of spatial sampling error in the infant and adult electroencephalogram. NeuroImage . 2004;21(4):1260–74. Available from:
https://www.sciencedirect.com/science/article/pii/S1053811903007705

224. George SR, Taylor MJ. Somatosensory evoked potentials in neonates and infants: developmental and normative data. Electroencephalography and Clinical Neurophysiology/Evoked Potentials Section . 1991;80(2):94–102. Available from:
https://www.sciencedirect.com/science/article/pii/016855979190146O

225. Taylor MJ, Boor R, Ekert PG. Preterm maturation of the somatosensory evoked potential. Electroencephalography and Clinical Neurophysiology/Evoked Potentials Section . 1996;100(5):448–52. Available from: http://www.sciencedirect.com/science/article/pii/0168559796952181

226. Pike AA, Marlow N, Dawson C. Posterior tibial somatosensory evoked potentials in very preterm infants. Early Human Development . 1997;47(1):71–84. Available from:
http://www.sciencedirect.com/science/article/pii/S0378378296017744

227. Isler JR, Grose-Fifer J, Fifer WP, Housman S, Stark RI, Grieve PG. Frequency Domain Analyses of Neonatal Flash VEP. Pediatric Research . 2007;62(5):581–5. Available from:
https://doi.org/10.1203/PDR.0b013e31815586a1

228. Leikos S, Tokariev A, Koolen N, Nevalainen P, Vanhatalo S. Cortical responses to tactile stimuli in preterm infants. European Journal of Neuroscience . 2020 Feb 1;51(4):1059–73. Available from:
https://doi.org/10.1111/ejn.14613

229. Maris E, Oostenveld R. Nonparametric statistical testing of EEG- and MEG-data. Journal of neuroscience methods. 2007 Aug;164(1):177–90.

230. Kayser J, Tenke CE. Optimizing PCA methodology for ERP component identification and measurement: theoretical rationale and empirical evaluation. Clinical Neurophysiology . 2003;114(12):2307–25. Available from: https://www.sciencedirect.com/science/article/pii/S1388245703002414

231. Harner RN. Singular Value Decomposition—A general linear model for analysis of multivariate structure in the electroencephalogram. Brain Topography . 1990;3(1):43–7. Available from: https://doi.org/10.1007/BF01128860

232. van Boxtel GJM. Computational and statistical methods for analyzing event-related potential data. Behavior Research Methods, Instruments, & Computers . 1998;30(1):87–102. Available from: https://doi.org/10.3758/BF03209419

233. Huang C-M, Lee S-H, Hsiao I-T, Kuan W-C, Wai Y-Y, Ko H-J, et al. Study-specific EPI template improves group analysis in functional MRI of young and older adults. Journal of neuroscience methods. 2010 Jun;189(2):257–66.

234. Slater R, Cornelissen L, Fabrizi L, Patten D, Yoxen J, Worley A, et al. Oral sucrose as an analgesic drug for procedural pain in newborn infants: a randomised controlled trial. The Lancet . 2010;376(9748):1225–32. Available from: http://www.sciencedirect.com/science/article/pii/S0140673610613037

235. Donchin E, Heffley E. Multivariate analysis of event-related potential data: A tutorial review. In: D Otto (Ed) Multidisciplinary perspectives in Event-Related brain potential research. Washington D.C.: U.S. Goverment Printing Office; 1978. p. 555–72.

236. Donchin E. A multivariate approach to the analysis of average evoked potentials. IEEE transactions on bio-medical engineering. 1966 Jul;13(3):131–9.

237. Woody CD. Characterization of an adaptive filter for the analysis of variable latency neuroelectric signals. Medical and biological engineering . 1967;5(6):539–54. Available from: https://doi.org/10.1007/BF02474247

238. Liu L, Oza S, Hogan D, Chu Y, Perin J, Zhu J, et al. Global, regional, and national causes of under-5 mortality in 2000–15: an updated systematic analysis with implications for the Sustainable Development Goals. The Lancet . 2016;388(10063):3027–35. Available from: https://www.sciencedirect.com/science/article/pii/S0140673616315938

239. Smith GC, Gutovich J, Smyser C, Pineda R, Newnham C, Tjoeng TH, et al. Neonatal intensive care unit stress is associated with brain development in preterm infants. Annals of neurology. 2011 Oct;70(4):541–9.

240. Leaf AA, Green CR, Escwk A, Costeloe KL, Prior PF. Maturation of electroretinograms and visual evoked potentials in preterm infants. Developmental Medicine & Child Neurology . 1995 Sep 1;37(9):814–26. Available from: https://doi.org/10.1111/j.1469-8749.1995.tb12065.x

241. Umezaki H, Morrell F. Developmental study of photic evoked responses in premature infants. Electroencephalography and Clinical Neurophysiology . 1970;28(1):55–63. Available from: http://www.sciencedirect.com/science/article/pii/0013469470900088

242. Pike AA, Marlow N, Reber C. Maturation of the flash visual evoked potential in preterm infants. Early Human Development . 1999;54(3):215–22. Available from: http://www.sciencedirect.com/science/article/pii/S0378378298000929

243. Tsuneishi S, Casaer P, Fock JM, Hirano S. Establishment of normal values for flash visual evoked potentials (VEPs) in preterm infants: a longitudinal study with special reference to two components of the N1 wave. Electroencephalography and Clinical Neurophysiology/Evoked Potentials Section . 1995;96(4):291–9. Available from: http://www.sciencedirect.com/science/article/pii/016855979500031M

244. Tsuneishi S, Casaer P. Stepwise decrease in VEP latencies and the process of myelination in the human visual pathway. Brain and Development . 1997;19(8):547–51. Available from: http://www.sciencedirect.com/science/article/pii/S0387760497000764

245. Moore CL, Kalil R, Richards W. Development of myelination in optic tract of the cat. Journal of Comparative Neurology . 1976 Jan 15;165(2):125–36. Available from: https://doi.org/10.1002/cne.901650202

246. Desmedt JE, Manil J. Somatosensory evoked potentials of the normal human neonate in REM sleep, in slow wave sleep and in waking. Electroencephalography and Clinical Neurophysiology . 1970;29(2):113–26. Available from: http://www.sciencedirect.com/science/article/pii/0013469470901148

247. Desmedt JE, Brunko E, Debecker J. Maturation of the somatosensory evoked potentials in normal infants and children, with special reference to the early N1 component. Electroencephalography and Clinical Neurophysiology . 1976;40(1):43–58. Available from: http://www.sciencedirect.com/science/article/pii/0013469476901784

248. Taylor MJ, Fagan ER. SEPs to median nerve stimulation: normative data for paediatrics. Electroencephalography and clinical neurophysiology. 1988;71(5):323–30.

249. Poduri A, Volpe JJ. Chapter 5 - Neuronal Proliferation. In: Volpe JJ, Inder TE, Darras BT, de Vries LS, du Plessis AJ, Neil JJ, et al., editors. Elsevier; 2018. p. 100-119.e6. Available from: http://www.sciencedirect.com/science/article/pii/B9780323428767000053

250. Gilles FH, Shankle W, Dooling EC. CHAPTER 12 - Myelinated tracts: Growth patterns. In: GILLES FH, LEVITON A, DOOLING ECBT-TDHB, editors. Butterworth-Heinemann; 1983. p. 117–83. Available from: https://www.sciencedirect.com/science/article/pii/B9780723670179500181

251. Gilles FH. Myelination in the neonatal brain. Human Pathology . 1976;7(3):244–8. Available from: https://www.sciencedirect.com/science/article/pii/S0046817776800354

252. Yakovlev P, Lecours AR. The myelogenetic cycles of regional maturation of the brain. In 1967.

253. Karniski W, Wyble L, Lease L, Blair RC. The late somatosensory evoked potential in premature and term infants. II. Topography and latency development. Electroencephalography and Clinical Neurophysiology/Evoked Potentials Section . 1992;84(1):44–54. Available from: http://www.sciencedirect.com/science/article/pii/016855979290067L

254. Pihko E, Lauronen L. Somatosensory processing in healthy newborns. Experimental Neurology . 2004;190:2–7. Available from: http://www.sciencedirect.com/science/article/pii/S0014488604000494

255. Tombini M, Pasqualetti P, Rizzo C, Zappasodi F, Dinatale A, Seminara M, et al. Extrauterine maturation of somatosensory pathways in preterm infants: a somatosensory evoked potential study. Clinical neurophysiology : official journal of the International Federation of Clinical Neurophysiology. 2009 Apr;120(4):783–9.

256. Rahkonen P, Nevalainen P, Lauronen L, Pihko E, Lano A, Vanhatalo S, et al. Cortical somatosensory processing measured by magnetoencephalography predicts neurodevelopment in extremely low-gestational-age infants. Pediatric Research . 2013;73(6):763–71. Available from: https://doi.org/10.1038/pr.2013.46

257. Nevalainen P, Rahkonen P, Pihko E, Lano A, Vanhatalo S, Andersson S, et al. Evaluation of somatosensory cortical processing in extremely preterm infants at term with MEG and EEG. Clinical Neurophysiology . 2015;126(2):275–83. Available from: http://www.sciencedirect.com/science/article/pii/S1388245714003216

258. Saby JN, Meltzoff AN, Marshall PJ. Beyond the N1: A review of late somatosensory evoked responses in human infants. International Journal of Psychophysiology . 2016;110:146–52. Available from: http://www.sciencedirect.com/science/article/pii/S0167876016306833

259. Martakis K, Hünseler C, Herkenrath P, Thangavelu K, Kribs A, Roth B. The flexion withdrawal reflex increases in premature infants at 22–26 weeks of

gestation due to changes in spinal cord excitability. Acta Paediatrica . 2017 Jul 1;106(7):1079–84. Available from: https://doi.org/10.1111/apa.13854

260. Ednick M, Cohen AP, McPhail GL, Beebe D, Simakajornboon N, Amin RS. A review of the effects of sleep during the first year of life on cognitive, psychomotor, and temperament development. Sleep . 2009 Nov;32(11):1449–58. Available from: https://pubmed.ncbi.nlm.nih.gov/19928384

261. Gilmore R, Brock J, Hermansen MC, Baumann R. Development of lumbar spinal cord and cortical evoked potentials after tibial nerve stimulation in the pre-term newborns: effects of gestational age and other factors. Electroencephalography and Clinical Neurophysiology/Evoked Potentials Section . 1987;68(1):28–39. Available from: http://www.sciencedirect.com/science/article/pii/0168559787900670

262. Tranier S, Bougle D, Pottier M, Venezia R. Maturation of peripheral nerves in preterm infants: proprioceptive and motor nerve conductions of tibial nerve. Brain & development. 1989;11(4):215–20.

263. Slater R, Fabrizi L, Worley A, Meek J, Boyd S, Fitzgerald M. Premature infants display increased noxious-evoked neuronal activity in the brain compared to healthy age-matched term-born infants. NeuroImage . 2010;52(2):583–9. Available from: http://www.sciencedirect.com/science/article/pii/S1053811910006658

264. Shepherd AJ, Saunders KJ, McCulloch DL, Dutton GN. Prognostic value of flash visual evoked potentials in preterm infants. Developmental medicine and child neurology. 1999 Jan;41(1):9–15.

265. Penfield W, Rasmussen T. The cerebral cortex of man; a clinical study of localization of function. The cerebral cortex of man; a clinical study of localization of function. Oxford, England: Macmillan; 1950. xv, 248–xv, 248.

266. Minami T, Gondo K, Nakayama H, Ueda K. Cortical somatosensory evoked potentials to posterior tibial nerve stimulation in newborn infants. Brain

and Development . 1996;18(4):294–8. Available from: http://www.sciencedirect.com/science/article/pii/0387760496000277

267. Keysers C, Kaas JH, Gazzola V. Somatosensation in social perception. Nature Reviews Neuroscience . 2010;11(6):417–28. Available from: https://doi.org/10.1038/nrn2833

268. Seggie J, Berry M. Ontogeny of interhemispheric evoked potentials in the rat: Significance of myelination of the corpus callosum. Experimental Neurology . 1972;35(2):215–32. Available from: http://www.sciencedirect.com/science/article/pii/0014488672901483

269. Vierck CJ, Whitsel BL, Favorov O v, Brown AW, Tommerdahl M. Role of primary somatosensory cortex in the coding of pain. Pain. 2013 Mar;154(3):334–44.

270. Iannetti GD, Baumgärtner U, Tracey I, Treede RD, Magerl W. Pinprick-evoked brain potentials: a novel tool to assess central sensitization of nociceptive pathways in humans. Journal of Neurophysiology . 2013 May 15;110(5):1107–16. Available from: https://doi.org/10.1152/jn.00774.2012

271. Schwindt E, Giordano V, Rona Z, Czaba-Hnizdo C, Olischar M, Waldhoer T, et al. The impact of extrauterine life on visual maturation in extremely preterm born infants. Pediatric Research . 2018;84(3):403–10. Available from: https://doi.org/10.1038/s41390-018-0084-y

272. Jandó G, Mikó-Baráth E, Markó K, Hollódy K, Török B, Kovacs I. Early-onset binocularity in preterm infants reveals experience-dependent visual development in humans. Proceedings of the National Academy of Sciences . 2012 Jul 3;109(27):11049 LP – 11052. Available from: http://www.pnas.org/content/109/27/11049.abstract

273. Romeo DM, Ricci D, Serrao F, Gallini F, Olivieri G, Cota F, et al. Visual function assessment in late-preterm newborns. Early Human Development . 2012;88(5):301–5. Available from: https://www.sciencedirect.com/science/article/pii/S0378378211002945

274. Weinacht S, Kind C, Mönting JS, Gottlob I. Visual development in preterm and full-term infants: a prospective masked study. Investigative Ophthalmology & Visual Science. 1999 Feb 1;40(2):346–53.

275. Bourgeois JP, Jastreboff PJ, Rakic P. Synaptogenesis in visual cortex of normal and preterm monkeys: evidence for intrinsic regulation of synaptic overproduction. Proceedings of the National Academy of Sciences . 1989 Jun 1;86(11):4297 LP – 4301. Available from: http://www.pnas.org/content/86/11/4297.abstract

276. Smyser CD, Inder TE, Shimony JS, Hill JE, Degnan AJ, Snyder AZ, et al. Longitudinal Analysis of Neural Network Development in Preterm Infants. Cerebral Cortex . 2010 Dec 1;20(12):2852–62. Available from: https://doi.org/10.1093/cercor/bhq035

277. Grunau RE. Neonatal pain in very preterm infants: long-term effects on brain, neurodevelopment and pain reactivity. Rambam Maimonides medical journal . 2013 Oct 29;4(4):e0025–e0025. Available from: https://pubmed.ncbi.nlm.nih.gov/24228168

278. Zwicker JG, Grunau RE, Adams E, Chau V, Brant R, Poskitt KJ, et al. Score for neonatal acute physiology-II and neonatal pain predict corticospinal tract development in premature newborns. Pediatric neurology. 2013 Feb;48(2):123-129.e1.

279. Boardman JP, Counsell SJ, Rueckert D, Hajnal J v, Bhatia KK, Srinivasan L, et al. Early growth in brain volume is preserved in the majority of preterm infants. Annals of Neurology . 2007 Aug 1;62(2):185–92. Available from: https://doi.org/10.1002/ana.21171

280. Bonifacio SL, Glass HC, Chau V, Berman JI, Xu D, Brant R, et al. Extreme Premature Birth is not Associated with Impaired Development of Brain Microstructure. The Journal of Pediatrics . 2010;157(5):726-732.e1. Available from: http://www.sciencedirect.com/science/article/pii/S0022347610004324

281. Als H, Duffy FH, McAnulty GB. Effectiveness of individualized neurodevelopmental care in the newborn intensive care unit (NICU). Acta Paediatrica . 1996 Oct 1;85(s416):21–30. Available from: https://doi.org/10.1111/j.1651-2227.1996.tb14273.x

282. Westrup B. Newborn Individualized Developmental Care and Assessment Program (NIDCAP). NeoReviews . 2005 Mar 1;6(3):e115 LP-e122. Available from: http://neoreviews.aappublications.org/content/6/3/e115.abstract

283. Hane AA, Myers MM, Hofer MA, Ludwig RJ, Halperin MS, Austin J, et al. Family nurture intervention improves the quality of maternal caregiving in the neonatal intensive care unit: evidence from a randomized controlled trial. Journal of developmental and behavioral pediatrics : JDBP. 2015 Apr;36(3):188–96.

284. Milgrom J, Newnham C, Anderson PJ, Doyle LW, Gemmill AW, Lee K, et al. Early sensitivity training for parents of preterm infants: impact on the developing brain. Pediatric research. 2010 Mar;67(3):330–5.

285. Pineda R, Guth R, Herring A, Reynolds L, Oberle S, Smith J. Enhancing sensory experiences for very preterm infants in the NICU: an integrative review. Journal of perinatology : official journal of the California Perinatal Association . 2016/10/20. 2017 Apr;37(4):323–32. Available from: https://pubmed.ncbi.nlm.nih.gov/27763631

286. Vandenberg KA. State systems development in high-risk newborns in the neonatal intensive care unit: identification and management of sleep, alertness, and crying. The Journal of perinatal & neonatal nursing. 2007;21(2):130–9.

287. Nordhov SM, Rønning JA, Dahl LB, Ulvund SE, Tunby J, Kaaresen PI. Early intervention improves cognitive outcomes for preterm infants: randomized controlled trial. Pediatrics. 2010 Nov;126(5):e1088-94.

288. Burke S. Systematic review of developmental care interventions in the neonatal intensive care unit since 2006. Journal of Child Health Care . 2018 Jan 12;22(2):269–86. Available from: https://doi.org/10.1177/1367493517753085

289. Montirosso R, del Prete A, Bellù R, Tronick E, Borgatti R. Level of NICU quality of developmental care and neurobehavioral performance in very preterm infants. Pediatrics. 2012 May;129(5):e1129-37.

290. Ortenstrand A, Westrup B, Broström EB, Sarman I, Akerström S, Brune T, et al. The Stockholm Neonatal Family Centered Care Study: effects on length of stay and infant morbidity. Pediatrics. 2010 Feb;125(2):e278-85.

291. Westrup B, Kleberg A, von Eichwald K, Stjernqvist K, Lagercrantz H. A Randomized, Controlled Trial to Evaluate the Effects of the Newborn Individualized Developmental Care and Assessment Program in a Swedish Setting. Pediatrics . 2000 Jan 1;105(1):66 LP – 72. Available from: http://pediatrics.aappublications.org/content/105/1/66.abstract

292. Volpe JJ. Dysmaturation of Premature Brain: Importance, Cellular Mechanisms, and Potential Interventions. Pediatric neurology. 2019 Jun;95:42–66.

293. Peterson BS, Vohr B, Staib LH, Cannistraci CJ, Dolberg A, Schneider KC, et al. Regional Brain Volume Abnormalities and Long-term Cognitive Outcome in Preterm Infants. JAMA . 2000 Oct 18;284(15):1939–47. Available from: https://doi.org/10.1001/jama.284.15.1939

294. Molloy EJ, Bearer C. Neonatal encephalopathy versus Hypoxic-Ischemic Encephalopathy. Pediatric Research . 2018;84(5):574. Available from: https://doi.org/10.1038/s41390-018-0169-7

295. The American College of Obstetricians and Gynecologists. Neonatal Encephalopathy and Neurologic Outcome (second edition). In 2019. Available from: https://www.acog.org/-/media/project/acog/acogorg/clinical/files/task-force-report/articles/2014/neonatal-encephalopathy-and-neurologic-outcome.pdf

296. Gale C, Statnikov Y, Jawad S, Uthaya SN, Modi N. Neonatal brain injuries in England: population-based incidence derived from routinely recorded clinical data held in the National Neonatal Research Database. Archives of Disease in Childhood - Fetal and Neonatal Edition . 2018 Jul 1;103(4):F301 LP-F306. Available from: http://fn.bmj.com/content/103/4/F301.abstract

297. Rice JE 3rd, Vannucci RC, Brierley JB. The influence of immaturity on hypoxic-ischemic brain damage in the rat. Annals of neurology. 1981 Feb;9(2):131–41.

298. Vannucci RC, Connor JR, Mauger DT, Palmer C, Smith MB, Towfighi J, et al. Rat model of perinatal hypoxic-ischemic brain damage. Journal of Neuroscience Research . 1999 Jan 15;55(2):158–63. Available from: https://doi.org/10.1002/(SICI)1097-4547(19990115)55:2%3C158::AID-JNR3%3E3.0.CO

299. Yager JY. Animal models of hypoxic-ischemic brain damage in the newborn. Seminars in Pediatric Neurology . 2004;11(1):31–46. Available from: http://www.sciencedirect.com/science/article/pii/S1071909104000075

300. Myers RE. Two patterns of perinatal brain damage and their conditions of occurrence. American Journal of Obstetrics and Gynecology . 1972;112(2):246–76. Available from: http://www.sciencedirect.com/science/article/pii/000293787290124X

301. de Haan HH, Gunn AJ, Williams CE, Gluckman PD. Brief Repeated Umbilical Cord Occlusions Cause Sustained Cytotoxic Cerebral Edema and Focal Infarcts in Near-Term Fetal Lambs. Pediatric Research . 1997;41(1):96–104. Available from: https://doi.org/10.1203/00006450-199701000-00015

302. Groenendaal F, Mishra OP, McGowan JE, Hoffman DJ, Delivoria-Papadopoulos M. Function of cell membranes in cerebral cortical tissue of newborn piglets after hypoxia and inhibition of nitric oxide synthase. Pediatric research. 1997 Aug;42(2):174–9.

303. Gunn AJ, Bennet L, Gunning MI, Gluckman PD, Gunn TR. Cerebral hypothermia is not neuroprotective when started after postischemic seizures in fetal sheep. Pediatric research. 1999 Sep;46(3):274–80.

304. Penrice J, Lorek A, Cady EB, Amess PN, Wylezinska M, Cooper CE, et al. Proton Magnetic Resonance Spectroscopy of the Brain during Acute Hypoxia-Ischemia and Delayed Cerebral Energy Failure in the Newborn Piglet. Pediatric Research . 1997;41(6):795–802. Available from: https://doi.org/10.1203/00006450-199706000-00001

305. Lorek A, Takei Y, Cady EB, Wyatt JS, Penrice J, Edwards AD, et al. Delayed ("secondary") cerebral energy failure after acute hypoxia-ischemia in the newborn piglet: continuous 48-hour studies by phosphorus magnetic resonance spectroscopy. Pediatric research. 1994 Dec;36(6):699–706.

306. de Vries LS, Groenendaal F. Patterns of neonatal hypoxic-ischaemic brain injury. Neuroradiology . 2010 Jun;52(6):555–66. Available from: https://pubmed.ncbi.nlm.nih.gov/20390260

307. Vannucci RC, Perlman JM. Interventions for perinatal hypoxic-ischemic encephalopathy. Pediatrics. 1997 Dec;100(6):1004–14.

308. Shankaran S. Neonatal encephalopathy: treatment with hypothermia. Journal of neurotrauma. 2009 Mar;26(3):437–43.

309. Higgins RD, Raju TNK, Perlman J, Azzopardi DV, Blackmon LR, Clark RH, et al. Hypothermia and perinatal asphyxia: executive summary of the National Institute of Child Health and Human Development workshop. The Journal of pediatrics. 2006 Feb;148(2):170–5.

310. Fleiss B, Gressens P. Tertiary mechanisms of brain damage: a new hope for treatment of cerebral palsy? The Lancet Neurology. 2012 Jun;11(6):556–66.

311. Gunn AJ, Gunn TR, de Haan HH, Williams CE, Gluckman PD. Dramatic neuronal rescue with prolonged selective head cooling after ischemia in fetal lambs. The Journal of clinical investigation. 1997 Jan;99(2):248–56.

312. Thoresen M, Penrice J, Lorek A, Cady EB, Wylezinska M, Kirkbride V, et al. Mild Hypothermia after Severe Transient Hypoxia-Ischemia Ameliorates Delayed Cerebral Energy Failure in the Newborn Piglet. Pediatric Research . 1995;37(5):667–70. Available from: https://doi.org/10.1203/00006450-199505000-00019

313. Bona E, Hagberg H, Løberg EM, Bågenholm R, Thoresen M. Protective Effects of Moderate Hypothermia after Neonatal Hypoxia-Ischemia: Short- and Long-Term Outcome. Pediatric Research . 1998;43(6):738–45. Available from: https://doi.org/10.1203/00006450-199806000-00005

314. Alonso-Alconada D, Broad KD, Bainbridge A, Chandrasekaran M, Faulkner SD, Kerenyi Á, et al. Brain cell death is reduced with cooling by 3.5°C to 5°C but increased with cooling by 8.5°C in a piglet asphyxia model. Stroke . 2015 Jan [cited 2020 Nov 29];46(1):275–8. Available from: https://www.ahajournals.org/doi/10.1161/STROKEAHA.114.007330

315. Kerenyi A, Kelen D, Faulkner SD, Bainbridge A, Chandrasekaran M, Cady EB, et al. Systemic effects of whole-body cooling to 35 °C, 33.5 °C, and 30 °C in a piglet model of perinatal asphyxia: implications for therapeutic hypothermia. Pediatric research. 2012 May;71(5):573–82.

316. Shankaran S, Laptook AR, Ehrenkranz RA, Tyson JE, McDonald SA, Donovan EF, et al. Whole-body hypothermia for neonates with hypoxic-ischemic encephalopathy. The New England journal of medicine. 2005 Oct;353(15):1574–84.

317. Azzopardi D, Robertson NJ, Cowan FM, Rutherford MA, Rampling M, Edwards AD. Pilot study of treatment with whole body hypothermia for neonatal encephalopathy. Pediatrics. 2000 Oct;106(4):684–94.

318. Jacobs SE, Berg M, Hunt R, Tarnow-Mordi WO, Inder TE, Davis PG. Cooling for newborns with hypoxic ischaemic encephalopathy. The Cochrane database of systematic reviews. 2013 Jan;2013(1):CD003311.

319. Azzopardi D, Brocklehurst P, Edwards D, Halliday H, Levene M, Thoresen M, et al. The TOBY Study. Whole body hypothermia for the treatment of perinatal asphyxial encephalopathy: a randomised controlled trial. BMC pediatrics. 2008 Apr;8:17.

320. Campbell H, Eddama O, Azzopardi D, Edwards AD, Strohm B, Rivero-Arias O. Hypothermia for perinatal asphyxia: trial-based quality of life at 6–7 years. Archives of Disease in Childhood . 2018 Jul 1;103(7):654 LP – 659. Available from: http://adc.bmj.com/content/103/7/654.abstract

321. Edwards AD, Brocklehurst P, Gunn AJ, Halliday H, Juszczak E, Levene M, et al. Neurological outcomes at 18 months of age after moderate hypothermia for perinatal hypoxic ischaemic encephalopathy: synthesis and meta-analysis of trial data. BMJ (Clinical research ed). 2010 Feb;340:c363.

322. Shankaran S, Pappas A, McDonald SA, Vohr BR, Hintz SR, Yolton K, et al. Childhood outcomes after hypothermia for neonatal encephalopathy. The New England journal of medicine. 2012 May;366(22):2085–92.

323. Conway JM, Walsh BH, Boylan GB, Murray DM. Mild hypoxic ischaemic encephalopathy and long term neurodevelopmental outcome - A systematic review. Early human development. 2018 May;120:80–7.

324. Kariholu U, Montaldo P, Markati T, Lally PJ, Pryce R, Teiserskas J, et al. Therapeutic hypothermia for mild neonatal encephalopathy: a systematic review and meta-analysis. Archives of disease in childhood Fetal and neonatal edition. 2020 Mar;105(2):225–8.

325. El-Dib M, Inder TE, Chalak LF, Massaro AN, Thoresen M, Gunn AJ. Should therapeutic hypothermia be offered to babies with mild neonatal encephalopathy in the first 6 h after birth? Pediatric research. 2019 Mar;85(4):442–8.

326. Gagne-Loranger M, Sheppard M, Ali N, Saint-Martin C, Wintermark P. Newborns Referred for Therapeutic Hypothermia: Association between Initial Degree of Encephalopathy and Severity of Brain Injury (What About the

Newborns with Mild Encephalopathy on Admission?). American journal of perinatology. 2016 Jan;33(2):195–202.

327. Walsh BH, Neil J, Morey J, Yang E, Silvera M v, Inder TE, et al. The Frequency and Severity of Magnetic Resonance Imaging Abnormalities in Infants with Mild Neonatal Encephalopathy. The Journal of pediatrics . 2017/05/04. 2017 Aug;187:26-33.e1. Available from: https://pubmed.ncbi.nlm.nih.gov/28479101

328. Montaldo P, Lally PJ, Oliveira V, Swamy R, Mendoza J, Atreja G, et al. Therapeutic hypothermia initiated within 6 hours of birth is associated with reduced brain injury on MR biomarkers in mild hypoxic-ischaemic encephalopathy: a non-randomised cohort study. Archives of Disease in Childhood - Fetal and Neonatal Edition . 2019 Sep 1;104(5):F515 LP-F520. Available from: http://fn.bmj.com/content/104/5/F515.abstract

329. Lally PJ, Montaldo P, Oliveira V, Soe A, Swamy R, Bassett P, et al. Magnetic resonance spectroscopy assessment of brain injury after moderate hypothermia in neonatal encephalopathy: a prospective multicentre cohort study. The Lancet Neurology . 2019 Jan 1 [cited 2021 Feb 10];18(1):35–45. Available from: http://www.ncbi.nlm.nih.gov/pubmed/30447969

330. Gunn AJ, Bennet L. Fetal hypoxia insults and patterns of brain injury: insights from animal models. Clinics in perinatology . 2009 Sep;36(3):579–93. Available from: https://pubmed.ncbi.nlm.nih.gov/19732615

331. Gunn AJ, Thoresen M. Animal studies of neonatal hypothermic neuroprotection have translated well in to practice. Resuscitation . 2015;97:88–90. Available from: http://www.sciencedirect.com/science/article/pii/S0300957215001628

332. Thoresen M, Tooley J, Liu X, Jary S, Fleming P, Luyt K, et al. Time is brain: starting therapeutic hypothermia within three hours after birth improves motor outcome in asphyxiated newborns. Neonatology. 2013;104(3):228–33.

333. Carreras N, Alsina M, Alarcon A, Arca-Díaz G, Agut T, García-Alix A. Efficacy of passive hypothermia and adverse events during transport of asphyxiated newborns according to the severity of hypoxic-ischemic encephalopathy. Jornal de pediatria. 2018;94(3):251–7.

334. Davidson JO, Wassink G, Yuill CA, Zhang FG, Bennet L, Gunn AJ. How long is too long for cerebral cooling after ischemia in fetal sheep? Journal of cerebral blood flow and metabolism : official journal of the International Society of Cerebral Blood Flow and Metabolism . 2015/01/21. 2015 May;35(5):751–8. Available from: https://pubmed.ncbi.nlm.nih.gov/25605291

335. Shankaran S, Laptook AR, Pappas A, McDonald SA, Das A, Tyson JE, et al. Effect of Depth and Duration of Cooling on Death or Disability at Age 18 Months Among Neonates With Hypoxic-Ischemic Encephalopathy: A Randomized Clinical Trial. JAMA. 2017 Jul;318(1):57–67.

336. Sarnat HB, Sarnat MS. Neonatal encephalopathy following fetal distress. A clinical and electroencephalographic study. Archives of neurology. 1976 Oct;33(10):696–705.

337. Sarnat HB, Flores-Sarnat L, Fajardo C, Leijser LM, Wusthoff C, Mohammad K. Sarnat Grading Scale for Neonatal Encephalopathy after 45 Years: An Update Proposal. Pediatric Neurology . 2020;113:75–9. Available from: http://www.sciencedirect.com/science/article/pii/S0887899420302976

338. Chalak LF, Sánchez PJ, Adams-Huet B, Laptook AR, Heyne RJ, Rosenfeld CR. Biomarkers for Severity of Neonatal Hypoxic-Ischemic Encephalopathy and Outcomes in Newborns Receiving Hypothermia Therapy. The Journal of Pediatrics . 2014;164(3):468-474.e1. Available from: http://www.sciencedirect.com/science/article/pii/S0022347613013632

339. Denihan NM, Kirwan JA, Walsh BH, Dunn WB, Broadhurst DI, Boylan GB, et al. Untargeted metabolomic analysis and pathway discovery in perinatal asphyxia and hypoxic-ischaemic encephalopathy. Journal of Cerebral Blood Flow & Metabolism . 2017 Aug 25;39(1):147–62. Available from: https://doi.org/10.1177/0271678X17726502

340. Ennen CS, Huisman TAGM, Savage WJ, Northington FJ, Jennings JM, Everett AD, et al. Glial fibrillary acidic protein as a biomarker for neonatal hypoxic-ischemic encephalopathy treated with whole-body cooling. American Journal of Obstetrics and Gynecology . 2011;205(3):251.e1-251.e7. Available from: http://www.sciencedirect.com/science/article/pii/S0002937811007538

341. Ennen C, Savage W, Northington F, Huisman T, Everett A, Graham E. 68: Serum glial fibrillary acidic protein as a biomarker in term neonates with hypoxic ischemic encephalopathy treated with total body cooling. American Journal of Obstetrics and Gynecology . 2011;204(1, Supplement):S38–9. Available from: http://www.sciencedirect.com/science/article/pii/S0002937810013402

342. León-Lozano M-Z, Arnaez J, Valls A, Arca G, Agut T, Alarcón A, et al. Cerebrospinal fluid levels of neuron-specific enolase predict the severity of brain damage in newborns with neonatal hypoxic-ischemic encephalopathy treated with hypothermia. PLOS ONE . 2020 Jun 1;15(6):e0234082. Available from: https://doi.org/10.1371/journal.pone.0234082

343. Ramaswamy V, Horton J, Vandermeer B, Buscemi N, Miller S, Yager J. Systematic Review of Biomarkers of Brain Injury in Term Neonatal Encephalopathy. Pediatric Neurology . 2009;40(3):215–26. Available from: http://www.sciencedirect.com/science/article/pii/S0887899408005961

344. Thoresen M. Supportive Care During Neuroprotective Hypothermia in the Term Newborn: Adverse Effects and Their Prevention. Clinics in Perinatology . 2008;35(4):749–63. Available from: http://www.sciencedirect.com/science/article/pii/S0095510808000614

345. Glass HC, Glidden D, Jeremy RJ, Barkovich AJ, Ferriero DM, Miller SP. Clinical Neonatal Seizures are Independently Associated with Outcome in Infants at Risk for Hypoxic-Ischemic Brain Injury. The Journal of Pediatrics . 2009;155(3):318–23. Available from: http://www.sciencedirect.com/science/article/pii/S0022347609003205

346. Foulkes T, Wood J. Mechanisms of Cold Pain. Channels . 2007 May 23;1(3):154–60. Available from: https://doi.org/10.4161/chan.4692

347. Stevens B, Riahi S, Cardoso R, Ballantyne M, Yamada J, Beyene J, et al. The influence of context on pain practices in the NICU: perceptions of health care professionals. Qualitative health research. 2011 Jun;21(6):757–70.

348. Oberlander TF, Grunau RE, Fitzgerald C, Whitfield MF. Does Parenchymal Brain Injury Affect Biobehavioral Pain Responses in Very Low Birth Weight Infants at 32 Weeks' Postconceptional Age? Pediatrics . 2002 Sep 1;110(3):570 LP – 576. Available from: http://pediatrics.aappublications.org/content/110/3/570.abstract

349. Liow N, Montaldo P, Lally PJ, Teiserskas J, Bassett P, Oliveira V, et al. Preemptive Morphine during Therapeutic Hypothermia after Neonatal Encephalopathy: A Secondary Analysis. Therapeutic Hypothermia and Temperature Management . 2020 Feb 26;10(1):45–52. Available from: https://doi.org/10.1089/ther.2018.0052

350. Thoresen M, Satas S, Løberg EM, Whitelaw A, Acolet D, Lindgren C, et al. Twenty-four hours of mild hypothermia in unsedated newborn pigs starting after a severe global hypoxic-ischemic insult is not neuroprotective. Pediatric research. 2001 Sep;50(3):405–11.

351. Angeles DM, Wycliffe N, Michelson D, Holshouser BA, Deming DD, Pearce WJ, et al. Use of opioids in asphyxiated term neonates: effects on neuroimaging and clinical outcome. Pediatric research. 2005 Jun;57(6):873–8.

352. Simbruner G, Mittal RA, Rohlmann F, Muche R. Systemic hypothermia after neonatal encephalopathy: outcomes of neo.nEURO.network RCT. Pediatrics. 2010 Oct;126(4):e771-8.

353. Natarajan G, Shankaran S, Laptook AR, McDonald SA, Pappas A, Hintz SR, et al. Association between sedation-analgesia and neurodevelopment outcomes in neonatal hypoxic-ischemic encephalopathy. Journal of

perinatology : official journal of the California Perinatal Association. 2018 Aug;38(8):1060–7.

354. Toet MC, Lemmers PMA, van Schelven LJ, van Bel F. Cerebral Oxygenation and Electrical Activity After Birth Asphyxia: Their Relation to Outcome. Pediatrics . 2006 Feb 1;117(2):333 LP – 339. Available from: http://pediatrics.aappublications.org/content/117/2/333.abstract

355. Martinez-Biarge M, Diez-Sebastian J, Rutherford MA, Cowan FM. Outcomes after central grey matter injury in term perinatal hypoxic-ischaemic encephalopathy. Early Human Development . 2010;86(11):675–82. Available from: http://www.sciencedirect.com/science/article/pii/S037837821000215X

356. Martinez-Biarge M, Bregant T, Wusthoff CJ, Chew ATM, Diez-Sebastian J, Rutherford MA, et al. White Matter and Cortical Injury in Hypoxic-Ischemic Encephalopathy: Antecedent Factors and 2-Year Outcome. The Journal of Pediatrics . 2012;161(5):799–807. Available from: http://www.sciencedirect.com/science/article/pii/S0022347612004908

357. Shankaran S, Barnes PD, Hintz SR, Laptook AR, Zaterka-Baxter KM, McDonald SA, et al. Brain injury following trial of hypothermia for neonatal hypoxic–ischaemic encephalopathy. Archives of Disease in Childhood - Fetal and Neonatal Edition . 2012 Nov 1;97(6):F398 LP-F404. Available from: http://fn.bmj.com/content/97/6/F398.abstract

358. Cheong JLY, Coleman L, Hunt RW, Lee KJ, Doyle LW, Inder TE, et al. Prognostic Utility of Magnetic Resonance Imaging in Neonatal Hypoxic-Ischemic Encephalopathy: Substudy of a Randomized Trial. Archives of Pediatrics & Adolescent Medicine . 2012 Jul 1;166(7):634–40. Available from: https://doi.org/10.1001/archpediatrics.2012.284

359. Rutherford M, Biarge MM, Allsop J, Counsell S, Cowan F. MRI of perinatal brain injury. Pediatric Radiology . 2010;40(6):819–33. Available from: https://doi.org/10.1007/s00247-010-1620-z

360. Shankaran S, Laptook AR, Tyson JE, Ehrenkranz RA, Bann CM, Das A, et al. Evolution of encephalopathy during whole body hypothermia for neonatal hypoxic-ischemic encephalopathy. The Journal of pediatrics. 2012 Apr;160(4):567-572.e3.

361. al Naqeeb N, Edwards AD, Cowan FM, Azzopardi D. Assessment of Neonatal Encephalopathy by Amplitude-integrated Electroencephalography. Pediatrics . 1999 Jun 1;103(6):1263 LP – 1271. Available from: http://pediatrics.aappublications.org/content/103/6/1263.abstract

362. Hellström-Westas L. Continuous Electroencephalography Monitoring of the Preterm Infant. Clinics in Perinatology . 2006;33(3):633–47. Available from: http://www.sciencedirect.com/science/article/pii/S0095510806000480

363. Spitzmiller RE, Phillips T, Meinzen-Derr J, Hoath SB. Amplitude-integrated EEG is useful in predicting neurodevelopmental outcome in full-term infants with hypoxic-ischemic encephalopathy: a meta-analysis. Journal of child neurology. 2007 Sep;22(9):1069–78.

364. van Rooij LGM, Toet MC, Osredkar D, van Huffelen AC, Groenendaal F, de Vries LS. Recovery of amplitude integrated electroencephalographic background patterns within 24 hours of perinatal asphyxia. Archives of Disease in Childhood - Fetal and Neonatal Edition . 2005 May 1;90(3):F245 LP-FF251. Available from: http://fn.bmj.com/content/90/3/F245.abstract

365. Thoresen M, Hellström-Westas L, Liu X, de Vries LS. Effect of Hypothermia on Amplitude-Integrated Electroencephalogram in Infants With Asphyxia. Pediatrics . 2010 Jul 1;126(1):e131 LP-e139. Available from: http://pediatrics.aappublications.org/content/126/1/e131.abstract

366. Monod N, Pajot N, Guidasci S. The neonatal EEG: Statistical studies and prognostic value in full-term and pre-term babies. Electroencephalography and Clinical Neurophysiology . 1972;32(5):529–44. Available from: http://www.sciencedirect.com/science/article/pii/0013469472900636

367. Holmes G, Rowe J, Hafford J, Schmidt R, Testa M, Zimmerman A. Prognostic value of the electroencephalogram in neonatal asphyxia. Electroencephalography and Clinical Neurophysiology . 1982;53(1):60–72. Available from: http://www.sciencedirect.com/science/article/pii/0013469482901067

368. Watanabe K, Miyazaki S, Hara K, Hakamada S. Behavioral state cycles, background EEGs and prognosis of newborns with perinatal hypoxia. Electroencephalography and Clinical Neurophysiology . 1980;49(5):618–25. Available from: http://www.sciencedirect.com/science/article/pii/0013469480904022

369. Murray DM, Boylan GB, Ryan CA, Connolly S. Early EEG Findings in Hypoxic-Ischemic Encephalopathy Predict Outcomes at 2 Years. Pediatrics . 2009 Sep 1;124(3):e459 LP-e467. Available from: http://pediatrics.aappublications.org/content/124/3/e459.abstract

370. Pressler RM, Boylan GB, Morton M, Binnie CD, Rennie JM. Early serial EEG in hypoxic ischaemic encephalopathy. Clinical Neurophysiology . 2001;112(1):31–7. Available from: http://www.sciencedirect.com/science/article/pii/S1388245700005174

371. Takeuchi T, Watanabe K. The EEG evolution and neurological prognosis of perinatal hypoxia neonates. Brain and Development . 1989;11(2):115–20. Available from: https://www.sciencedirect.com/science/article/pii/S0387760489800798

372. Wertheim D, Mercuri E, Faundez J, Rutherford M, Acolet D, Dubowitz L. Prognostic value of continuous electroencephalographic recording in full term infants with hypoxic ischaemic encephalopathy. Archives of Disease in Childhood - Fetal and Neonatal Edition . 1994 Sep 1;71(2):F97 LP-F102. Available from: http://fn.bmj.com/content/71/2/F97.abstract

373. Zeinstra E, Fock JM, Begeer JH, van Weerden TW, Maurits NM, Zweens MJ. The prognostic value of serial EEG recordings following acute neonatal asphyxia in full-term infants. European Journal of Paediatric Neurology .

2001;5(4):155–60. Available from: https://www.sciencedirect.com/science/article/pii/S1090379801904969

374. Boylan GB, Panerai RB, Rennie JM, Evans DH, Rabe-Hesketh S, Binnie CD. Cerebral blood flow velocity during neonatal seizures. Archives of Disease in Childhood - Fetal and Neonatal Edition . 1999 Mar 1;80(2):F105 LP-F110. Available from: http://fn.bmj.com/content/80/2/F105.abstract

375. Miller SP, Weiss J, Barnwell A, Ferriero DM, Latal-Hajnal B, Ferrer-Rogers A, et al. Seizure-associated brain injury in term newborns with perinatal asphyxia. Neurology. 2002 Feb;58(4):542–8.

376. Kwon JM, Guillet R, Shankaran S, Laptook AR, McDonald SA, Ehrenkranz RA, et al. Clinical Seizures in Neonatal Hypoxic-Ischemic Encephalopathy Have No Independent Impact on Neurodevelopmental Outcome: Secondary Analyses of Data from the Neonatal Research Network Hypothermia Trial. Journal of Child Neurology . 2010 Oct 4;26(3):322–8. Available from: https://doi.org/10.1177/0883073810380915

377. Pisani F, Copioli C, di Gioia C, Turco E, Sisti L. Neonatal Seizures: Relation of Ictal Video-Electroencephalography (EEG) Findings With Neurodevelopmental Outcome. Journal of Child Neurology . 2008 Apr 1;23(4):394–8. Available from: https://doi.org/10.1177/0883073807309253

378. Pisani F, Spagnoli C. Monitoring of newborns at high risk for brain injury. Italian Journal of Pediatrics . 2016;42(1):48. Available from: https://doi.org/10.1186/s13052-016-0261-8

379. Filan P, Boylan GB, Chorley G, Davies A, Fox GF, Pressler R, et al. The relationship between the onset of electrographic seizure activity after birth and the time of cerebral injury in utero. BJOG: An International Journal of Obstetrics & Gynaecology . 2005 Apr 1;112(4):504–7. Available from: https://doi.org/10.1111/j.1471-0528.2004.00476.x

380. Isaeva E, Isaev D, Savrasova A, Khazipov R, Holmes GL. Recurrent neonatal seizures result in long-term increases in neuronal network excitability

in the rat neocortex. European Journal of Neuroscience . 2010 Apr 1;31(8):1446–55. Available from: https://doi.org/10.1111/j.1460-9568.2010.07179.x

381. McCabe BK, Silveira DC, Cilio MR, Cha BH, Liu X, Sogawa Y, et al. Reduced Neurogenesis after Neonatal Seizures. The Journal of Neuroscience . 2001 Mar 15;21(6):2094 LP – 2103. Available from: http://www.jneurosci.org/content/21/6/2094.abstract

382. Glass HC, Hong KJ, Rogers EE, Jeremy RJ, Bonifacio SL, Sullivan JE, et al. Risk factors for epilepsy in children with neonatal encephalopathy. Pediatric research . 2011 Nov;70(5):535–40. Available from: https://pubmed.ncbi.nlm.nih.gov/21796017

383. Dammann O. Persistent neuro-inflammation in cerebral palsy: a therapeutic window of opportunity? Acta paediatrica (Oslo, Norway : 1992). 2007 Jan;96(1):6–7.

384. Malone A, Ryan CA, Fitzgerald A, Burgoyne L, Connolly S, Boylan GB. Interobserver agreement in neonatal seizure identification. Epilepsia. 2009 Sep;50(9):2097–101.

385. Rennie JM, de Vries LS, Blennow M, Foran A, Shah DK, Livingstone V, et al. Characterisation of neonatal seizures and their treatment using continuous EEG monitoring: a multicentre experience. Archives of Disease in Childhood - Fetal and Neonatal Edition . 2019 Sep 1;104(5):F493 LP-F501. Available from: http://fn.bmj.com/content/104/5/F493.abstract

386. Mizrahi EM, Kellaway P. Characterization and classification of neonatal seizures. Neurology . 1987 Dec 1;37(12):1837 LP – 1837. Available from: http://n.neurology.org/content/37/12/1837.abstract

387. Weiner SP, Painter MJ, Geva D, Guthrie RD, Scher MS. Neonatal seizures: Electroclinical dissociation. Pediatric Neurology . 1991;7(5):363–8. Available from: https://www.sciencedirect.com/science/article/pii/088789949190067U

388. Nash KB, Bonifacio SL, Glass HC, Sullivan JE, Barkovich AJ, Ferriero DM, et al. Video-EEG monitoring in newborns with hypoxic-ischemic encephalopathy treated with hypothermia. Neurology . 2011 Feb 8;76(6):556–62. Available from: https://pubmed.ncbi.nlm.nih.gov/21300971

389. Mariani E, Scelsa B, Pogliani L, Introvini P, Lista G. Prognostic Value of Electroencephalograms in Asphyxiated Newborns Treated With Hypothermia. Pediatric Neurology . 2008;39(5):317–24. Available from: https://www.sciencedirect.com/science/article/pii/S0887899408003731

390. Hamelin S, Delnard N, Cneude F, Debillon T, Vercueil L. Influence of hypothermia on the prognostic value of early EEG in full-term neonates with hypoxic ischemic encephalopathy. Neurophysiologie Clinique/Clinical Neurophysiology . 2011;41(1):19–27. Available from: http://www.sciencedirect.com/science/article/pii/S0987705310001097

391. Drury PP, Gunn ER, Bennet L, Gunn AJ. Mechanisms of Hypothermic Neuroprotection. Clinics in Perinatology . 2014;41(1):161–75. Available from: http://www.sciencedirect.com/science/article/pii/S0095510813001310

392. Low E, Boylan GB, Mathieson SR, Murray DM, Korotchikova I, Stevenson NJ, et al. Cooling and seizure burden in term neonates: an observational study. Archives of Disease in Childhood - Fetal and Neonatal Edition . 2012 Jul 1;97(4):F267 LP-F272. Available from: http://fn.bmj.com/content/97/4/F267.abstract

393. Wusthoff CJ, Dlugos DJ, Gutierrez-Colina A, Wang A, Cook N, Donnelly M, et al. Electrographic seizures during therapeutic hypothermia for neonatal hypoxic-ischemic encephalopathy. Journal of child neurology . 2011/03/29. 2011 Jun;26(6):724–8. Available from: https://pubmed.ncbi.nlm.nih.gov/21447810

394. Elshorbagy HH, Azab AA, Kamal NM, Barseem NF, Bassiouny MM, Elsayed MA, et al. Value of electroencephalographic monitoring in newborns with hypoxic-ischemic encephalopathy treated with hypothermia. Journal of

pediatric neurosciences . 2016;11(4):309–15. Available from: https://pubmed.ncbi.nlm.nih.gov/28217152

395. Murray DM, Boylan GB, Ali I, Ryan CA, Murphy BP, Connolly S. Defining the gap between electrographic seizure burden, clinical expression and staff recognition of neonatal seizures. Archives of disease in childhood Fetal and neonatal edition. 2008 May;93(3):F187-91.

396. Kharoshankaya L, Stevenson NJ, Livingstone V, Murray DM, Murphy BP, Ahearne CE, et al. Seizure burden and neurodevelopmental outcome in neonates with hypoxic–ischemic encephalopathy. Developmental Medicine & Child Neurology . 2016 Dec 1;58(12):1242–8. Available from: https://doi.org/10.1111/dmcn.13215

397. van Laerhoven H, de Haan TR, Offringa M, Post B, van der Lee JH. Prognostic Tests in Term Neonates With Hypoxic-Ischemic Encephalopathy: A Systematic Review. Pediatrics . 2013 Jan 1;131(1):88 LP – 98. Available from: http://pediatrics.aappublications.org/content/131/1/88.abstract

398. Gambi D, Rossini PM, Albertini G, Sollazzo D, Torriolo MG, Polidori GC. Follow-up of visual evoked potential in full-term and pre-term control newborns and in subjects who suffered from perinatal respiratory distress. Electroencephalography and Clinical Neurophysiology . 1980;48(5):509–16. Available from: http://www.sciencedirect.com/science/article/pii/0013469480902862

399. Hakamada S, Watanabe K, Hara K, Miyazaki S. The evolution of visual and auditory evoked potentials in infants with perinatal disorder. Brain & development. 1981;3(4):339–44.

400. Suppiej A, Cappellari A, Franzoi M, Traverso A, Ermani M, Zanardo V. Bilateral loss of cortical somatosensory evoked potential at birth predicts cerebral palsy in term and near-term newborns. Early Human Development . 2010;86(2):93–8. Available from: http://www.sciencedirect.com/science/article/pii/S0378378210000356

401. Reilly EL, Kondo C, Brunberg JA, Doty DB. Visual evoked potentials during hypothermia and prolonged circulatory arrest. Electroencephalography and Clinical Neurophysiology . 1978;45(1):100–6. Available from: http://www.sciencedirect.com/science/article/pii/0013469478903462

402. Markand ON, Warren CH, Moorthy SS, Stoelting RK, King RD. Monitoring of multimodality evoked potentials during open heart surgery under hypothermia. Electroencephalography and Clinical Neurophysiology/Evoked Potentials Section . 1984;59(6):432–40. Available from: http://www.sciencedirect.com/science/article/pii/0168559784900029

403. FitzGibbon T, Hayward JS, Walker D. EEG and visual evoked potentials of conscious man during moderate hypothermia. Electroencephalography and Clinical Neurophysiology . 1984;58(1):48–54. Available from: http://www.sciencedirect.com/science/article/pii/0013469484901998

404. Takaki O, Kuro M, Nakajima T, Hayashi Y, Kitaguchi K, Shibata H. [Effects of hypothermia with cardiopulmonary bypass on posterior tibial nerve somatosensory evoked potentials in man]. Masui The Japanese journal of anesthesiology. 1992 Jul;41(7):1119–24.

405. Zanatta P, Bosco E, Comin A, Mazzarolo AP, di Pasquale P, Forti A, et al. Effect of mild hypothermic cardiopulmonary bypass on the amplitude of somatosensory-evoked potentials. Journal of neurosurgical anesthesiology. 2014 Apr;26(2):161–6.

406. Agatiello M, Toffoli E, Scarabel F, Tessari L, Traverso A, Bizzaro E, et al. 17. Somatosensory evoked potentials recorded during hypothermia in neonatal hypoxic–ischemic encephalopathy. Clinical Neurophysiology . 2015;126(1):e5. Available from: http://www.sciencedirect.com/science/article/pii/S1388245714005768

407. Nevalainen P, Marchi V, Metsäranta M, Lönnqvist T, Toiviainen-Salo S, Vanhatalo S, et al. Evoked potentials recorded during routine EEG predict outcome after perinatal asphyxia. Clinical Neurophysiology . 2017;128(7):1337–

43. Available from:
http://www.sciencedirect.com/science/article/pii/S1388245717301815

408. Cainelli E, Trevisanuto D, Cavallin F, Manara R, Suppiej A. Evoked potentials predict psychomotor development in neonates with normal MRI after hypothermia for hypoxic-ischemic encephalopathy. Clinical Neurophysiology . 2018;129(6):1300–6. Available from:
http://www.sciencedirect.com/science/article/pii/S1388245718306588

409. Garfinkle J, Wintermark P, Shevell MI, Platt RW, Oskoui M, Andersen J, et al. Cerebral Palsy after Neonatal Encephalopathy: How Much Is Preventable? The Journal of Pediatrics . 2015;167(1):58-63.e1. Available from:
http://www.sciencedirect.com/science/article/pii/S0022347615001559

410. Banoub M, Tetzlaff JE, Schubert A. Pharmacologic and Physiologic Influences Affecting Sensory Evoked Potentials: Implications for Perioperative Monitoring. Anesthesiology . 2003 Sep 1;99(3):716–37. Available from:
https://doi.org/10.1097/00000542-200309000-00029

411. Russ W, Sticher J, Scheld H, Hempelmann G. Effects of hypothermia on somatosensory evoked responses in man. British journal of anaesthesia. 1987 Dec;59(12):1484–91.

412. Madhok J, Wu D, Xiong W, Geocadin RG, Jia X. Hypothermia amplifies somatosensory-evoked potentials in uninjured rats. Journal of neurosurgical anesthesiology . 2012 Jul;24(3):197–202. Available from:
https://pubmed.ncbi.nlm.nih.gov/22441433

413. Montaldo P, Vakharia A, Ivain P, Mendoza J, Oliveira V, Markati T, et al. Pre-emptive opioid sedation during therapeutic hypothermia. Archives of Disease in Childhood - Fetal and Neonatal Edition . 2020 Jan 1;105(1):108 LP – 109. Available from: http://fn.bmj.com/content/105/1/108.abstract

414. Róka A, Melinda KT, Vásárhelyi B, Machay T, Azzopardi D, Szabó M. Elevated morphine concentrations in neonates treated with morphine and prolonged hypothermia for hypoxic ischemic encephalopathy. Pediatrics . 2008

Apr 1;121(4):e844 LP-e849. Available from: http://pediatrics.aappublications.org/content/121/4/e844.abstract

415. Frymoyer A, Bonifacio SL, Drover DR, Su F, Wustoff CJ, van Meurs KP. Decreased Morphine Clearance in Neonates With Hypoxic Ischemic Encephalopathy Receiving Hypothermia. Journal of Clinical Pharmacology. 2017 Jan;57(1):64–76.

416. Knibbe CAJ, Krekels EHJ, van den Anker JN, DeJongh J, Santen GWE, van Dijk M, et al. Morphine glucuronidation in preterm neonates, infants and children younger than 3 years. Clinical pharmacokinetics. 2009;48(6):371–85.

417. Favié LMA, Groenendaal F, van den Broek MPH, Rademaker CMA, de Haan TR, van Straaten HLM, et al. Pharmacokinetics of morphine in encephalopathic neonates treated with therapeutic hypothermia. PLoS ONE . 2019 Feb 14;14(2):e0211910. Available from: https://doi.org/10.1371/journal.pone.0211910

418. Pacifici GM. Clinical Pharmacology of Phenobarbital in Neonates: Effects, Metabolism and Pharmacokinetics. Current pediatric reviews. 2016;12(1):48–54.

419. Favié LMA, Groenendaal F, van den Broek MPH, Rademaker CMA, de Haan TR, van Straaten HLM, et al. Phenobarbital, Midazolam Pharmacokinetics, Effectiveness, and Drug-Drug Interaction in Asphyxiated Neonates Undergoing Therapeutic Hypothermia. Neonatology . 2019;116(2):154–62. Available from: https://www.karger.com/DOI/10.1159/000499330

420. Filippi L, la Marca G, Cavallaro G, Fiorini P, Favelli F, Malvagia S, et al. Phenobarbital for neonatal seizures in hypoxic ischemic encephalopathy: a pharmacokinetic study during whole body hypothermia. Epilepsia. 2011 Apr;52(4):794–801.

421. Lutz IC, Allegaert K, de Hoon JN, Marynissen H. Pharmacokinetics during therapeutic hypothermia for neonatal hypoxic ischaemic

encephalopathy: A literature review. BMJ Paediatrics Open . 2020 Jun 1;4(1):e000685. Available from: http://bmjpaedsopen.bmj.com/content/4/1/e000685.abstract

422. Pokorná P, Posch L, Šíma M, Klement P, Slanar O, van den Anker J, et al. Severity of asphyxia is a covariate of phenobarbital clearance in newborns undergoing hypothermia. The journal of maternal-fetal & neonatal medicine : the official journal of the European Association of Perinatal Medicine, the Federation of Asia and Oceania Perinatal Societies, the International Society of Perinatal Obstetricians. 2019 Jul;32(14):2302–9.

423. Shellhaas RA, Ng CM, Dillon CH, Barks JDE, Bhatt-Mehta V. Population pharmacokinetics of phenobarbital in infants with neonatal encephalopathy treated with therapeutic hypothermia. Pediatric critical care medicine : a journal of the Society of Critical Care Medicine and the World Federation of Pediatric Intensive and Critical Care Societies. 2013 Feb;14(2):194–202.

424. Šíma M, Pokorná P, Hronová K, Slanař O. Effect of co-medication on the pharmacokinetic parameters of phenobarbital in asphyxiated newborns. Physiological research. 2015;64(Suppl 4):S513-9.

425. van den Broek MPH, Groenendaal F, Toet MC, van Straaten HLM, van Hasselt JGC, Huitema ADR, et al. Pharmacokinetics and clinical efficacy of phenobarbital in asphyxiated newborns treated with hypothermia: a thermopharmacological approach. Clinical pharmacokinetics. 2012 Oct;51(10):671–9.

426. Salama H, Young GB, da Silva OP. Effect of Continuous Infusion of Morphine on the Electroencephalogram of Infants, Admitted to a Neonatal Intensive Care Unit. Pediatric Research . 1999;45(7):347. Available from: https://doi.org/10.1203/00006450-199904020-02061

427. Young GB, da Silva OP. Effects of morphine on the electroencephalograms of neonates: a prospective, observational study. Clinical

Neurophysiology . 2000;111(11):1955–60. Available from: http://www.sciencedirect.com/science/article/pii/S1388245700004338

428. Pathak KS, Brown RH, Cascorbi HF, Nash CLJ. Effects of fentanyl and morphine on intraoperative somatosensory cortical-evoked potentials. Anesthesia and analgesia. 1984 Sep;63(9):833–7.

429. Murray DM, O'Connor CM, Ryan CA, Korotchikova I, Boylan GB. Early EEG Grade and Outcome at 5 Years After Mild Neonatal Hypoxic Ischemic Encephalopathy. Pediatrics . 2016 Oct 1;138(4):e20160659. Available from: http://pediatrics.aappublications.org/content/138/4/e20160659.abstract

430. Robertson CM, Finer NN. Long-term follow-up of term neonates with perinatal asphyxia. Clinics in perinatology. 1993 Jun;20(2):483–500.

431. Chalak LF, Nguyen K-A, Prempunpong C, Heyne R, Thayyil S, Shankaran S, et al. Prospective research in infants with mild encephalopathy identified in the first six hours of life: neurodevelopmental outcomes at 18–22 months. Pediatric Research . 2018;84(6):861–8. Available from: https://doi.org/10.1038/s41390-018-0174-x

432. Gibbins S, Stevens BJ, Yamada J, Dionne K, Campbell-Yeo M, Lee G, et al. Validation of the Premature Infant Pain Profile-Revised (PIPP-R). Early Human Development . 2014;90(4):189–93. Available from: http://www.sciencedirect.com/science/article/pii/S0378378214000140

433. Huang XZ, Li L, Zhou J, He F, Zhong CX, Wang B. Evaluation of three pain assessment scales used for ventilated neonates. Journal of Clinical Nursing . 2018 Oct 1;27(19–20):3522–9. Available from: https://doi.org/10.1111/jocn.14585

434. Ferree TC. Spherical splines and average referencing in scalp electroencephalography. Brain Topogr. 2006 Winter;19(1-2):43-52. doi: 10.1007/s10548-006-0011-0. PMID: 17019635.

435. Mele G, Cavaliere C, Alfano V, Orsini M, Salvatore M and Aiello M. Simultaneous EEG-fMRI for Functional Neurological Assessment. Front. Neurol. 2019 10:848. doi: 10.3389/fneur.2019.00848

436. Deffieux T, Demene C, Pernot M, Tanter M. Functional ultrasound neuroimaging: a review of the preclinical and clinical state of the art. Curr Opin Neurobiol. 2018 Jun; 50:128-135. doi: 10.1016/j.conb.2018.02.001. Epub 2018 Feb 22. PMID: 29477979

Chapter 8 APPENDICES

Appendix 1 Participant information leaflets 235

Appendix 2 Patient consent forms 245

APPENDIX 1 PARTICIPANT INFORMATION LEAFLETS

Patient Information Leaflet for studies involving a clinical procedure



Your child is, or may be, eligible to take part in a research study. Before you decide, it is important that you understand why the research is being done and what it involves. Please read the following information carefully and ask us if anything is unclear or if you would like more information.

1. Study title: Investigating pain in the developing human brain

2. What is the purpose of the study?

Infants in hospital often need to have many procedures as part of their routine medical treatment, which may cause discomfort. As they cannot tell us how much these procedures hurt, it is difficult to know how much pain they are feeling and to make sure that they receive the right medicines. We know that infants can process discomfort and pain in their brains and we have developed a method of assessing this pain-related brain activity. We also know that infants show that they are in pain using different behaviours. These may be indicated by changes in heart rate and breathing rate in response to pain.

The aim of this research is to understand more about how infants experience pain, so that better ways of treating pain can be developed. We are also interested in how infants respond to different stimuli from their environment, such as light and sound, and how this might change across development.

3. Does my child have to take part?

No, it is your decision whether or not your child takes part. If you decide to allow your child to take part, you will be asked to sign a consent form. If you decide you do not want your child to take part, this will not affect your child's care.

If you decide you would like your child to take part, you can change your mind at any time and withdraw your child from the study by telling the research team. You do not have to give a reason. You will be asked if we can use the data/images that have already been collected for analysis (all data) and for publication of results (anonymised data only).

4. What is involved in the study?

In this study we would like to understand how infants respond to clinically-required procedures, such as blood tests. **No clinical procedures will be carried out solely for research purposes.** We will only study your child during a procedure that is needed for clinical purposes.

As we are interested in how infants respond to different stimuli in their environment, we may also ask to study your baby during a control procedure and in response to sharp touch. These do not pierce the skin, but they stimulate the receptors that we are interested in generally without waking or upsetting the infant.

We will assess your child's response by measuring their activity. We may also video your child's face, and measure other responses such as muscle activity, heart rate and oxygen saturations. We will monitor your child before, during and after the clinical procedure. On rare occasions we may ask to monitor your child for up to 24 hours before and/or after the clinical procedure.

Clinical procedures will be completed in the routine way. The study will not interfere with your child's clinical care, nor will there be any delay if an emergency procedure is required. We may also explore the impact of pain relief and comfort measures on your child.

As we are interested in how your child's response to pain changes as they grow, we may ask if

we can study your child more than once during their stay in hospital. We will also ask you if we can contact you in the future, to ask if you would be happy for your child to take part in other research studies. If you agree that we can contact you in the future about other research studies, we will also record your contact details. Your contact details will not be passed onto anyone outside of the research team. You can opt-out of this at any point by contacting Prof Rebeccah Slater (details below). Your agreement for us to contact you does not form any obligation to participate in future research.

We may use the following recording measures for your child:

Measuring brain activity

Electroencephalography (EEG): EEG is a portable imaging system to measure brain activity. It involves gently placing electrodes (small metal discs) on the head using a paste that can be washed off with soap and water. EEG is routinely used on the neonatal unit, children's wards and clinics.

Near Infrared Spectroscopy (NIRS): NIRS is a non-invasive technique to measure brain activity. It involves placing lights and detectors on the head to record changes in blood and tissue oxygen levels.

Measuring other responses

Electromyography (EMG): EMG is a safe non-invasive technique to record muscle activity. Small electrodes will be placed on the skin over the muscle to see if your child pulls away during the stimulation (and clinical procedure if relevant).

Vital sign monitoring: Small adhesive electrodes may be placed on your child's chest to measure changes in heart rate (this is called an ECG) and breathing rate. A small probe may also be wrapped around your child's foot to measure changes in blood oxygen levels.

Videoing your child: We may also video your child during the study. This is so that we can assess changes in facial expression and body movements, and to record the exact timing of the stimulation or clinical procedure.

We may also approach you to ask if you are happy for us to use these images for teaching, publicity and/or scientific journals. If you agree, we will take separate consent for this as your child's face would be visible in the video footage. This is not a mandatory part of the study. If you choose not to allow us to use the images in this way, this will not affect your child's care or prevent your child from participating in this research.

5. Are there any additional risks or benefits for my child?

Obtaining video footage of your child is non-invasive and does not present any risk to your child. EEG, EMG and ECG have been used clinically for over 20 years without any adverse effects. All studies have a dedicated team of healthcare professionals and researchers that will ensure the safety of your child at all times. We are not aware of any risks for your child taking part in this study.

The data collected are for research, so will not be reviewed by a doctor routinely. If any clinically significant findings are identified at the time of the study then the research team will report these to the clinical care team to handle as appropriate.

There are no direct benefits of participating in this research. This study is designed to gather information, to help guide improvements in care for infants in the future. If your child becomes

distressed, the research study will be paused or stopped. Any clinically required procedures will still go ahead if the treating clinician feels that this is appropriate.

6. What information will be collected about my child?

We will collect clinical information about your child, for example their gestational age at birth and any medications they have received. This information helps us to determine which factors may influence the way an infant copes with pain. We will also collect information about your child's brain activity, and may collect information about your child's muscle activity, vital signs (such as heart rate and breathing rate), and recordings of their facial expressions and body movements.

All information and videos that are collected during this research study will be kept strictly confidential. Each infant will be allocated a study number which will be used to label all data. This study has been registered with the data protection registration office and forms part of an educational programme.

7. What will happen to the results?

Results will be analysed and published in a journal. All publications will be made available on our website **https://neuroimaging.paediatrics.ox.ac.uk**. The findings may also be used for teaching or academic research presentations. No identifying information will be presented about you or your child, unless you have provided specific consent for us to use videos/images of your child in this way.

8. What will happen to my child's data?

We will be using information collected from your child and their medical records in order to conduct this study. Research is a task that we perform in the public interest. The University of Oxford, as Sponsor, is the data controller. This means that we, as University of Oxford researchers, are responsible for looking after the information collected and using it properly. We will use the minimum personally-identifiable information possible. We will keep identifiable information about your child for up to 5 years after the study has finished. This excludes any research documents with personal information, such as consent forms, which will be held securely at the University of Oxford for 25 years after the end of the study.

Data protection regulation provides you with control over your personal data and how it is used. When you agree to your information being used in research, however, some of those rights may be limited in order for the research to be reliable and accurate. Further information about your rights with respect to your personal data is available at
http://www.admin.ox.ac.uk/councilsec/compliance/gdpr/individualrights/

You can find out more about how we use your information from the contacts in section 12.

Research data may be shared with other researchers doing similar work, both here and abroad. Responsible members of the University of Oxford or the Oxford University Hospitals NHS Trust may be given access to data for monitoring and/or audit of the study to ensure we are complying with regulations.

9. Who is organising and funding this research?

This study is sponsored by University of Oxford and has been funded by The Wellcome Trust. Your doctor will not be paid for including you in this study.

10. Who has reviewed the study?

All research that involves NHS patients has to be approved by a Research Ethics Committee. Approval means that the Committee is satisfied that yours and your child's rights will be respected, that any risks have been reduced to a minimum and balanced against possible benefits, and that you have been given sufficient information on which to make an informed decision about whether to take part. The South Central Oxford C Research Ethics Committee has reviewed and approved this study.

11. Comments or concerns during the study

The University has arrangements in place to provide for harm arising from participation in thestudy for which the University is the Research Sponsor. NHS indemnity operates in respect of the clinical treatment with which your child is provided. If you wish to complain about any aspect of the way in which you have been approached or treated during the course of this study, you should contact Prof Rebeccah Slater (details below) or the University of Oxford Clinical Trials and Research Governance (CTRG) office (tel: 01865 (6)16480, email: ctrg@admin.ox.ac.uk).

12. Contact for further information

Prof Rebeccah Slater (Study Lead)
Professor of Paediatric Neuroscience
University of Oxford
01865 234537
rebeccah.slater@paediatrics.ox.ac.uk

Dr Eleri Adams (Clinical Lead)
Consultant Neonatologist
Oxford University Hospitals NHS Trust
01865 221356
eleri.adams@ouh.nhs.uk



Picture shows example of an EEG study.

Thank you for reading this information leaflet.

Patient information leaflet for studies involving experimental stimuli



Your child is, or may be, eligible to take part in a research study. Before you decide, it is important that you understand why the research is being done and what it involves. Please read the following information carefully and ask us if anything is unclear or if you would like more information.

1. Study title: Investigating pain in the developing human brain

2. What is the purpose of the study?

Infants in hospital often need to have many procedures like blood tests as part of their routine medical treatment, which may cause discomfort. As they cannot tell us how much these procedures hurt, it is difficult to know how much pain they are feeling and to make sure that they receive the right medicines. We know that infants can process discomfort and pain in their brains and we have developed a method of assessing this pain-related brain activity. We also know that infants show that they are in pain using different behaviours. These may be indicated by changes in heart rate and breathing rate in response to pain.

The aim of this research is to understand more about how infants experience pain, so that better ways of treating pain can be developed. We are also interested in how infants respond to different stimuli from their environment, such as light and sound, and how this might change across development.

3. Does my child have to take part?

No, it is your decision whether or not your child takes part. If you decide to allow your child to take part, you will be asked to sign a consent form. If you decide you do not want your child to take part, this will not affect your child's care.

If you decide you would like your child to take part, you can change your mind at any time and withdraw your child from the study by telling the research team. You do not have to give a reason. You will be asked if we can use the data/images that have already been collected for analysis (all data) and for publication of results (anonymised data only).

4. What is involved in the study?

In this study we would like to understand how infants respond to different sensory stimuli, for example:

- Light touch
- Sharp touch (this does not pierce the skin, but does stimulate the receptors that we are interested in without generally waking or upsetting the infant)
- Sound
- Light

We will assess your child's response by measuring their brain activity. We may also video your child's face, and measure other responses such as muscle activity, heart rate and oxygen saturations.

If your child requires a clinical procedure (such as a blood test), we may ask if we can also study their response to this. Clinical procedures will be completed in the routine way. The study will not interfere with your child's clinical care, nor will there be any delay if an emergency procedure is required. We may also ask to study your child during a control procedure (this will not pierce the skin). We may also explore the impact of pain relief and comfort measures on your child.

No clinical procedures will be carried out solely for research purposes. We may monitor

your child before, during and after the stimulus (and clinical procedure if your child requires one). On rare occasions we may ask to monitor your child for up to 24 hours before and/or after the experimental stimulus/clinical procedure if your child requires one.

As we are interested in how your child's response to pain changes as they grow, we may ask if we can study your child more than once during their stay in hospital. We will also ask you if we can contact you in the future, to ask if you would be happy for your child to take part in other research studies. If you agree that we can contact you in the future about other research studies, we will also record your contact details. Your contact details will not be passed onto anyone outside of the research team. You can opt-out of this at any point by contacting Dr Rebeccah Slater (details below). Your agreement for us to contact you does not form any obligation to participate in future research.

We may use the following recording measures for your child:

Measuring brain activity

Electroencephalography (EEG): EEG is a portable imaging system to measure brain activity. It involves gently placing electrodes (small metal discs) on the head using a paste that can be washed off with soap and water. EEG is routinely used on the neonatal unit, children's wards and clinics.

Near Infrared Spectroscopy (NIRS): NIRS is a non-invasive technique to measure brain activity. It involves placing lights and detectors on the head to record changes in blood and tissue oxygen levels.

Measuring other responses

Electromyography (EMG): EMG is a safe non-invasive technique to record muscle activity. Small electrodes will be placed on the skin over the muscle to see if your child pulls away during the stimulation (and clinical procedure if relevant).

Vital sign monitoring: Small adhesive electrodes may be placed on your child's chest to measure changes in heart rate (this is called an ECG) and breathing rate. A small probe may also be wrapped around your child's foot to measure changes in blood oxygen levels.

Videoing your child: We may also video your child during the study. This is so that we can assess changes in facial expression and body movements, and to record the exact timing of the stimulation or clinical procedure.

We may also approach you to ask if you are happy for us to use these images for teaching, publicity and/or scientific journals. If you agree, we will take separate consent for this as your child's face would be visible in the video footage. This is not a mandatory part of the study. If you choose not to allow us to use the images in this way, this will not affect your child's care or prevent your child from participating in this research.

5. Are there any additional risks or benefits for my child?

Obtaining video footage of your child is non-invasive and does not present any risk to your child. EEG, EMG and ECG have been used clinically for over 20 years without any adverse effects. The stimuli which will be applied to your child have been used in many other patient groups. All studies have a dedicated team of healthcare professionals and researchers that will ensure the safety of your child at all times. We are not aware of any risks for your child taking part in this study.

The data collected are for research, so will not be reviewed by a doctor routinely. If any clinically significant findings are identified at the time of the study then the research team will report these to the clinical care team to handle as appropriate.

There are no direct benefits of participating in this research. This study is designed to gather information, to help guide improvements in care for infants in the future. If your child becomes distressed, the research study will be paused or stopped. Any clinically required procedures will still go ahead if the treating clinician feels that this is appropriate.

6. What information will be collected about my child?

We will collect clinical information about your child, for example their gestational age at birth and any medications they have received. This information helps us to determine which factors may influence the way an infant copes with pain. We will also collect information about your child's brain activity, and may collect information about your child's muscle activity, vital signs (such as heart rate and breathing rate), and recordings of their facial expressions and body movements.

All information and videos that are collected during this research study will be kept strictly confidential. Each infant will be allocated a study number which will be used to label all data. This study has been registered with the data protection registration office and forms part of an educational programme.

7. What will happen to the results?

Results will be analysed and published in a journal. All publications will be made available on our website **https://neuroimaging.paediatrics.ox.ac.uk**. The findings may also be used for teaching or academic research presentations. No identifying information will be presented about you or your child, unless you have provided specific consent for us to use videos/images of your child in this way.

8. What will happen to my child's data?

We will be using information collected from your child and their medical records in order to conduct this study. Research is a task that we perform in the public interest. The University of Oxford, as Sponsor, is the data controller. This means that we, as University of Oxford researchers, are responsible for looking after the information collected and using it properly. We will use the minimum personally-identifiable information possible. We will keep identifiable information about your child for up to 5 years after the study has finished. This excludes any research documents with personal information, such as consent forms, which will be held securely at the University of Oxford for 25 years after the end of the study.

Data protection regulation provides you with control over your personal data and how it is used. When you agree to your information being used in research, however, some of those rights may be limited in order for the research to be reliable and accurate. Further information about your rights with respect to your personal data is available at
http://www.admin.ox.ac.uk/councilsec/compliance/gdpr/individualrights/

You can find out more about how we use your information from the contacts in section 12.

Research data may be shared with other researchers doing similar work, both here and abroad. Responsible members of the University of Oxford or the Oxford University Hospitals NHS Trust may be given access to data for monitoring and/or audit of the study to ensure we are complying with regulations.

9. Who is organising and funding this research?

This study is sponsored by University of Oxford and has been funded by The Wellcome Trust. Your doctor will not be paid for including you in this study.

10. Who has reviewed the study?

All research that involves NHS patients has to be approved by a Research Ethics Committee. Approval means that the Committee is satisfied that yours and your child's rights will be respected, that any risks have been reduced to a minimum and balanced against possible benefits, and that you have been given sufficient information on which to make an informed decision about whether to take part. The South Central Oxford C Research Ethics Committee has reviewed and approved this study.

11. Comments or concerns during the study

The University has arrangements in place to provide for harm arising from participation in the study for which the University is the Research Sponsor. NHS indemnity operates in respect of the clinical treatment with which your child is provided. If you wish to complain about any aspect of the way in which you have been approached or treated during the course of this study, you should contact Prof Rebeccah Slater (details below) or the University of Oxford Clinical Trials and Research Governance (CTRG) office (01865 (6)16480, ctrg@admin.ox.ac.uk).

12. Contact for further information

Prof Rebeccah Slater (Study Lead)
Professor of Paediatric Neuroscience
University of Oxford
01865 234537
rebeccah.slater@paediatrics.ox.ac.uk

Dr Eleri Adams (Clinical Lead)
Consultant Neonatologist
Oxford University Hospitals NHS Trust
01865 221356
eleri.adams@ouh.nhs.uk



Picture shows example of an EEG study.

Thank you for reading this information leaflet.

APPENDIX 2 PATIENT CONSENT FORMS

Consent form (clinical procedure)

Consent Form

Study ID: | | | | | | | | |

Infant's name:

Study Title: Investigating pain in the developing human brain

Chief Investigator: Prof Rebeccah Slater (01865 234537, rebeccah.slater@paediatrics.ox.ac.uk)

Please initial each box

Please complete in black ballpoint pen.

1	I confirm that I have read and understood the information sheet (clinical procedures) (**v9.0, dated 31/05/2019)**, for the above study. I have had the opportunity to ask questions and have had these answered satisfactorily.	☐
2	I understand that my child's participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my child's medical care or legal rights being affected.	☐
3	I understand that relevant sections of my child's medical notes and data collected during the study may be looked at by individuals from the University of Oxford or Oxford University Hospitals NHS Trust, where it is relevant to my child's taking part in this research. I give permission for these individuals to access to my child's records.	☐
4	I agree to my child being videoed during the study. I understand that recorded images will not be used for public use, only analysis. No identifiable information, including video recordings or imaging, will be used in any publications/presentations. Only anonymised data will be published or presented at meetings.	☐
5	I agree for the collected data to be used for teaching or academic research presentations.	☐
6	I agree to my child taking part in the above study.	☐
	OPTIONAL	
7	I agree to my child being studied on more than one occasion, up to a maximum of 5 occasions.	☐
8	I consent to being approached in the future about other research studies that my child may be eligible for.	☐
9	I agree to the images/videos of my child recorded during this study being used for publications and presentations.	☐

Name of parent: _____

Relationship to baby: _____

Signature: _____

Date: _____

Name of investigator taking consent: _____

Signature: _____

Date: _____

1 to be kept as part of the study documentation (original)
1 form for parent
1 to be kept with hospital notes

NIPI Consent Form: clinical procedures v9.0 31/05/2019
REC Ref: 12/SC/0447 Tel: 01865 227988 ***Newborn Care Unit, John Radcliffe,*** *Headley Way, Headington, Oxford, OX3 9DU*

Consent form (experimental procedure)

Consent Form

Study ID: | | | | | | | | |

Infant's name:

Study Title: Investigating pain in the developing human brain

Chief Investigator: Prof Rebeccah Slater (01865 234537, rebeccah.slater@paediatrics.ox.ac.uk)

Please initial each box

Please complete in black ballpoint pen.

1	I confirm that I have read and understood the information sheet (experimental stimuli) (**v9.0, dated 31/05/2019**), for the above study. I have had the opportunity to ask questions and have had these answered satisfactorily.	☐
2	I understand that my child's participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my child's medical care or legal rights being affected.	☐
3	If my child requires a procedure for clinical reasons, I consent to my child being studied for this procedure.	☐
4	I understand that relevant sections of my child's medical notes and data collected during the study may be looked at by individuals from the University of Oxford or Oxford University Hospitals NHS Trust, where it is relevant to my child's taking part in this research. I give permission for these individuals to access to my child's records.	☐
5	I agree to my child being videoed during the study. I understand that recorded images will not be used for public use, only analysis. No identifiable information, including video recordings or imaging, will be used in any publications/presentations. Only anonymised data will be published or presented at meetings.	☐
6	I agree for the collected data to be used for teaching or academic research presentations.	☐
7	I agree to my child taking part in the above study.	☐
	OPTIONAL	
8	I agree to my child being studied on more than one occasion, up to a maximum of 5 occasions.	☐
9	I consent to being approached in the future about other research studies for which my child may be eligible.	☐
10	I agree to the images/videos of my child recorded during this study being used for publications and presentations.	☐

Name of parent:

Relationship to baby

Signature:

Date:

Name of investigator taking consent:

Signature:

Date:

1 to be kept as part of the study documentation (original)
1 form for parent